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FOREWORD

The material presented in this 11th volume of the transactions of the scientific collective of the Tomsk NIIVS [Scientific Research Institute for Vaccines and Sera] is essentially a summary of its activity during the year 1957.

The gradual limitation of the scientific scope of the Tomsk NIIVS has had certain results. Two basic problems, the epidemiology and microbiology of the kray, now constitute 75-80% of the entire scientific work of the institute, special attention being paid to the study of natural-nidus diseases and the development of a scientific basis for the production of bacterial preparations.

In analyzing the contents of this book one is struck by a second fact characteristic of our scientific corrective, i.e., the creative collaboration of science and technology both within and without the institute.

These undoubtedly fruitful creative ties developed as various problems were worked out under the general supervision of the Tomsk NIIVS, in conjunction with a whole series of the departments and clinics of the Tomsk Medical Institute, the oblast epidemiological and public health station, and other organizations. This book also contains individual fragments of doctoral and graduate dissertations prepared at the Stalinsk GIDUV [State Institute for the Specialization and Advanced Training of Physicians] and other laboratories and institutions working in these fields under the supervision of the Tomsk

NIIVS and, in particular, its scientific supervisor, Prof. S.P. Karpov, Corresponding Member of the Academy of Medical Sciences USSR.

A mutually beneficial collaboration also exists between science and our specific manufacturing duties and has led to, for example, the production of an improved preparation for diagnosing tick-borne encephalitis. On the whole, our virologists are now producing a rather complete range of preparations for the diagnosis, treatment, and prophylaxis of tick-borne encephalitis, thus making a definite contribution to the prevention of this disease, a very important one in Siberia.

Studies of new associative vaccines and their influence on the vaccinated organism are in a rather well-developed state.

It must be supposed that the works presented herein have certain inadequacies. The editorial board and the authors' collective will be very grateful for all critical comments which may be made on the material in this book.

B.G. Trukhmanov, Director, Tomsk NIIVS

PROBLEMS OF EPIDEMIOLOGY

AND MICROBIOLOGY

NIDI OF TICK-BORNE ENCEPHALITIS IN WESTERN SIBERIA AND PROBLEMS INVOLVED IN ELIMINATING THEM

S.P. Karpov, V.M. Popov, and A.G. Kolmakova

Tick-borne encephalitis was first diagnosed in Western Siberia in 1939, by N.V. Shubin in the city of Tomsk. During the period 1940-1946 it was discovered in Novosibirskaya, Kemerovskaya, and Omskaya oblasts and Altayskiy kray.

Western Siberia basically occupies the Western Siberian plain, bordering on the so-called Altay uplands on the southeast.

Western Siberia may conditionally be divided into the following topographical-climatic zones: mountainous, steppe, wooded steppe, wooded, wooded tundra, and tundra. The greatest portion of Western Siberia is occupied by the wooded zone, while the wooded steppes cover the least territory. The steppe zone occupies the southern portion of the Western Siberian plain (Altayskiy kray).

The natural nidi of tick-borne encephalitis lie principally in the wooded zone, since the principal reservoir and carrier of the infection, the wood tick Ixodes persulcatus, is found here.

The belief that tick-borne encephalitis occurs only in sparsely-populated forested areas is now being reexamined. Observations have shown that the majority of cases of this disease occur in heavily-populated areas. The natural nidi of Western Siberia may serve as an example of this. In addition to the sparsely-populated forested regions (Tomskaya and Omskaya oblasts and Gornyy Altay), a large number

of tick-borne encephalitis nidi lie in heavily-populated areas and in the environs of large industrial centers (Tomsk, Prokop'yevsk, Stalinsk, Anzhero-Sudzhensk, Gur'yevsk, Belovo, etc.).

Nidi of this disease in Western Siberia have been recorded in the wooded steppe zone as well as in the wooded zone (L.I. Dobrynina, Ye.N. Levkovich, and Ye.S. Sarmanova, 1956; L.I. Dobrynina, 1957).

The natural nidi in the different topographical-climatic zones have their own epidemiological characteristics. First of all, the quantitative ratio among various species, the presence of hosts, and the extent to which the populace is in contact with the out-of-doors are of importance in faunistics of the Ixodidae.

The species of Ixodidae present in each zone are now as follows: wooded zone - I. persulcatus; wooded steppes - I. persulcatus, D. silvarum, D. pictus, and H. concinna; steppes - D. marginatus and, in the northern region, D. pictus and I. persulcatus.

I. persulcatus predominates in all of the tick-borne encephaliti nidi of the wooded and wooded steppe zones and the presence of the infection results from spreading of this species of tick. The investigations of P.A. Petrishcheva, Ye.N. Levkovich, Ye.D. Ron'zhina, and M.K. Tyushnyakova have shown that this type of tick is spontaneously infected with tick-borne encephalitis virus; however, the importance of the other species of ticks in the epidemiology of tick-borne encephalitis in Western Siberia is not clear. It may be assumed by analogy with the natural nidi in other regions of the Soviet Union that at least some of these species are of epidemiological significance in Western Siberia.

Thus, L.I. Dobrynina (1957) reported from Kuznetskiy rayon of Kemerovskaya oblast that the incidence of tick-borne encephalitis increases in the wooded steppe zone during hot years. She also pointed

have rather complete systematic lists of the ixodid ticks encountered

that D varum and H. concinna occur there in addition to I. persulcatus. We should not exclude the possibility that these species of ticks may be carriers of the infection during years especially favorable for their development. M.S. Davydova (1955) showed that H. concinna plays a definite role in the transmission of tick-borne encephalitis in the wooded steppe nidi of Krasnoyarskiy kray, where it is the basic vector in certain nidi. By analogy with other species of this genus, D. silvarum may be a vector of tick-borne encephalitis during the autumn months.

According to the data of T.N. Zakorkina (1955), the nidi of tick-borne encephalitis in Omskaya oblast occur in areas where D. pictus is encountered. Considering the possibility that tick-borne encephalitis virus is transmitted by ticks of the genus Dermacentor, it may be assumed that this species may also be a vector of the infection in question under favorable conditions.

The biology of the wood tick I. persulcatus in Western Siberia has been studied rather thoroughly by V.M. Popov and A.A. Shipova.

The curve expressing the number of native ticks has two peaks, one in May and one in June; however, in certain years the second peak may be lacking or there may be three peaks. Forest-dwelling rodents and birds are the hosts of the preimaginal stages, but no species specific in this respect have been discovered. In individual years the basic hosts are those animals and birds which are numerically predominant.

The hosts of the adult ticks are large wild and domestic animals. In a heavily-populated nidus the only hosts for the adult ticks are domestic animals, since the wild animals have been displaced by man (the Tomsk nidus, V.M. Popov, 1949). In Altay, in the mountainous dark-conifer taiga, the hosts are Siberian deer, roe deer, bears, etc.

species of these mites in Siberia; they are predominantly new species

(V.V. Kryzhanovskaya, 1956).

Virological investigations have established that there are many small mammals and birds, tick hosts, which may be natural vectors of the infection.Thus, in the Tomsk nidus tick-borne encephalitis virus was isolated from chipmunks, common hamsters, narrow-headed voles, shrews, northern mice, and common and cropland voles, as well as from birds – tree pippets, yellow buntings, fieldfares, and chaffinches (Ye.D. Ron'zhina, M.K. Tyushnyakova, and Yu.V. Fedorov). The investigations which Yu.V. Fedorov and N.I. Igolkin conducted in 1957 in the sparsely-populated taiga and wooded steppes in one of the nidi of Kemerovskaya oblast revealed encephalitis virus in bank voles, red-cheeked susliks, wood hens, and tree pippets.

According to the data obtained in the investigations of M.K. Tyushnyakova, the incidence of encephalitis virus among wood ticks in the same nidus varies from year to year, also differing in various nidi during the same season. According to the aggregate data of this author, encephalitis virus occurred in from 1.6 to 3.2% of the ticks in Tomskaya oblast, its incidence being considerably higher in the southern areas than the northern regions.

Ye.N. Levkovich and Ye.S. Sarmanova (1956) established that the incidence of encephalitis virus in certain nidi of Kemerovskaya Oblast ranges up to 40%. T.N. Zakorkina (1956) noted a high incidence of infection among the wood ticks of Omskaya Oblast, where encephalitis virus was discovered in 11 of the 13 batches of ticks which she investigated.

The data which has been amassed on the epidemiology of tick-borne encephalitis shows that this disease is closely related to the activity of the wood tick.

The first cases of tick-borne encephalitis are usually recorded

epizootiology of plague, in which they play a large role. However,

during the first ten days of May. The disease reaches its maximum incidence at the middle and end of June or the beginning of July, depending on the meteorological conditions which obtain during the epidemic season. The incidence curve then gradually drops, individual cases being recorded during the first ten days of September.

Contact between the populace and carriers of tick-borne encephalitis in nidi of this disease results principally from brief or extended stays in the forest. Contact differs in nature; it may take the form of gardening, work in the woods, collecting wood, hay-cutting, or gathering flowers, berries, garlic, etc.

The anamnesis of the majority of patients show tick bites. In 1956 Ye.I. Levkovich and L.I. Dobrynina observed cases of encephalitis among family groups in Kemerovskaya Oblast resulting from consumption of goat's milk.

In the majority of large nidi the disease occurs equally among the rural and urban inhabitants, both transient and permanent. In certain nidi 50-70% of all cases of encephalitis occur among urban residents (Tomsk agricultural region). The clinical characteristics of the disease have recently changed, the incidents of mild forms increasing. Thus, until 1954 tick-borne encephalitis diagnosed in the Tomsk nidus consisted primarily of severe forms, the poliomyelitic type constituting 34.5% and the cerebral type 31.7% of all cases. In recent years tick-borne encephalitis has been of the lethargic type in 48.0-57.6% and the meningeal type in 35.9-40.5% of all cases. An analogous picture is observed in the natural nidi of other oblasts of Western Siberia (Kemerovskaya and Novosibirskaya Oblasts).

This has resulted to a considerable extent from an improvement in the diagnosis of encephalitis, but the evolution of the disease has also been important. It is observed to occur in two phases in

10-25% of all cases. Death occurs in individual cases (0.6% in 1954, 0.5% in 1955, 0.2% in 1956, and 0.96% in 1957 in the Tomsk nidus and 0.7% in 1956 in the Omsk nidus).

In order to reduce the incidents of tick-borne encephalitis and eliminate its natural nidi such prophylactic measures as extermination of ticks, vaccination, seroprophylaxis, and dissemination of medical information were taken.

Anti-tick measures were taken in two directions; the area was treated with hexachlorane and DDT preparations and cattle were dusted with DDT. The first experimental extermination measures in Western Siberia were carried out in the Tomsk nidus by A.A. Shipova and V. M. Popov in 1951. Similar work was undertaken in Kemerovskaya Oblast in 1953 by N.N. Gorchakovskaya, I.V. Tarasevich, S.A. Shipova, and Ye.D. Chigirik. It was at this same time that planning for the prevention of ticks with the aid of DDT and hexachlorocyclohexane applied by the surface method (in quanties of 50 kg/ha) was begun in the Tomsk nidus.

Observations showed that before treatment of the area the maximum number of ticks in one of the experimental sections during the second ten days of May reached 7 ticks per man-hour and per flag-hour [sic] taken together; after treatment there was 1 tick/man-hour, i.e., a decrease of 85.8% in the number of ticks. No ticks were found after a second treatment the following year.

In a second section there were 2.5 ticks per man-nour and flag-hour before treatment and 0.5 ticks per man-hour and flag-hour after treatment (a decrease of 80%).

No ticks were found in the center of the treated section after a second treatment the following year, but there were 1.5 ticks per man-hour and flag-hour at its boundary with the untreated area. Du-

As a result of the expeditions carried out by the Zoological

ring the third year this section was treated with 45 kg/ha of insecticide from the air. Only 1 tick was found during the entire season. No ticks were found from the beginning of the observation period in the fourth year.

The number of ticks remained high in the untreated control section, varying from 9.5 to 22.5 ticks per man-hour and flag-hour together in individual years.

The effectiveness of tick extermination by aerial dusting of the area with insecticides in quantities of 50 kg/ha was studied in the spring of 1957 in the vicinity of the city of Tomsk. In one of the experimental areas there were 20.4 ticks per man-hour before dusting and 2.25 per man-hour after dusting (a decrease of 89%); in a second area there were 2.6 ticks per man-hour before treatment and no ticks after treatment.

Summing up our observations on the effectiveness of tick extermination with hexachlorocyclohexane and DDT preparations, it may be affirmed that surface application has a stable parasitological effect when the treatment is repeated twice. Aerial dusting is highly effective only when surface signaling is well organized and dosing is correctly maintained.

Simultaneously with a study of the effectiveness of tick extermination in the area from 1953 onward, an investigation of the effectiveness of tick prevention by dusting domestic animals with 10% DDT was organized in the Tomsk nidus, a dose of 75-100 g being used for cattle. The effectiveness of this measure was studied over the period 1953-1956 on the animals of a single isolated farm and those in the pastures where the treated animals grazed.

The number of ticks on the treated animals gradually decreased. Thus, during the year in which treatment was begun (1953) the fre-

repellents, primarily dimethylphthalate, have been successfully tes-

quency of occurrence was 4.3 and the incidence 0.6; in 1955 these figures were 0.8 and 0.08 respectively, while in 1957 no ticks were detected during the entire season.

The number of ticks in the pasture in which the treated animals grazed also decreased from year to year. Thus, while the frequency of occurrence in the pasture was 4.9 and the incidence 0.06 during the year in which treatment was begun (1953), in 1955 these figures were 2.8 and 0.03 respectively, while in 1957 no ticks were detected during the entire season; at the same time, in the control pasture, where antitick measures were not taken, the number of ticks increased continuously (in 1953 the frequency of occurrence was 6.7 and the incidents 0.07, while in 1956 these figures were 30.2 and 0.34 respectively).

The effectiveness of vaccine prophylaxis was studied over a period of years (1951-1957). According to the data of A.R. Yav'ya and G.T. Postoyeva (Tomsk nidus), the incidents of encephalitis among vaccinated animals was one-fourth that among unvaccinated animals.

The first time that seroprophylaxis was tested in Western Siberia among persons who had been bitten by ticks was in the Tomsk nidus in 1954 (P.Ye. Boltenko, T.P. Karmanova, 1954; A.R. Yav'ya, 1956).

Tick-borne encephalitis was observed in 1.7% of the persons who received antiencephalitis serum,while it occurred in 5.5% of the persons for whom this type of prophylaxis was not carried out. Seroprophylaxis was later used in other areas of Western Siberia.

Dissemination of medical information among the populace is of great importance in the prevention of tick-borne encephalitis. It leads to an increase in the number of persons requesting seroprophylaxis, to earlier and more timely hospitalization and, consequently, to timely specific therapy; the populace is more willing to go for

Siberian "mosquito problem," the sandflies (family Heleidae), as

vaccination prophylaxis, etc.

We believe that the most important factor in the complex of prophylactic measures employed for tick-borne encephalitis is extermination of ticks by treating the area and domestic animals with DDT and hexachlorocyclohexane preparations. Observations made in this direction in the Tomsk, Kemerovskaya, and other nidi over a period of years have shown its parasitological and epidemiological effectiveness. Thus, in 1957 the incidents of encephalitis in the Tomsk nidus had dropped by 50.4% in comparison with the 1954 level, the decrease amounting to 93.8% in certain micronidi. In Kemerovskaya Oblast the decrease in the incidents of encephalitis since 1956 reached 85% in individual rayons.

Familiarity with the incidents of tick-borne encephalitis in various areas shows that, despite a number of prophylactic measures, its rate of occurrence varies sharply. It depends not only on the completeness and quality of the measures taken, but also on a knowledge of local biocenotic, meteorological, and epidemiological characteristics, as well as on the nature of the natural nidus.

The data available on this subject make it possible to differentiate 3 basic topographical types of nidi (wooded, wooded steppe, and steppe) in Western Siberia.

The wooded type of nidus may in turn be divided into 2 subtypes: a) nidi in dense uninhabited or sparsely-inhabited taiga and b) nidi in heavily-populated taiga. Although there is a common basic encephalitis vector, the wood tick, for these types of nidi, in planning measures to be taken in them it is necessary to take into account the number and activity of the vector during various periods of the epidemic season, the incidence of virus among the vector, which determines the incidence of the disease, and the extent and character of contact

under the supervision of A.V. Fedyushin (K.S. Rastegayeva).

between the populace and the vector.

For nidi of the wooded type it is necessary to plan an entire complex of measures, the first of which is an antitick program involving treatment of both the area and domestic livestock.

It is wise to carry out tick extermination on domestic animals in heavily-populated nidi in which single diseases are present and where the basic hosts of the imaginal stages of the vector are the animals in question. In those nidi where the disease has a high incidence it is necessary to plan to treat the area containing the greatest portion of the populace in order to achieve the desired result during the first year. Careful treatment of farm animals must be carried out later in order to reinforce the results achieved (this treatment must be timely, during the entire period of tick activity, and the necessary dosage of DDT dust must be used).

During the following year adjacent areas are treated in order to expand the disease-free territory. Vaccination and obligatory seroprophylaxis come later.

In the uninhabited taiga it is necessary to exterminate the ticks in the area, primarily in the workers' settlements run by lumbering enterprises and in timbered-over areas, to vaccinate the most susceptible groups of inhabitants, to carry out seroprophylaxis, and to take measures which will keep ticks away. For nidi of the wooded steppe and steppe types the basic measures are specific prophylaxis for the most susceptible groups of inhabitants, seroprophylaxis, extermination of ticks by treatment of farm animals with DDT dust, and antitick measures on the grounds of pediatric institutions.

Dissemination of information on tick-borne encephalitis should be carried out in all types of natural encephalitis nidi.

Tomsk Scientific Research Institute for Vaccines and Sera

(1953) in Novosibirskaya Oblast

Tomsk Medical Institute

TICK ENCEPHALITIS NIDUSES IN WEST SIBERIA AND THE QUESTIONS OF THEIR SANITATION

The characteristic of tick encephalitis niduses of West Siberia on landscape-geographic zones was given. Epidemiologic peculiarities of infection in the forest zone and the ways of niduses sanitation were shown.

being and domestic animals were received for the last 40 years.

SUMMARY OF RESEARCH ON THE BLOOD-SUCKING ARTHROPODS OF SIBERIA AND PROBLEMS IN THIS FIELD

V.M. Popov

Systematic study of the blood-sucking ticks and insects of Siberia was begun in the nineteen-twenties and was at first concentrated in the local antimaleria stations. Depending on the practical requirements of these institutions, it was limited to determining the biological characteristics and distribution of maleria carriers and, to some extent, blood-sucking mosquitoes of other genera.

The blood-sucking arthropods of Siberia were later studied by a series of expeditions carried out by central scientific institutions, as well as by the antitularemia stations and institutes of epidemiology and microbiology which had been newly organized in certain oblasts. As a result, the investigation compassed the whole of the vast territory of Siberia, from the Urals to the shores of the Pacific Ocean, and almost all of the systematic groups of blood-suckers were studied to a greater or lesser extent.

Despite the large scope of the work in the sense of territory covered, the blood-sucking fauna of individual oblasts of Siberia have not as yet been sufficiently well studied. The composition by species and epidemiological importance of the blood-sucking mosquitoes and ixodid ticks of Primorskaya Oblast are the most well established. A new species was discovered among the latter and was described by B.I. Pomerantsev under the name Ixodes pavlovskiy.

There are isolated works from the Amur River region, the Transbaykal region, and Eastern Siberia on ixodid and gamasid ticks and the anopheles mosquito. The fleas of the Transbaykal region have been comparatively well studied (I.G. Ioff, Z.M. Vovchinskaya, O.I. Skalon, etal.). In addition, I.A. Rubtsov carried out a great deal of work on the classification and ecology of the midges of Eastern Siberia, obtaining much new information on this group of diptera.

The faunistics of the blood-suckers of Krasnoyarskiy Kray have hardly been studied at all. The only published works are on the ixodid ticks, which were studied in connection with their role in the epizootiology of equine haemosporidiosis and in the epidemiology of tick-borne typhus and tick-borne encephalitis. There are two works on mosquitoes and the other groups of blood-suckers in this region of Siberia remain almost completely uninvestigated.

During the past 10-15 years an intensified and well-organized study has been made of the blood-sucking ticks and insects of Western Siberia. Blood-sucking mosquitoes have now been thoroughly investigated and the biology of maleria carriers has been especially well elucidated. The faunistics of the ixodid ticks parasitic on domestic animals have been almost exhaustively determined. The ecology of the wood and meadow ticks has been studied especially thoroughly.

The study of gamasid ticks has been successfully developed in recent times and the composition by species, distribution, and ecology of horseflies have been exhaustively investigated. A great deal of work has been done on the faunistics of fleas. There is a large-scale work, still the only one, by D.I. Blagoveshchenskiy on the bird lice of the divers of the Barabinsk wooded steppe.

The faunistics of the individual systematic groups of blood-suckers encountered in Siberia have not been uniformly studied. We now

have rather complete systematic lists of the ixodid ticks encountered in various oblasts of Siberia, among which have been found species which are the carriers and transmitters of diseases of man and his domestic animals. A successful battle is already being waged against them on the basis of investigations of their local ecology.

Further study of the faunistics of the ixodid ticks of Siberia must proceed in the direction of a more detailed elucidation of the ecological characteristics of individual species as a function of local conditions and, in accordance with the data obtained, the metho employed for tick-extermination measures and the times at which these measures are taken must be made more precise. It is also necessary to continue the multifaceted study of the faunistics of specific species native to a given area, paying special attention to determining their importance in the maintenance of natural nidi of infection.

The faunistics of the gamasid ticks have been intensively studied in recent years; we now know the specific composition of the most widely and frequently encountered species, have determined their hosts and have some idea of their geographic distribution in Siberia.

Because of the slow development of the classification of this group of ticks we still have no reliable data on the epidemiological and epizootological importance of individual species. We consequently believe that the most immediate task in the study of gamasid ticks is to continue investigating their specific composition in Siberia, to study the species most widely encountered in nidi of infection in order to determine which of them are most dangerous in terms of human pathology, to investigate their ecology and distribution, and to develop measures to be taken to prevent them. Very little research has been done on the thrombiculid ticks (family Thrombidiidae). We have only fragmentary information on the occurrence of approximately 10

species of these mites in Siberia; they are predominantly new species and were first described by Ye.G. Shluger (1947). Further study of the thrombiculid mites of Siberia is the most immediate task of parasitological research in this area.

In addition to ticks, blood-sucking insects, primarily fleas (Aphaniptera), are of considerable importance among the ectoparasites of wild animals and birds as transmitters and carriers of infection. The classification of Siberian fleas has now been studied comparatively thoroughly. According to the complete summary compiled by I.G. Ioff and O.I. Skalon (1954) 127 species and 15 subspecies of fleas are encountered in Siberia.

In studying this group of ectoparasites great importance attaches to the work done by the workers of the Irkutsk Antiplague Institute of Siberia and the Far East (O.I. Skalon, Z.M. Vovchinskaya, etal.) under the supervision of the late I.G. Ioff, the leading Soviet specialist on these blood-suckers. The authors described a number of new species collected in Siberia and the Far East. The workers of the Siberian Antitularemia Stations (V.M. Popov, O.N. Sazonova, and A.D. Lebedev) had less to do and consequently made a rather complete study of the composition by species and the hosts of the fleas of Western Siberia and described one new subspecies.

In addition to studying the specific composition of the fleas, the authors established their hosts and the change in the numbers of individual species by season and year.

As a result of a great deal of painstaking work we now not only have lists of the specific composition of the fleas of Siberia and their hosts, but we also know their basic geographic distribution in individual oblasts.

As we know, fleas attracted attention in connection with the

epizootiology of plague, in which they play a large role. However, the works of Yu.V. Fedorov and N.I. Igolkin (1957) of the Tomsk NIIVS on two groups of fleas collected from bank voles and shrews in a nidus of tick-borne encephalitis recently revealed two strains of neurotropic viruses, of which one proved to be the virus of tick-borne encephalitis and the second that of lymphocytic choriomeningitis.

In further research on the fleas of Siberia attention must be paid to their ecology and to elucidating the role of individual species in the epizootiology and epidemiology of various infections.

Among the mammalian ectoparasites the lice (Anoplura) and bird lice (Mallophaga) have not as yet been studied at all. The literature contains only very short lists of species of lice found on man and animals in the Far East (A.I. Shpringol'ts-Schmidt, 1935) and in Western Siberia (V.M. Popov, 1953). These lists contain approximately 10 species collected accidentally and naturally does not in any way reflect the specific composition of these blood-suckers in Siberia.

A more thorough investigation has been made of the bird lice (Mallophaga) of the divers on Lake Chany (D.I. Blagoveshchenskiy, 1948). The author found more than 100 species here, 18 of which he was the first to describe.

These two orders are thus the least studied groups of Siberian parasites and are awaiting their investigators.

As a result of the large amount of work being done to develop virgin lands and to build new enterprises in Siberia the "mosquito problem," whose detrimental import is well known, has now become especially pressing. Among these insects are the blood-sucking mosquitoes (family Culicidae), of which the most important in terms of local pathology is the anopheles mosquito.

As a result of work carried out primarily by the staffs of the

local antimalaria stations (V.V. Vnukovskiy, Ye.A. Pletnov, A.A. Shipova, L.V. Ferri, V.M. Popov, I.A. Tarabukhin, G.I. Netskiy, etal.) and partly by the departments of biology of certain higher educational institutions such as Tomsk University (M.D. Ruzskiy and Ye.F. Kiseleva), the Khabarovsk Medical Institute (A.V. Maslov), and the Novosibirsk Medical Institute (N.M. Vlasenko etal.) and central scientific research institutions such as the Zoology Institute of the Academy of Sciences USSR (A.A. Shtakel'berg and A.S. Monchadskiy), the Academy of Military Medicine of Soviet Armenia (A.V. Gutsevich), the Central Malaria Institute of the Academy of Medical Sciences USSR (V.N. Beklemishev, O.F. Buyanova, S.N. Zvyagintsev, etal.), the Institute of Epidemiology and Microbiology imeni N.F. Gamaleya of the Academy of Medical Sciences USSR (P.A. Petrishcheva), and others it may be said that the composition by species and subspecies of malaria carriers has been exhaustively determined during the past 40 years. They are represented in Siberia by two species, the common malarial mosquito (Anopheles maculipennis Mg.) and two of its subspecies (An. m. messeae Fal. and An. m. maculipennis Mg.) and the Pallasov malarial mosquito (An. hyrcanus Pall.). The geographic distribution and biological characteristics of the common malarial mosquito have been satisfactorily determined in all of the Siberian oblasts.

On the basis of the data obtained it has been possible to eliminate massive outbreaks of malaria in Siberia and to gather information for developing practical measures which will ultimately free the territory of this infection.

As for research on the remaining species of this family, the so-called "simple mosquitoes" (tribe Culicini), data have been obtained which make it possible to draw up a systematic list including 36 species.

As a result of the expeditions carried out by the Zoological Institute of the Academy of Sciences USSR, the Department of General Biology of the Academy of Military Medicine of Soviet Armenia, and the Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR under the supervision of Ye.N. Pavlovskiy (A.V. Gutsevich, P.A. Petrishcheva, A.S. Monchadskiy, etal.), as well as of the work of the Department of Biology of the Khabarovsk Medical Institute (A.V. Maslov) the Culicini of the Far East have been studied quite thoroughly. A total of 33 species were found and it was discovered that certain of them are carriers of Japanese encephalitis; they are the species Aedes togoi Theob., A. esoensis Jam. (cinerens Meig.), Culex tritaeniorhynchus Giles and C. Bitaeniorhynchus Giles. P.A. Petrishcheva and a number of her colleagues studied the biology of these insects and worked out a complex of measures to be taken against them in the various topographical zones of Primor'ye.

Study of the Culicini in the remaining oblasts of Siberia, primarily its western portion (V.M. Popov, and I.A. Tarabukhin) showed that there are far fewer species in this region than in the Far East. Among them are a number of species, principally of the genus Culex, which are carriers of Japanese encephalitis. In addition, as the investigations of S.P. Karpov, V.M. Popov, O.K. Kupressova, etal. showed, species of Aedes which carry the causitive agent of tularemia are encountered here. Data have also been obtained which indicate that it is possible that certain species of the communis group and of the genus Mansonia carry pathogenic viruses (investigations conducted by V.M. Popov and Ye.D. Ron'zhina, G.I. Netskiy, and A.V. Gagarina).

Little research has been done on the biology of this group of mosquitoes in general, particularly under Siberian conditions, and methods of combating them must consequently still be developed. Various

repellents, primarily dimethylphthalate, have been successfully tested and are now being used in Siberia (A.V. Maslov).

A second important factor in the "mosquito problem" in Siberia is the blackflies (family Simuliidae). Despite the fact that these insects form the overwhelming majority of the flying blood-suckers during the season when the latter are active, almost no research has been done on their faunistics in Siberia, except in certain areas (the Lake Baykal region and Primor'ye).

According to the data obtained in the investigations which I.A. Rubtsov, A.V. Gutsevich, and others conducted in Eastern Siberia, the Transbaykal region, and the Far East, there are 39 species and three subspecies of blackflies in these areas, 32 of which were first described by I.A. Rubtsov from his collecting work in Siberia. His thorough study of their biology in Eastern Siberia is insufficient, since the extreme diversity of hydrological conditions in the various oblasts of Siberia forces us to assume that their composition by species and phenology differs from area to area.

Despite the fact that this group of blood-suckers has not been sufficiently well studied, the public health service of the construction authority for the Bratsk Hydroelectric Power Station, under the supervision of S.G. Grebel'skiy, is doing a great deal of work on combating them. The results of this work have still not been published, but they will undoubtedly be of great importance in enlarging the scope of mosquito prevention in Siberia.

The most immediate task in the study of blackflies in Siberia is faunistic research in Western Siberia to determine which of the widespread species are the most dangerous to man and ecological research to enable us to organize the most effective means for combating them.

Certain data have now been obtained on the next component of the

Siberian "mosquito problem," the sandflies (family Heleidae), as a result of the work of A.V. Gutsevich (1937, 1939) and S.P. Karpov, V.M. Popov, and A.G. Slinkina (1945).

According to the data of A.V. Gutsevich (1956), approximately 50 species of sandflies have been recorded in the USSR, of which only about 15 are found in Siberia. Little research has thus been done on the sandflies of Siberia, although they are undoubtedly important as carriers of infection, as the investigations which we conducted jointly with S.P. Karpov and A.G. Slinkina in Tomskaya Oblast showed. This points up the necessity of turning our attention to an immediate multifaceted study of Siberian sandflies, individual species of which attack man and animals in large number.

Finally, the last factor in our problem is the most numerous representatives of the mosquito family, the horseflies. This group of blood-sucking flying diptera has also been studied to different extents in individual oblasts.

Thanks to the work of N.G. Olsuf'yev (1936) the horseflies of Western Siberia are the most completely, perhaps exhaustively, studied. A total of 61 species have been recorded in this area. According to the data of A.V. Gutsevich (1947), 58 species are encountered in the Transbaykal region and in the Far East.

There are no works on the horseflies of Krasnoyarskiy Kray and Eastern Siberia. Nevertheless, it must be assumed that the number of species of horseflies found in Siberia will hardly be found to be considerably larger. As for the incidence, phenology, and seasonal activity of individual species of horseflies, these problems were studied in Tomskaya Oblast by the Department of Vertebrate Zoology of Tomsk University (Ye.F. Kiseleva and T.G. Shavkunova) and in Omskaya Oblast by the Department of Zoology of the Omsk Agricultural Institute,

under the supervision of A.V. Fedyushin (K.S. Rastegayeva).

The data obtained in the aforementioned work make it possible to draw up a first approximation of the geographic distribution of horseflies in Siberia by topographical zones and existing administrative regions, as well as furnishing us with preliminary information on the phenology of the most widespread species, the latter being very important for organizing protection against these blood-suckers, which are a burden to man and especially to domestic animals.

As for the role of horseflies as carriers of infection, we may consider it to be established that they transmit anthrax (N. Olsuf'yev and P. Lelep) and probably tularemia under the conditions which obtain in Siberia.

The epidemiological observations which we made in Western Siberia (S.P. Karpov, V.M. Popov, O.K. Kupressova, etal.) show that certain species of the genera Chrysops and Chryzosona may be carriers of this disease, but we have not obtained any bacterial confirmation of this.

Pressing problems in the further study of the horseflies of Siberia are systematic observations of their phenology in various oblasts and elucidation of their importance in spreading diseases with natural nidi.

The greatest difficulty in studying the blood-suckers of Siberia is the lack of works on blood-sucking flies (families Musidae and Hyppoboscidae). There are no works on their biology and even their specific composition in Siberia has remained completely uninvestigated. They include such extremely important carriers of human and animal diseases as the stable fly (Stomoxys calcitrans). This species was not known in Siberia before 1946, but in this year it was discovered simultaneously by us in Tomskaya Oblast and by I.F. Zhovtyy

(1953) in Novosibirskaya Oblast.

The study of blood-sucking flies is a very pressing problem of parasitological research in Siberia, since there are indications in the literature that these flies, together with horseflies, may be the mechanical vectors of anthrax and poliomyelitis.

Summing up the material presented here, it must be noted that there is much that we still do not know about the blood-suckers of Siberia, despite undoubted large advances. This is true both of the faunistics and ecology of individual groups of ticks and insects and of individual oblasts of this region of the Soviet Union.

Fulfilling the directives of the 20th Congress of the Communist Party of the USSR, Soviet citizens are successfully working toward utilization of the natural resources of Siberia and it is the duty of medical workers to preserve their health in every way possible. This requires us to have a deep and multifaceted knowledge of the natural conditions which obtain in this region, especially the negative factors which exist there, including blood-sucking ticks and insects, which must be combated with every means at our disposal. The problems of studying these insects consequently are of especially great importance under Siberian conditions and the gaps in our knowledge must be filled in as soon as possible.

Tomsk Scientific Research Institute for Vaccines and Sera

THE RESULT OF STUDYING OF BLOOD-SUCTORIAL ARTHROPODA OF SIBERIA AND THE PROBLEMS IN THIS FIELD

Popov V.M.

Data with respect of specific composition and geographic distribution in Siberia almost of all groups of blood - suctorial ticks and insects being germ carriers of transmission sicknesses of human

being and domestic animals were received for the last 40 years.

A number of new for the science species were described and germ carriers of some infections unknown before found out. It is noted that not all regions of Siberia and not all groups of germ carriers were studied equally completely.

The necessity of continuing the study of the fauna of blood-suckers in separate districts and regions, the intensification of studying the ecology of found out vermins of human beings health is stressed.

DATA ON THE FAUNISTICS OF THE GAMASID TICKS OF THE EASTERN PORTION OF WESTERN SIBERIA

V.M. Popov and N.I. Igolkin

Virusological and bacteriological research conducted on gamasid ticks by a number of investigators studying natural nidi of tularemia and tick-borne encephalitis in Western Siberia (Ye.I. Kleytman, 1956; S.N. Levkovich and A.A. Tagil'tsev, 1956) have established that the ticks are spontaneously infected with the causitive agents of these diseases.

Similar investigations carried out by other authors in the European portion of the Soviet Union (A. Vol'ferts, S. Kolpakova, and A. Flegontova, 1934; N.K. Grzhebina, 1939; Yu.A. Myasnikov, 1954; Ye. N. Nel'zina and I.P. Barkov, 1951; and a whole series of others) also showed that spontaneously infected gamasid ticks are present in the natural nidi of various infections, it being possible to observe transmission of the causitive agent from sick to healthy animals in certain cases, as in tularemia (Ye.N. Nel'zina and V.P. Romanova, 1950; etal.).

The data cited above give us reason to assume that gamasid ticks are of definite importance in the epizootiology of infections such as tularemia, tick-borne encephalitis, and perhaps certain others.

As things now stand the classification of this group of ticks has been worked out poorly, as a result of which little research has been done on the gamasid fauna of Siberia in general (and of its western portion in particular). The literature contains only a few works devoted to descriptions of the gamasid fauna of individual Siberian oblasts. These include the works of N.G. Bregetova (1953) on the Far East, V.I. Alifanov (1954) on Omskaya Oblast, and A.A. Goncharova (1957) on Eastern Siberia. There are no published works on the remaining oblasts of Siberia, but it is known that data on certain of them have been collected and partially processed (Novosibirskaya Oblast and part of Altayskiy Kray).

During the past 10 years we have gathered a considerable amount of material on gamasid ticks parasitic on various rodents in Novosibirskaya and Tomskaya Oblasts (V.M. Popov) and have also made wide collections of gamasid ticks from the nests of small mammals in Tomskaya and Kemerovskaya Oblasts (N.I. Igolkin).

The processing of these data now enables us to augment our information on the gamasid ticks which live on small mammals in the eastern portion of Western Siberia. Of the 24 families which have now been established for gamasid ticks 12 are encountered in the USSR. According to the data obtained in our collecting work representatives of all of these families are found in the eastern portion of Western Siberia, there being 33 species belonging to 15 genera. In addition, we have collected representatives of at least another 10 genera which cannot be precisely classified given the current state of gamasid classification. We do not exclude the possibility that we may later be able to find new species among them.

The data which we obtained are naturally only preliminary and they are undoubtedly far from a complete representation of the composition of the gamasid ticks of our region.

Despite this, we may now note certain peculiarities which they exhibit in comparison with the faunistics of the gamasid ticks of other areas of Siberia. Of the 33 species found in the eastern portion of Western Siberia five (Euryparasitus emarginatus, Poecilochirus necrofori, Eulaelaps stabularis, Haemolaelaps glasglowi, and Haemogamasus ambulans) are encountered in all areas of Siberia. This small number of species common to all areas of Siberia apparently does not indicate a considerable difference in the specific composition of gamasids in individual regions of the territory with which we are concerned, but rather reflects the fact that they have not been sufficiently well studied. Our hypothesis that the specific composition of the gamasid ticks of individual areas of Siberia, with the exception of the Transbaykal region and the Far East, should not differ considerably is confirmed by the fact that the eastern portion of Western Siberia, which is more heavily wooded than Omskaya Oblast, the latter

lying almost completely in the wooded steppe zone and even partially in the steppe region, has 30 species of gamasid in common with it.

The specific composition of this group of ticks differs considerably between Primor'ye and the eastern portion of Western Siberia. According to our data, these regions have only 12 species in common. However, this difference seems to be only apparent, since the lists for Primor'ye Oblasts note species which are parasitic not only on small mammals and birds, but also within the body cavities of the latter. We do not have such information from other areas of Siberia.

The wooded eastern portion of Western Siberia has 17 species of gamasid in common with Altayskiy Kray and the Transbaykal region, which are to a considerable extent steppe areas. It must be taken into account that further study of the gamasids of the Transbaykal region and Altayskiy Kray, especially their wooded areas, will increase this number and the specific composition of the gamasid ticks of the eastern portion of Western Siberia will thus no longer seem so different from that of the gamasids of these other regions.

Individual species of gamasid ticks differ considerably in their ecology. There are both free-living ticks and ticks parasitic on various vertebrates and invertebrates. The parasites include both endoparasites which inhabit various body cavities and ectoparasites. The latter may be permanent or temporary.

The range of hosts on which gamasid ticks are parasitic is extremely wide; they include representatives of all classes and many orders of animals. The collections which we made in the eastern portion of Western Siberia contain representatives of all of the ecological groups of gamasids. Thus, of the 33 species found here 5 were encountered only on animals (ectoparasites) and 8 were found only in their nests; 20 species were encountered both on animals and in their nests

We cannot make any definite statement at this time about the epizootiological and epidemiological importance of gamasid ticks in the eastern portion of Western Siberia. As was indicated above, the available microbiological data show that gamasid ticks may carry tularemia (Ye.I. Kleytman, 1956) and tick-borne encephalitis (Ye.N. Levkovich and A.A. Tagil'tsev, 1956) under the conditions which obtain here. However, we still do not know which of the true species are the carriers of the causitive agents of these infections, since the authors made their investigations without systematically classifying the ticks which they were studying.

Further research should be directed toward elucidating the roles of individual species of gamasid ticks in transmitting and maintaining the causitive agents of diseases with natural nidi.

Tomsk Scientific Research Institute for Vaccines and Sera

MATERIALS TO THE FAUNA OF TICKS OF THE SUPERFAMILY OF GAMASOIDEA REUTER OF THE EASTERN PART OF WEST SIBERIA

Popov V.M., Igolkin N.I.

On the base of working up own collection of ticks of superfamily of Gamasoidea Reuter made from small mammals and literary data it is noted that there are above 30 species of them belonging to 12 families in the eastern part of West Siberia. The comparison of specific composition of ticks of superfamily of Gamasoides Reuter of separate regions of Siberia has shown that there is no great difference between them that this is due to the insufficient study of the fauna of superfamily of Gamasoidea Reuter of separate parts of Siberia and insufficient working out systematism of this group of parasites.

DATA OBTAINED IN AN INVESTIGATION OF LYMPHOCYTIC CHORIOMENINGITIS IN TOMSKAYA OBLAST

M.K. Tyushnyakova and M.S. Zagromova

The part which lymphocytic choriomeningitis (LKhM) virus takes in damaging the central nervous system has now been described by many researchers in all parts of the world, excluding the Scandinavian countries.

LKhM was first shown to be present in the Soviet Union by V.M. Zhdanov and M.I. Levi, then by D.K. Lunev and others. The recent literature has contained a rather large number of reports (Smadel, Schied, Shilkenecht, and others) on the existence of various clinical forms of lymphocytic choriomeningitis with damage localized in a number of organs and systems of the body; this refutes the hypothesis that its causitive agent is strictly neurotropic.

We detected LKhM in Tomskaya Oblast while studying the etiology of serous meningitis and meningoencephalitis. We were able to isolate three strains of LKhM virus from the spinal fluid during the acute period of the infection.

The first strain, S, was isolated from a 25-year-old patient diagnosed as having meningoencephalitis who suddenly became ill in September 1957.

The second strain, V, was obtained from a 58-year-old patient diagnosed as having arachnoiditis who became ill in November 1948.

The third strain, Zh, was isolated from the spinal fluid of a

19-year-old patient diagnosed as having meningoencephalitis who suddenly fell ill in November 1957.

All of the patients denied having been bitten by ticks or other blood-sucking arthropods. Housemice were found in the home of only one patient.

These strains passed freely through the No.1 and 2 filters of the Rublevo water supply system and a Zeitz membrane. They withstood being stored in 50% glycerine and in a dried state, but proved to be less stable under these conditions than tick-borne encephalitis virus.

All of the strains were isolated by intracerebral culturing of material from the patients in white mice. The incubation period in the inoculated animals was most frequently 4-6 days, but it occasionally was shortened to 2 days or prolonged to as much as 18 days.

The clinical picture of the experimental encephalitis which developed in the white mice was characterized by photophobia, tremors, and clonic spasms. All of the sick animals were sluggish, their coats were disheveled, and they moved unwillingly with spastically extended limbs; however, no paralysis could be detected.

In subsequent experiments considerable fluctuations were observed in the incidence of disease and percentage mortality among the mice. There were also substantial variations in the titre of virulent virus.

Strain Zh is the most thoroughly studied in this respect. Its titre varies from 4.0 to 6.2. These properties serve as something of a distinction between the local strains and the Armstrong strain. The unusual characteristics of different strains of lymphocytic choriomeningitis have been described by Revo, Rivers, Khel'fis, Khel'mer, Gibs, and others.

In investigating various organs of the experimentally inoculated mice LKhM virus was isolated from the brain, blood, spleen, lungs,

kidneys, and urine. No virus could be obtained from the liver. The highest titre of virus occurred in the brain, followed by the spleen and the lungs.

A histological examination performed by N.V. Shubin on the brains of white mice inoculated intranasally with strains S and Zh showed that there was a proliferation of the endothelium of the lateral ventricles and a lymphocytic infiltration along the lateral vascular networks and the meninges.

The mice which survived the experimental infection remained carriers of the virus for a prolonged period (the observation time was 75 days).

Many foreign and Soviet authors (Traub, Scott, M.I. Levi, and others) believed that mice which have survived LKhM frequently remain carriers of the virus throughout their lives. This fact is very important both from the standpoint of epidemiology and in differentiating the virus.

In studying isolated strains of LKhM virus and the standard strains YaS-3376 and 325 we (just as other authors who previously studied lymphocytic choriomeningitis) were able to infect white mice intracerebrally, subcutaneously, intranasally, and intraperitoneally. In addition, we were successful in inducing an experimental infection in mice by feeding them organs from sick animals. The possibility of employing this means of inoculation was denied by K. Slyakova, Levi, and others. However, A.A. Medvedkova was also able to infect mice by feeding them infected material.

We were also successful in infecting healthy animals by bringing them into direct contact with mice inoculated intranasally and intracerebrally.

In conducting these experiments we excluded the possibility of

transmission, carefully treating the experimental animals beforehand and grooming them by DDT dusting and frequent combings. All of the isolated strains were found to be apathogenic for rabbits and white rats. Strain Zh was highly virulent for both white mice and guinea pigs. It increased considerably in virulence when passivated in guinea pigs. Strains S and V proved to be only slightly virulent for guinea pigs after prolonged passivation in white mice.

Table 1 shows the biological properties of these strains.

Despite the fact that many wild animals have been found to serve as reservoirs for LKhM virus, many investigators (Traub, Armstrong, Levi, and others) believe that house mice are the basic reservoir of this microorganism. It has been proved that they play an active role in releasing this virus into the environment by means of their nasal mucus, feces, and urine. However, the mechanism by which the virus is transmitted in a nidus and the means by which humans are infected remain controversal.

The observations made by various investigators enable us to recognize that there are several possible ways in which LKhM virus may be transmitted from animals to humans (Findleay and Stern, Armstrong, Traub, M.I. Levi, and others).

Thus, in 1938 Traub concluded that the principal means of transmission for mice is intraplacental. Komrower and others have described cases of diplacental infection of newborn young. M.I. Levi and L.N. Kislyakova believe that the basic means of transfer in nature and in man is transmissive. On the other hand, Armstrong, Milzer, and others believe that this mode is not very probable for man.

Lepin and Soutter (1938) found that it is quite possible for LKhM virus to enter the bodies of laboratory animals and humans through undamaged skin and mucosae. Shaughnessy and Zichis were able to infect

a guinea pig by applying virus to an apparently undamaged portion of its skin. Armstrong (1942) and Smadel showed that it is possible for contact infection to occur among humans.

TABLE 1

Pathogenicity of Isolated Strains of Lymphocytic Choriomeningitis for White Mice When Administered by Various Methods (brain emulsion)

A Наименов. штамма	B Способ заражения	C К-во заражен. животных	D К-во заболевших животных	E Дни инкубации	F Титр вируса	G Обнаружение вируса в органах: H мозг	I селезенка	J печень	K легкое	L кровь	M моча	N почки
"С" "S"	O Интрацеребрально	190	149	2–6	от S до T 4,6 – 5,2	+	+	–	+	+	+	–
	P Интраназально	23	14	4–8		+	–	–	+	+	0	0
	Q Подкожно	24	13	4–8		+	–	–	–	+	0	0
	R Внутрибрюшинно	8	5	4–8		+	0	0	0	0	0	0
"В" "V"	O Интрацеребрально	40	26	2–6	4.0	+	+	–	+	–	–	–
	P Интраназально	7	4	3–8		0	0	0	0	0	0	0
"Ж" "Z"	O Интрацеребрально	212	192	3–12	от 4,0 до 6,8	+	+	–	+	–	–	+
	Q Подкожно	13	8	5–18		+	+	–	+	–	–	–
	P Интраназально	13	8	4–6		+	+	–	–	–	–	–
	R Внутрибрюшинно	16	12	9		+	+	–	+	–	–	+

Note: The plus signs indicate that virus was detected, the minus signs that no virus was found, and the zeros that no investigation was conducted.

A) Designation of strain; B) mode of infection; C) number of infected animals; D) number of animals contracting disease; E) days of incubation; F) titre of virus; G) organs in which virus was detected; H) brain; I) spleen; J) liver; K) lungs; L) blood; M) urine; N) kidneys; O) intracerebrally; P) intranasally; Q) subcutaneously; R) intraperitoneally; S) from; T) to.

MacCallum and Findleay (1939), Duncan, and Thomas (1951) detected LKhM virus in the nasopharynx and Lepin and Soutter (1935) found it

in the urine.

The problem of the mechanism by which this virus circulates in nature and the ways in which it is transmitted to man thus remains very pressing, but still unsolved. On the basis of the data which we obtained we concluded that the alimentary and contact modes of transfer (among both animals and humans) cannot be excluded. Further careful experimental and epidemiological observations are necessary.

The antigenic relationship of the strains which we isolated to LKhM virus was shown by the positive results obtained in cross complement-fixation reactions with hyperimmune guinea pig sera and antigens prepared from both local and standard (YaS-3376 and 325) strains of the virus.

For immunizing the guinea pigs we used the active virus, in the form of a 10% suspension of cerebral matter from infected mice. The antigens were at first prepared only from cerebral matter, by Casals' method, and the suspension was made in ether by A.A. Smorodintsev's method. Suspensions of the spleens, lungs, and livers of infected guinea pigs and white mice were then used for obtaining specific antigens. Just as many other authors (Baid, Wall, A.A. Medvedkova, R.M. Shur, etal.), we found that splenic antigens have the highest specific activity and contain the fewest ballast substances.

Table 2 gives the results of the complement-fixation reactions with the antigens and hyperimmune sera.

These experiments involving the complement-fixation reaction give us a basis for computing that the Tomsk strains of LKhM virus are antigenically and immunogenically identical to the standard strains YaS-3376 and 325.

The data on the presence of virus-neutralizing antibodies in the blood sera of persons suffering from or convalescing from LKhM are

contradictory. Thus, Lepin, Mollaret, and Soutter found that virus-neutralizing antibodies cannot always be detected, even in persons with manifest infections.

We did not obtain any clear results from the neutralization reaction in analyzing either the blood sera of hyperimmune animals or sera from persons suffering from or convalescing from LKhM.

The complement-fixation reaction is the generally-accepted method of diagnosing LKhM. We ourselves have used it widely for diagnosing this disease. In setting up the experiments on human blood sera we used specific antibodies prepared in our laboratory, as well as antigens obtained from the Khar'kov Institute of Vaccines and Sera. With the aid of this reaction we were able to diagnos LKhM in eighteen patients during both the acute and chronic stages of the disease.

There are various data on the changes in the quantities of complement-fixing antibodies during LKhM. According to the data of Schied, Shur, and others, complement-fixing antibodies can be detected during the 3rd-4th week of illness. Smadel noted their presence in the blood serum during the 2nd-3rd week. Nelson observed a rapid increase in the titre of antibodies up to the 6th week, this being followed by a continuous drop.

In constrast to these results, MacCallum observed the blood serum to have a specific activity until the 117th day of convalescence, while Milzer and Levinson found that it lasts up to 26 months and Levi and others observed activity for 3-4 years.

However, Smadel and Wall did not detect antibodies in the blood sera of 5 convalescent patients examined after one year.

All of these data indicate that the content of specific antibodies in the blood sera of convalescent patients varies in magnitude and duration.

In one patient we were able to detect complement-fixing antibodies in a titre of 1:16 on the 10th day of illness.

We observed the highest specific activity during the 2nd month of convalesence, a gradual decrease then being noted in certain cases; however, in some patients clearly positive results were obtained for a period of 2 years, this being especially marked in patients whose infections were relapsing or chronic.

The titres of antibodies in our patients varied from 1:4 to 1:16.

The specific diagnostic methods (virusological and complement-fixation) which we employed made it possible to diagnose LKhM in 20 patients. All of them were residents of the city of Tomsk or of one of two regions (Asino or Kozhevnikovo) of Tomskaya Oblast.

CONCLUSION

By means of specific diagnostic methods it has been established that there are sporadic cases of lymphocytic choriomeningitis among the residents of the city of Tomsk and the Asino and Kozhevnikovo regions of Tomskaya Oblast.

The incidence of LKhM was markedly higher in the fall. The affected persons were predominantly young or middle-aged.

Three strains of virus isolated from the spinal fluid of the patients during the acute period of the infection proved to be identical antigenically to the standard strains YaS-3376 and 325 of LKhM virus.

A peculiarity of the Tomsk strains is the wide variation in their virulence and incubation periods in infected animals.

It was established experimentally that the virus can be transmitted to white mice by the contact and alimentary modes.

With the aid of the complement-fixation reaction we were able to detect specific antibodies in the blood sera of persons suffering from

TABLE 2

Results of Cross Complement-Fixation Reactions With Hyperimmune Guinea Pig Sera and Antigens From Strains YaS-3376, 325, S, V, and Zh of Lymphocytic Choriomeningitis Virus

A Антиген из штамма	B Сыворотка крови шт. „С"				D КС	E КА	C Сыворотка крови шт. "ЯС—3376"				КС	КА
	1/4	1/8	1/16	1/32			1/4	1/8	1/16	1/32		
C	+++	+++	++	++	−	−	++++	+++	++	+	−	−
F Антиген из штамма	G Сыворотка крови шт. "В"						C Сыворотка крови шт. "ЯС—3376"					
B	+++	+++	++	±	−	−	++++	+++	++	+	−	−
Антиген из штамма	I Сыворотка крови шт. "Ж"						J Сыворотка крови шт. "325"					
H Ж	++++	+++	+++	++	−	−	+++	++	++	±	−	−
Антиген из штамма	J Сыворотка крови шт „325"						Сыворотка крови шт. I "Ж"					
K 325	++	++	++	+	−	−	+++	++	++	−	−	−

A) Antigen from strain S; B) blood serum of strain S; C) blood serum of strain YaS-3376; D) complement-serum; E) complement antigen; F) antigen from strain V; G) blood serum of strain V; H) antigen from strain Zh; I) blood serum of strain Zh; J) blood serum of strain 325; K) antigen from strain 325.

and convalescing from LKhM on the 10th day of illness and for two years thereafter.

These investigations point up the need for a thorough study of the natural vector point of this illness under the conditions prevailing in the Tomsk region.

Tomsk Scientific Research Institute for Vaccines and Sera

MATERIALS OF RESEARCH ON LYMPHOCYTIC CHORIOMENINGTIS IN TOMSK REGION

Tyushnyakova M.K., Zagromova M.S.

The presence of lymphocytic choriomeningitis among citizens of Tomsk region was found out by using microbiologic methods of research.

The possibility of contact and per os ways of infection of white mice by the virus of lymphocytic choriomeningitis was experimentally established.

VECTORS OF TICK-BORNE ENCEPHALITIS VIRUS IN THE TOMSK NIDUS OF INFECTION

V.M. Popov, N.I. Igolkin, and Yu. V. Federov

The vectors of the causitive agent of tick-borne encephalitis in natural nidi of this disease are many species of mammals and birds among which the virus circulates as a result of its transmission by certain species of ixodid ticks. These ticks are parasitic on vertebrates and are the only vector of this infection for man.

Determination of the ways in which the causitive agent of tick-borne encephalitis circulates among vertebrates and arthropods will make it possible to plan more effective measures for combating this disease in the nidus in question and, in certain cases, to find ways of completely eliminating it.

Considering the importance of investigations of this type, we mad the appropriate observations on tick-borne encephalitis in the Tomsk nidus.

As for its natural conditions, the nidus is a hilly area covered with a mixture of aspen and birch with occasional coniferous species. The coniferous forest (spruce, cedar, fir, and pine) is the residue of the Taiga which once spread over a wide area but is now encountered only in places. The open wooded sections and old cut-over areas are covered with scrub and high grass. Sections of this type are used primarily for pasture.

In order to make a detailed study of the nidus we conducted sys-

tematic investigations of its zoology, parasitology, and virusology from 1945 onward.

It was established that there are 30 species of wild mammals which permanently inhabit the nidus and 2-3 species which may be found there from time to time. The former include 20 species of the order Rodentia, 7 species of the order Insectivora, and 3 species of the order Carnivora. One always encounters domestic cattle in the environs of populated sections and there are horses, dogs, and other animals. The occasionally-encountered species are large wild animals, including moose, roe deer, and comparatively few foxes and hares.

In addition to these mammals, approximately one and one-half times as many species of birds live in the nidus. Of the other groups of animals which inhabit it, the various vertebrate ectoparasites are of interest to us.

Research conducted in this direction has made it possible to establish that there are 5 species of ixodid ticks, no less than 30 species of gamasid ticks, and 19 species of fleas parasitic on the animals and birds. Lice, bird lice, and blood-sucking flies have also been detected.

The flying blood-suckers are represented in the nidus by 30 species of mosquitoes (family Culicidae), 26 species of horseflies (family Tabanidae), 7 species of sandflies (family Heleidae), several species of blackflies (family Simuliidae), and 3 species of blood-sucking flies (family Muscidae).

The presence of tick-borne encephalitis virus in the nidus was first established by M.P. Chumakov (1940) and later repeatedly confirmed by Ye.D. Ron'zhina, M.K. Tyushnyakova, and Yu.V. Fedorov. These investigations established that the carriers of tick-borne encephalitis in the Tomsk nidus are 7 species of small mammals: the common,

narrow-headed, and meadow voles, the wood mouse, the chipmunk, the shrew, and the common hamster.

Several strains of tick-borne encephalitis were isolated in 1954-1956, in investigations of 1159 birds belonging to 39 species; the virus was found in the brains of the tree pippet, yellow bunting, and chaffinch, as well as in the blood of the fieldfare. In addition, antibodies which neutralize tick-borne encephalitis virus were detected in blood serum of the birds. A total of 117 serum samples from 12 species of birds were examined. Antibodies were found in 5 species: the European bullfinch, the fieldfare, the yellow bunting, the tree pippet, and the nutcracker.

Investigations of ectoparasites established that the causitive agent of tick-borne encephalitis is encountered in ixodid ticks. The virus has not as yet been detected in gamasid ticks and fleas in the Tomsk nidus.

At one time a neurotropic virus pathogenic for white mice was isolated from mosquitoes (of the genus Aedes), but its nature has not been studied.

There are three factors which combine in maintaining a natural nidus: 1) the numbers of the virus-carrying population; 2) the existence of biocenotic relationships between these carriers and the other inhabitants of the nidus and the character of these relationships; 3) the incidence of the virus among the various species of animals, especially the basic carrier of the causitive agent of the infection.

As was noted above, a knowledge of the ways in which the virus circulates in a given area is of extremely great importance in working out measures for eliminating natural nidi of infection. Unfortunately, in describing natural nidi the majority of authors limit themselves to stating that certain species of animals are present and elucidating

the spontaneous incidence of virus among them, not making any attempt to give a concrete representation of the structure of the nidus under study, using the biocenotic relationships between the members of its biocenosis, including the causitive agent, as a basis.

Our observations over a period of years have shown that the permanent members of the biocenosis of the Tomsk nidus are chipmunks, bank voles, shrews, thrushes, nutcrackers, yellow buntings, tree pippets, and domestic animals. The individual populations of these animals did not vary substantially over the entire observation period.

The mode of life of all of these species is closely tied to the earth, as is that of the wood tick. It is consequently quite understandable that small mammals and birds are the basic hosts for the preimaginal stages of the wood tick, while its imaginal stage feeds on domestic animals.

The study which M.K. Tyushnyakova and Yu.V. Fedorov made of the incidence of encephalitis virus among various species of animals, birds, and ticks showed that the number of infected animals varies from year to year and from season to season. Thus, according to Tyushnyakova's data, the percentage of virus-carrying ticks in the Tomsk nidus was 3% in 1950, 1.6% in 1951, 3.2% in 1952, 2.1% in 1953, and 2.35% in 1954. According to these same data, which were confirmed by Yu. V. Fedorov, the virus was most frequently detected in I. persulcatus during the spring and in mammals and birds during the latter half of the summer and the fall.

It is obvious that under the conditions which obtain in the nidus the virus spends the winter in ticks, which must be considered the principal natural host of the infection, especially if we take into account the existence of transovarian transmission of the virus.

The data cited show that the nucleus of the biocenosis of the

Tomsk tick-borne encephalitis nidus, which ensures its continuation, is composed of the basic host of the virus, the wood tick, and its hosts during various stages of its development (chipmunks, shrews, bank voles, domestic animals, thrushes, yellow buntings, and tree pippets).

In addition to these species of animals and birds, there are others in the nidus which may serve as hosts for the wood tick and carriers of encephalitis virus. However, because of their low population or the mode of their lives, they take the part of supplemental virus carriers; however, in years when they are present in larger numbers the basic nucleus of the biocenosis becomes enlarged, so that the nidus becomes highly saturated with the virus. These supplemental species include wood mice, common hamsters, squirrels, field and forest mice, hares, narrow-headed voles, European bullfinches, jays, and chaffinches.

Finally, a third group of 12 species of animals and birds are accidental members of the biocenosis and are of still less importance in maintaining the natural nidus of infection. These are the ferret, ermine, weasel, water vole, water shrew, mole, harvest mouse, matron vole (Microtus oeconomius), meadow vole, nutcracker, magpie, and titmouse. In speaking of the biocenosis of the Tomsk nidus of tick-borne encephalitis we may note that it contains a unique closed biocenosis, the nesting ground of the sand martin. The nests of these birds are located along the steep banks of a stream, deep in the earth. The sand martin lives exclusively along the banks of streams.

A study of the parasites of these birds showed that their nests are inhabited by ticks of the genus Ixodes of the group Crenulatus. The specific classification of these ticks has not as yet been precisely established and various authors assign them to I. lividus or

I. plumbeus. All developmental stages of these ticks were found in the nests. It is obvious that they are a specific parasite of the nests of sand martins and are not encountered on other animals or birds in the Tomsk nidus. Yu.V. Fedorov nd M.K. Tyushnyakova (1957) isolated a strain of neurotropic virus from these ticks, there being indications that it is the causitive agent of tick-borne encephalitis.

The importance of the closed biocenosis, the nesting ground of the sand martin, which we observed in the Tomsk nidus of tick-borne encephalitis and in which a virus identical in biological properties to tick-borne encephalitis virus circulates, is still unclear and requires further study.

Analysis of the structure of this nidus will make it possible for us to draw up a more rational plan for prophylactic measures to combat the various carriers of encephalitis virus which constitute the basic nucleus of the biocenosis of the nidus. Specifically, it enables us to recommend:

1) extermination of the wood tick in areas where its imaginal stage concentrates on domestic animals;

2) isolation of the comparatively small range of basic hosts for the preimaginal stages so that preventive measures can be taken.

Tomsk Scientific Research Institute for Vaccines and Sera

CARRIERS OF VIRUS OF TICK ENCEPHALITIS IN TOMSK NIDUS OF INFECTION

Popov V.M., Igolkin N.I., Feodorov Y.V.

In Tomsk nidus of tick encephalitis there was established specific composition of animals among which virus is circulating a long time and continually. The main nucleus of cenosis are domestic animals on which the ticks are feeded at imago stage of germ carrier.

"Ixodes persulcatus" and small mammals and birds (Entamias sibiricus Laxm., gen Clethrionomys tiles, gen Sorex, Emberiza citrinella L., Anthus trivialis L., gen Turdus L.) on which the ticks are feeded in the stages before imago of the growth of the tick.

The rest species of animals in maintaining the infection in nature have secondary meaning.

ROLE OF SMALL MAMMALS IN THE CREATION OF NATURAL NIDI OF INFECTION IN WESTERN SIBERIA

N.I. Igolkin

According to Ye.N. Pavlovskiy's teachings on natural nidi, "the causitive agent of a disease, its carriers, and the animals which serve as donors and recipients are members of a biocenosis which has evolved independently of man in certain geographic areas with appropriate flora. These areas are natural nidi of the disease in question." The existence of the causitive agent of any disease depends on its circulation among mammals. The latter play the part of reservoirs of the infection in the nidus and are the hosts for the arthropods which serve as carriers.

In a number of diseases small mammals are the direct sources of infection. Contaminating water, grains, and, in certain cases, food products with their excreta mammals cause epidemic outbreaks of diseases such as leptospirosis, tularemia, listerellosis, etc. among the populace.

The principal hosts for the causitive agents of the majority of infections are small mammals present in large and basically stable numbers. Other mammals inhabiting the same biotopes are of secondary importance, but may be involved in epizootics and maintain them for some time. N.P. Naumov has stated that there is not a single mammalian species which is not associated with some infection dangerous to man.

Attention was consequently drawn to mammals and the appearance of

epizootics among them during the first stages of research on diseases with natural nidi. A vast amount of material has now been amassed on the role of small mammals in the nidi of various topographic-geographic zones of the Soviet Union, including Western Siberia. Of the diseases with natural nidi, it has been established that tularemia, tick-borne encephalitis, tick-borne Northern Asiatic typhus, Eastern Russian hemorrhagic fever, Q fever and leptospirosis are present.

TULAREMIA

Tularemia is one of the most markedly zoonotic diseases. The basic role in the formation of nidi of this disease is played by small mammals, principally rodents, which are highly susceptible to it. It is consequently quite understandable that in studying the epidemiology of tularemia special attention must be paid to elucidating the roles of individual species of small mammals.

In 1928 G.I. Zarkhi was the first to observe spontaneous infection of the water vole in Western Siberia. This fact was later confirmed by S.P. Karpov, A.F. Komarova, and O.K. Kupressova.

The expeditions of the VIEM [All Union Institute of Experimental Medicine] and the TIEM [Tomsk Institute of Epidemiology and Microbiology] in 1940 isolated the causitive agent of tularemia from matron voles, narrow-headed voles, and house mice. In 1936 S.P. Karpov and N.I. Antonov proved that chipmunks and white hares are reservoirs of tularemia. In 1940 A.F. Komarova established that tularemia occurs among muskrats. In 1941 A.A. Selezneva succeeded in culturing the causitive agent of tularemia from the suslik Citellus eversmanni Brandt in the Oyrotskaya Autonomous Oblast. O.K. Kupressova cultured this microorganism from rat and sable sera.

In 1950 V.B. Plakhovoy examined 19 different species of mammals in one enzootic nidus. Bacterialogical investigation established that

tularemia occurs in the water vole, the wood lemming, the wood mouse, the meadow vole, the water shrew, the common shrew, and the ermine. Its causitive agent was simultaneously cultured from a batch of fleas taken from water shrews.

Prolonged systematic observations have been made of cultures of the causitive agent of tularemia taken from water voles, ermine, weasels, hooded rats, moles, and chipmunks in Tomskaya Oblast by the antitularemia station (Ye.I. Kositsina and V.M. Popov, 1956). Bacterium tularense was most frequently isolated from the water vole.

According to the observations which we(V.M. Popov, Ye.I. Kleytman, and N.I. Igolkin) made in 1955-1956, nidal tularemia is most frequently diagnosed in water voles, matron voles, bank voles, and shrews.

Of the 29 species of rodents encountered in Omskaya Oblast, 16 have been found to be susceptible to tularemia. However, as O.V. Ravilonikas (1952) notes, only five play any substantial role in spreading the disease. These are the water vole, muskrat, house mouse, narrow-headed vole, and white hare.

A detailed study of a tularemia nidus (A.F. Komarova, 1945) established that tularemia epidemics among humans are parallel to epizootics of this disease among water voles. Thus, in 1937, 81% of the cases of tularemia which occurred in Vasyuganskiy Rayon during 1937 were due to direct contact with rodents (water vole bites incurred while fishing), 8% resulted from drinking contaminated water, and 10% resulted from transmission. There is no doubt that the two latter modes of infection are directly linked to diseased water voles.

During the period of the epizootic the percentage of infected water voles is very high, reaching 10-20%, as A.F. Komarova showed (1945); it does not exceed 1.85% during the interepizootic period.

The development of an epizootic is usually preceded by intensive

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multiplication among highly-susceptible mammals, a phenomenon which always brings with it the danger of an epizootic.

The beginning of an epizootic coincides with a severe deterioration of the climatic conditions which affect the animals. When tularemia develops among one species so as to have a high incidence, it involves other species of susceptible mammals with similar feeding habits or other cenotic relationships.

There is now a great deal of interest in the maintenance of the tularemic infection during the interepizootic period. A number of authors (V.P. Dzhanpaladova, 1937; S.P. Karpov, A.F. Komarova, and V.I. Seredina, 1941; S.P. Karpov and V.M. Popov, 1949; V.G. Pilipenko, 1953; G.A. Kondrashkin, 1955) assign the principal role in this process to rodents, the water vole playing this part under the conditions which obtain in Western Siberia.

It has been recognized that under certain conditions some rodents become latently ill, remaining bacteria carriers for a long time. The infection manifests itself when the organism's resistance drops, the disease taking an acute course.

The causitive agent of tularemia may be maintained by ixodid or gamasid ticks during the interepizootic period, but again as a result of their biocenotic relationships with small mammals, their hosts. There is no doubt that where there are only small numbers of rodents highly susceptible to tularemia there is no danger of an epidemic.

TICK-BORNE ENCEPHALITIS

Nidi of tick-borne encephalitis are associated with spreading of the tick Ixodes persulcatus Sch. and appears where the causitive agent circulates by the root carrier-mammal-carrier. In a nidus of tick-borne encephalitis the numerous small mammals not only serve as reservoirs for the causitive agent, but also play the part of hosts for

the larval and nymphal stages of the ixodid ticks.

According to the data of V.M. Popov (1949), chipmunks, field mice, hamsters, and hares are the most important hosts in the Tomsk nidus of tick-borne encephalitis. He showed that the larvae are parasitic on very small mammals such as bank voles, wood voles, and mice; the nymphs are parasitic on larger mammals such as chipmunks and hamsters.

V.V. Kryzhanovskaya (1956) notes 29 species of small mammals common to Western Siberia. The larval, nymphal, or imaginal stages of ixodes persulcatus were found on all of these species. The percentage of tick-infested animals varied from 12 to 50%, there being from 1 to 16 larvae and nymphs per animal.

Ye.D. Ron'zhina began research on small mammals in Western Siberia to prove that they harbor the causitive agent of tick-borne encephalitis. In 1946 he isolated strains of virus from hamsters and narrow-headed voles from the Tomsk tick-borne encephalitis nidus. Virological investigations were conducted by M.K. Tyushnyakova (1956) and led to the detection of tick-borne encephalitis virus in shrews, northern mice, common voles, and meadow voles (in addition to hamsters and narrow-headed voles).

In 1955 the same author, working in conjunction with Yu.V. Fedorov and N.I. Igolkin, isolated 4 strains of virus from chipmunks, establishing that there was a high degree of tick infestation throughout the entire fall; in 1957 they established that the red-cheeked suslik is a virus carrier at the boundaries of its habitat.

In one of the nidi of tick-borne encephalitis in Kemerovskaya Oblast Ye.N. Levkovich and A.A. Tagil'tsev (1956) were able in two cases to isolate tick-borne encephalitis virus from gamasid ticks taken from the nests of bank voles and shrews. The authors concluded that "the presence of additional carriers and reservoirs of tick-borne

encephalitis virus in a natural nidus may promote an increase in the number of forest animals with virus in their blood; this in turn leads to the infection of a large number of ixodid ticks and thus increases the incidence of infection in the nidus."

Thus, considering the capacity of mammals to serve as virus carriers, their enormous role as hosts for the larval and nymphal stages of ixodid ticks, as well as for gamasid ticks in their nests, gives small mammals an enormous importance in maintaining the infection in nidi of tick-borne encephalitis. In Western Siberia the most important role in such nidi is played by chipmunks, bank voles, wood voles, and shrews.

NORTHERN ASIATIC TICK-BORNE TYPHUS

Since 1942 an unusual ricketsial disease, tick-borne typhus, has been recorded in Altay. In studying the spontaneous infection of mammals with the causitive agent of this disease the infection was detected in field mice and narrow-headed voles (S.M. Kulagin, O.S. Korshunova, and N.I. Alfeyev, 1947). M.A. Mastenitsa (1946) isolated ricketsia from narrow-headed voles, long-tailed susliks, and common hamsters in Kemerovskaya Oblast.

EASTERN RUSSIAN HEMORRHAGIC FEVER

A group of authors (A.V. Fedyushin, M.P. Chumakov, A.A. Avakyan, A.V. Gagarina, O.V. Ravdonikas, etal.) studied a disease of virus etiology in the wooded steppe zone of Western Siberia, in Omskaya Oblast; they conditionally named it Omsk hemorrhagic fever, to indicate that it differs from tick-borne encephalitis. Its principal carrier was found to be the tick Dermacentor pictus. The search for mammals infected with the virus was unsuccessful for some time, although the experimental investigations conducted by A.V. Gagarina (1952) established that hamsters, susliks, narrow-headed voles, hedgehogs,

and muskrats are susceptible to the disease. It took an acute course in hamsters, muskrats, and narrow-headed voles and terminated in death.

In 1956 A.V. Gagarina, O.V. Ravdonikas, and V.Ye. Zimina wrote a report on the isolation of three strains of Omsk hemorrhagic fever virus from the brains of muskrats which died natural deaths. The data obtained make it possible to conclude that small mammals are additional sources of the fever, although only during the brief time when it is in its acute stage. The only way in which this infection is passed on to man is through transmission, by the ticks Dermacentor pictus and D. marginatus.

LEPTOSPIROSIS

Leptospirosis of the swamp fever type is very closely associated with farm animals and its nidi have a rather marked antipurgic character. Under natural conditions, as in the nidi of the European portion of the Soviet Union, the reservoirs of infection are most frequently species of small mammals with damp, swampy habitats; these include matron voles, water voles, water shrews, and field mice (Ye. V. Karaseva and V.V. Anan'in, 1954).

The literature notes cases of leptospirosis among silver-gray foxes, arctic foxes, and hooded rats.

V.V. Anan'in (1955) has shown that leptospirae circulate in a vole's blood for 5-10 days after infection, then becoming localized in the convoluted tubules of the kidneys, whence they are excreted in the urine. Healthy animals are obviously infected under natural conditions as a result of direct contact with infected animals or by drinking contaminated water. The disease is chronic among rodents. Sick rodents remain sources of infection throughout their entire lives. Water infected by their excreta causes the disease among farm animals

and man.

In 1950-1951 V.I. Seredina conducted microbiological investigations of 10 species of mammals in Western Siberia, in Altayskiy Kray. Strains of leptospirae were isolated from two of them, the common hamster and the Altay mole. The disease was simultaneously detected among farm animals.

In 1953-1954 V.N. Novikova found leptospirae carriers among cattle in Tomskaya Oblast. In 1956 the same author, working in conjunction with L.P. Sagaydak and N.I. Igolkin, isolated leptospirae from narrow-headed voles and Altay moles; in 1957 they obtained this microorganism from bank voles, matron voles, and harvest mice.

The nidi of leptospirosis in Western Siberia are obviously basically no different from the better-studied nidi of the European portion of the Soviet Union, where small mammals are of rather great importance.

Investigation of mammals in Western Siberia as reservoirs of the causitive agents of a whole series of other infections has just begun. Among these infections is Q fever. This disease was noted principally among farm animals and persons who came into contact with infected animals in some fashion.

In his summary of the epidemiology of this disease S.M. Kulagin (1956) notes the following as naturally-infected animals in the USSR: Central Asian gazelles, hares, the suslik Spermophilopis leptodactylus, greater gerbils, and gray hamsters. The corresponding animals in Czechoslovakia were foxes, Norwegian rats, water voles, house mice, wood mice, golden-throated mice, etc. Many of these mammals such as water voles, Norwegian rats, house mice, and foxes are also encountered in Western Siberia. Under natural conditions these animals may be important carriers of infection. The following list is of mammals which are proved or probable sources of infections with natural nidi in Western

Siberia.

LIST

of Mammals of Western Siberia Which Carry Infections With Natural Nidi

	A Вид млекопитающего	B Заболевание						
		C клещевой энцефалит	D туляремия	E клещевой сыпной тиф	F омская геморраг. лихорадка	G лептоспирозы	H лихорадка Ку	
1	Водяная полевка		+			?	?	
2	Полевка-экономка		+			+		
3	Узкочерепная полевка	+	+	+	?	+		
4	Пашенная полевка	+	+					
5	Обыкновенная полевка	+	?					
6	Рыжие полевки (род Clethrionomys)	+	+			+		
7	Ондатра		+		+			
8	Домовая мышь		+				?	
9	Мышь-малютка					+		
10	Серая крыса		+			?	?	
11	Полевая мышь			+		?		
12	Хомяк обыкновенный	+		+	?	+		
13	Заяц-беляк	?	+					
14	Бурундук	+	+					
15	Белка	?						
16	Суслик длиннохвостый		+	+	?			
17	Лемминг лесной		+					
18	Лесная мышовка	+	+					
19	Землеройка (род Sorex)	+	+					
20	Кутора		+			?		
21	Крот		+			+		
22	Еж				?			
23	Горностай		+					
24	Ласка		+					
25	Лиса							
26	Соболь		+		?	?		

Note: A plus sign indicates a proven carrier and a question mark a possible carrier.

A) Species of mammal; B) disease; C) tick-borne encephalitis; D) tularemia; E) tick-borne typhus; F) Omsk hemorrhagic fever; G) leptospirosis; H) Q fever. 1) Water vole; 2) matron vole; 3) narrow-headed vole; 4) meadow vole; 5) common vole; 6) bank vole (genus Clethrionomys); 7) muskrat; 8) house mouse; 9) harvest mouse; 10) hooded rat; 11) field mouse; 12) common hamster; 13) white hare; 14) chipmunk; 15) squirrel; 16) long-tailed suslik; 17) wood lemming; 18) wood mouse; 19) shrew (genus Sorex); 20) water shrew; 21) mole; 22) hedgehog; 23) ermine; 24) weasel; 25) fox; 26) sable.

Further investigation of mammals under natural conditions in the presence of various infections will make it possible to give a more complete evaluation of their role in given nidi.

The study of spontaneous infection in mammals is dictated by the necessity of establishing the boundaries of natural nidi, which will ultimately make it possible to solve a number of problems associated with the prophylaxis of disease in these areas.

Tomsk Scientific Research Institute for Vaccines and Sera

THE ROLE OF SMALL MAMMALS IN FORMATION OF NATURAL NIDUSES OF INFECTION IN WEST SIBERIA

Igolkin N.I.

From the number of sicknesses with natural nidus in West Siberia there were found out: tularemia by 19 kinds of mammals (the main carrier is Arvicola terrestris); tick encephalitis by 8 species, the most important carriers being Eutamias sibiricus, gen. Clethrionomys, gen. Sorex, Tick typhus of North Asia by 4 species: Stenocranius gregalis, Apodemus agrarius, Cricetus, cricetus Citellus undulatus; haemorrhaged fever of east districts of the USSR is only by Ondatra zibethica; leptospira by six species (the most important apparently are Microtus oeconomus and Stenocranius gregalis).

Among mammals of West Siberia the Q-fever has not yet been found out.

EPIDEMIOLOGY AND PROPHYLAXIS OF TICK-BORNE ENCEPHALITIS IN THE TOMSK NIDUS DURING THE 1957 SEASON

S.P. Karpov and A.R. Yav'ya

TABLE 1

Incidence of Tick-borne Encephalitis in the Tomsk Nidus During 1956-1957

Год A	Май B		Июнь C		Июль D		Август E		Сентябрь F		За сезон G
	К-во случаев H	%	К-во случаев	%	К-во случаев	%	К-во случаев	%	К-во случаев	%	К-во случаев
1956	36	12,0	138	46,2	85	28,2	38	12,6	3	1,0	300
1957	14	9,7	55	38,2	60	41,7	14	9,7	1	0,7	144

A) Year; B) May; C) June; D) July; E) August; F) September; G) season; H) number of cases.

Over a period of years (1955-1958) we investigated the epidemiology of tick-borne encephalitis in the Tomsk nidus and the effectiveness of prophylactic measures taken against it. This report describes the results of the observations made during the 1957 season.

It is known that the population of the basic reservoir and only carrier of tick-borne encephalitis in the Tomsk nidus, the wood tick, depends on meteorological conditions. The snow cover at the beginning of the 1956-1957 winter was small for an extended period, while comparatively low temperatures set in early. This was apparently first reflected in the tick population during the 1957 season.

The first ticks appeared or 15 April, but they remained few in number until the end of the month. This resulted from the low soil temperature, which reached $+3^{\circ}$ only during the last five days of April. The tick population began to increase during the first five days of

May, reaching its maximum during the third five-day period of the month. The number of ticks then gradually decreased until the second ten days of June, the low population (5.5-2 ticks per man/flag/hr) persisting from that time until the middle of July. Only isolated ticks were encountered at the end of July and during August. The change in tick population during 1957 differed little from that which occurred during 1956, but the total number of ticks was only approximately one-sixth as great.

The first cases of tick-borne encephalitis appeared during the first ten days of May. May saw 9.7% of the total number of cases in the nidus (12% in 1956). Cases were almost uniformly distributed over all of the ten-day periods in June and July (ranging from 11.8 to 13.2% of the total number of cases for the season), only the second ten days of July reaching 16.7%. The monthly figures as percentages of the total number of cases for the season were 38.2% for June, 41.7% for July, 9.7% for August, and 0.7% for September. Table 1 gives comparative data on the incidence of the disease in the nidus during 1956-1957

It may be seen from the material in Table 1 that the maximum incidence of encephalitis in 1956 was during June, while in 1957 it was during July, although the change in tick population differed only numerically between the two seasons, as was indicated above. This phenomenon is explained by the difference in meteorological conditions. Thus, favorable weather, which increases the contact between the populace and the out-of-doors, set in later in 1957 than in 1956. At the same time, it must be pointed out that the total incidence of disease in the nidus during 1957 was 52% less than in 1956. This was undoubtedly determined by two factors: 1) the quantitative decrease in the tick population resulting from the meteorological conditions

and 2) the intensification of prophylactic measures, especially tick extermination, from year to year.

Of the persons taken ill in the nidus 48.2% were residents of the city of Tomsk and 51.8% were residents of Tomskiy Rayon. Infection occurred in 58 micronidi and partially in a number of micronidi in adjacent Tuganskiy Rayon.

It must be pointed out that the Tuganskiy nidus is the second most important epidemiologically in Tomskaya Oblast. Thus, in 1957 68.2% of all cases of tick-borne encephalitis in the oblast occurred in the Tomsk nidus, 19.4% occurred in the Tuganskiy nidus and only 12.4% in the 7 remaining rayons.

Cases of tick-borne encephalitis were observed among all age groups, but the greatest incidence in the urban populace was among persons 15 to 30 years of age (52.2%), while 46.3% of the cases in Tuganskiy Rayon were among persons under 15 years of age.

TABLE 2

Incidence of Tick-borne Encephalitis by Age Group (in %)

B \ A Возраст / Территория	До 2 лет C	2–6 лет D	7–14 лет	15–19 лет	20–29 лет	30–39 лет	40–49 лет	50–59 лет	60 лет и выше E
г. Томск F	—	1,4	9,9	25,4	26,8	16,8	15,5	1,4	2,8
Томский район G	—	2,7	13,5	8,1	23,1	32,4	9,5	8,0	2,7
Томский очаг H	—	2,0	11,7	16,1	24,8	24,7	12,4	5,0	2,8
Туганский район I	2,4	14,6	29,4	7,3	14,6	12,2	12,2	7,3	—

A) Age; B) area; C) up to 2 years; D) years; E) 60 years or more; F) city of Tomsk; G) Tomskiy Rayon; H) Tomsk nidus; I) Tuganskiy Rayon.

Table 2 gives data on the incidence of tick-borne encephalitis by age groups in Tomsk and the agricultural regions.

From the material in Table 2 it may be seen that in Tuganskiy Rayon, where there was a sharp increase in the incidence of tick-borne

TABLE 3

Incidence of Tick-Borne Encephalitis By Profession (in %)

Профессия / Территория	Дошкольники C	Школьники D	Студенты E	Рабоч. фабрик и заводов F	Рабоч. пром. предприятий G	Рабоч. ЛПХ H	Служащие I	Колхозники J	Животноводы K	Домохозяйки L	Прочие M
Томск N	14,2	14,0	14,0	14,0	12,8	1,4	12,8	—	—	15,5	11,3
Томский район O	2,7	13,4	—	—	15,1	4,0	10,8	17,5	14,9	12,2	9,4
Туганский район P	21,1	31,6	—	—	8,0	10,5	2,6	10,5	2,6	10,5	2,6

A) Profession; B) area; C) preschool-age children; D) school-age children; E) students; F) workers in factories and plants; G) workers in industrial enterprises; H) workers in lumber industry enterprises; I) workers in service industries; J) collective farm workers; K) cattle raisers; L) housewives; M) others; N) Tomsk; O) Tomskiy Rayon; P) Tuganskiy Rayon.

TABLE 4

Types of Contact Between Persons Contracting Tick-Borne Encephalitis and a Natural Nidus of Infection (in %)

A Виды контакта	B Жители		
	C Томска	Томского сельского района D	Туганского района E
Работа в лесу и поле F	12,5	21,5	16,2
Работа на огороде G	18,7	1,6	—
Работа в экспедиции H	1,6	—	—
Временное пребывание в лесу I	10,8	19,7	13,5
Заготовка леса и дров J	7,8	4,9	5,4
Заготовка сена K	12,5	16,4	—
Пастьба скота L	—	16,4	2,7
Прогулка в лес, сбор цветов M	32,9	17,9	56,8
Поездка на охоту, рыбную ловлю N	1,6	—	—
Занос клещей и заражение в населенном пункте O	1,6	1,6	5,4

A) Types of contact; B) residents; C) Tomsk; D) Tomsk agricultural region; E) Tuganskiy Rayon; F) work in woods and fields; G) gardeni H) expeditions; I) short stays in woods; J) cutting timber and firewood; K) haying; L) cattle herding; M) walks in the woods, flower gathering; N) hunting and fishing trips; O) occurrence of ticks and infection in a populated area.

encephalitis during the second year, the disease exhibits a shift toward the younger age groups in comparison with the other rayons of the oblast (with the exception of Tomskiy Rayon); this is not observed in Tomskiy Rayon, where this disease has been a danger for many years.

Analysis of the incidence of encephalitis by profession shows that workers in plants and industrial enterprises, students at higher educational institutions, school children, workers in service occupations, and housewives predominate among urban residents who contract the disease. Among the rural populace school children, collective farm workers, cattle raisers, and workers in timber enterprises constitute the greatest percentage of encephalitis sufferers. In Tuganskiy Rayon the greatest percentage of cases (21.1%) is among preschool children.

Table 3 gives data characterizing the incidence of tick-borne encephalitis by profession.

In 92.2% of all cases the disease results from tick bites.

Table 4 shows the types of contact between persons who contract encephalitis and the natural nidi of the infection.

It may be seen from the material in Table 4 that the basic forms of contact with natural nidi for urban residents were walks in the woods and flower gathering (32.9%), gardening (18.7%), work in woods and fields (12.5%), and haying (12.5%); for residents of the two agricultural regions nearest Tomsk the principal forms of contact were work and short stays in woods and fields, haying, and cattle herding. A large percentage of cases, particularly in Tuganskiy Rayon, resulted from walks in the woods and flower gathering (56.8%). This type of contact with the out-of-doors was a substantial cause of infection in children; thus, in Tuganskiy Rayon it accounted for 46.7% of the

cases in children less than 14 years old. In the majority of the affected persons the incubation period was less than 10 days (65.4%); 32% of the patients exhibited incubation periods of 1-3 days, 14.7% of 4-5 days, 18.7% of 6-10 days, 12.9% of 11-15 days, 9.0% of 16-20 days, 7.6% of 21-25 days, 4.5% of 26-30 days, and 0.6% of 31-35 days.

TABLE 5

Clinical Forms of Tick-borne Encephalitis (in %)

Формы / Территория (A / B)	стертая (C)	менингеальная (D)	абортивная (E)	церебральная (F)	полиомиелитическая (G)	энцефалополиомиелитич. (H)	понтинная (I)	миелоэнцефалогеморрагическая (J)
Томск (K)	47,4	38,5	8,8	1,8	3,5	—	—	—
Томский район (L)	32,7	47,8	9,0	6,0	1,5	1,5	1,5	—
В целом по Томскому очагу (M)	39,4	43,7	8,8	4,0	2,3	0,9	0,9	—
Туганский район (N)	50,0	46,7	3,3	—	—	—	—	—
Остальные районы области (O)	18,9	37,5	6,2	25,0	6,2	—	—	6,2
В целом по области (P)	39,4	43,5	7,6	5,3	2,4	0,6	0,6	0,6

A) Form; B) area; C) lethargic; D) meningeal; E) abortive; F) cerebral; G) policmyelitic; H) encephalopoliomyelitic; I) pontine; J) myelo-encephalohemorrhagic; K) Tomsk; L) Tomskiy Rayon; M) total for the Tomsk nidus; N) Tuganskiy Rayon; O) remaining rayons of the oblast; P) total for the oblast.

Table 5 gives data on the clinical forms of tick-borne encephalitis in the Tomsk nidus and a number of the agricultural regions of the oblast.

It may be seen from the material in Table 5 that tick-borne encephalitis occurs principally in the lethargic, meningeal, and abortive forms. Thus, these forms accounted for 91.9% of all cases in the Tomsk nidus and all cases in Tuganskiy Rayon; their incidence was considerably lower (62.6%) in the more northern rayons of the oblast, cerebral forms constituting a considerably higher percentage (25%). This latter phenomenon can be explained only by inadequate diagnosis

of the lethargic, meningeal, and abortive forms in rayons far from Tomsk. The three rural patients who died of tick-borne encephalitis had the pol'omyelitic, encephalopoliomyelitic, and myeloencephalo-hemorrhagic forms.

The same prophylactic measures were employed as in past years, with the exception of vaccination. A broad campaign to eliminate ticks from the area and from domestic animals was carried out. A territory comprising 18,040 ha was treated with DDT and hexachlorane, 14,365 ha by aerial dusting. The area encompassed by the Tomsk natural nidus and a part (1820 ha) of Tuganskiy Rayon were treated. The same treatment was given to 29, 510 cattle, 6518 horses, and 4128 sheep.

The study begun in 1953 (S.P. Karpov, V.M. Popov, V.G. Kazanskaya, A.G. Kolmakova, and T.A. Vershinina) of the effectiveness of tick extermination by treatment of domestic animals with 10% DDT dust was finished up during this season. The investigations showed that the number of ticks in the pastures where the treated animals grazed systematically decreased (from an incidence of 4.9 in 1953 to 0 in 1957) and the ticks disappeared after four years, while the tick population in the control area increased continuously and reached a high level in 1956 (an incidence of 30.2).

Vaccination against tick-borne encephalitis was not carried out during this season, although persons bitten by ticks were given seroprophylaxis at the city polyclinics. A total of 366 individuals received antiencephalitis serum, 8 dying (2.1%).

In summing up the observations which were made it must be pointed out that there was a considerable decrease in the incidence of tick-borne encephalitis between 1956 and 1957. Thus, the incidence for the oblast as a whol dropped by 52.7% and that for the Tomsk nidus by 52%. As before, the Tomsk nidus, in which 68.2% of all of the cases

of tick-borne encephalitis in the oblast occurred, was of principal epidemiological importance. The incidence of encephalitis, elevated in 1956, in Tuganskiy Rayon, which lies extremely close to this nidus, continued to contribute substantially (19.4%) to the incidence for the oblast as a whole. There is every reason to believe that a nidus has formed in the inhabited taiga in Tuganskiy Rayon as a result of the agricultural activity there.

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EPIDEMIOLOGY AND PROPHYLAXIS OF TICK ENCEPHALITIS IN TOMSK NIDUS IN THE SEASON OF 1957

Karpov S.P., Yavya A.R.

Epidemiology of tick encephalitis in Tomsk nidus in the season of 1957 and the measures of its prophylaxis were characterized.

DATA ON THE GUR'YEVSK NIDUS OF TICK-BORNE ENCEPHALITIS

A.R. Yav'ya, N.I. Igolkin, and Yu.V. Fedorov

The Gur'yevsk nidus of tick-borne encephalitis must be considered to include Gur'yevskiy Rayon and the southwestern portion of Belovskiy Rayon in Kemerovskaya Oblast. This area may be divided into two basic topographic zones, the forested zone occupying the greater portion of it and the wooded steppe zone. As for the botanic-geographic classification of the area, the forest zone lies in the Salair forest region, while the wooded steppe zone lies in the central wooded steppe region of the Kuznetsk basin.

The Salairskiy Ridge, which has lost almost all of the morphological characteristics of a mountain range, runs from the northwest to the southeast of the Gur'yevsk nidus. Its mean height varies from 420 to 470 m above sea level. The eastern boundary of the Salair forest region runs somewhat to the east of the city of Salair and, further on, to the east of the settlement of Biryulya, to the west of Ursk, and on to Vaganovo and Zhuravlevo.

This region differs climatically from the wooded steppes to the east and west and serves as an area of condensation for the moisture carried by the southwest winds.

The western slopes of the Salair Ridge receive the greatest amount of precipitation, the eastern area receiving less. The eastern portion of the nidus is a transitional zone and receives the least precipitation of all the areas of the oblast.

The principal sylvan formation is a deciduous fir-aspen taiga with a variety of thick undergrowth.

The wooded steppe zone is a rolling elevated plain which generally slopes from the southeast to the northwest. The sylvan formations are not very characteristic of this type of zone, taking the form of small spans of birch. This area has a maximal amount of tillable soil and grain raising has consequently developed there.

Along the eastern boundary of the forest region, which is a broad strip of foothills, there is a transition to the deciduous taiga of the wooded steppes, i.e., a subsylvan transitional belt which is thickly populated and used for plowland and hayfields because of its virgin areas.

The incidence of tick-borne encephalitis in the Gur'yevsk nidus increased by a factor of 5 from the 1955 to 1956 seasons, its specific weight for the oblast as a whole increasing from 5.4% in 1955 to 20.8% in 1956. As a result, we were faced with the problem of studying the natural and epidemiological conditions which led to its high endemic incidence in this territory.

Stationary observations were made in the Gur'yevsk tick-borne encephalitis nidus from May to August of 1957, in the vicinity of the settlements of Tokovaya and Gornyy, which are located on the border between the two aforementioned topographic zones, in the southwest portion of Belovskiy Rayon. This territory had not previously been aerially dusted.

A wealth and diversity of animals inhabit this section of the nidus, there being 30 species of mammals, 106 species of birds, 3 species of ixodid ticks, more than 19 species of gamasid ticks, and 14 species of fleas. During the working period from 18 May to 6 August 1998 warmblooded animals (1529 mammals and 469 birds) were

trapped or shot and studied.

Active mature ticks appeared at the beginning of the second ten days of April in the wooded steppe zone of the nidus and during the third ten days of the month in the forest zone. Regular observations of the change in tick population were carried out from the 3rd ten-day-period in May until the 2nd ten-day-period in August in 5 biotopes: 1) the dark-conifer taiga; 2) mixed woods with a predominance of broad-leaved trees; 3) aspen woods of moderate age; 4) the sorga (swampy mixed woods); 5) pine woods with undergrowth.

The greatest number of ticks was observed during the 3rd ten-day-period of May in all biotopes, as shown in Fig. 1. The decrease in tick population was approximately the same in all biotopes until the first ten days of July, single specimens being encountered during the 3rd ten days of the month. The greatest tick population was observed in the dark conifer taiga, where it reached 44.7 ticks per man-hour at its maximum. Approximately the same figure was recorded at the forest station in the settlement of Osipovka. According to observations made by the workers of the parasitological department of the Gur'yevsk Rayon Public Health and Epidemiological Station at a point in the wooded steppe zone (M. Salairka), the tick population was considerably (14 specimens per man-hour) and their active season was shorter (from the 2nd ten-day-period of April to the 1st ten-day-period of July).

Considering the enormous role which small mammals play as hosts for the preimaginal stages of ixodid ticks, we studied the change in the extent of infestation of these animals with ticks and in the tick population at stations in the forest zone. The change in the small mammal population was studied by determining their population every ten days by the track-day method; the data obtained are shown in Fig. 2.

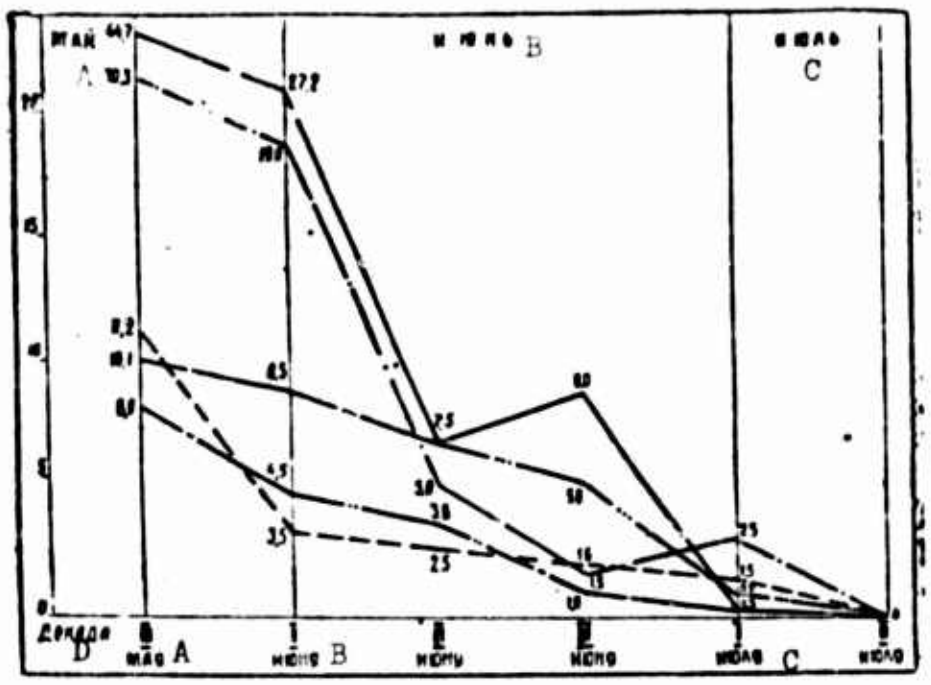

Fig. 1. Change in tick population in various biotopes (Tokovaya). The graph was compiled on the basis of the number of ticks collected per man-hour. Conventional symbols: – deciduous taiga; – · – mixed woods with a predominance of broad-leaved trees; – – – aspen woods of moderate age; – ·· – pine woods with undergrowth; – ··· – sorga (swampy mixed woods). A) May; B) June; C) July; D) ten-day-period.

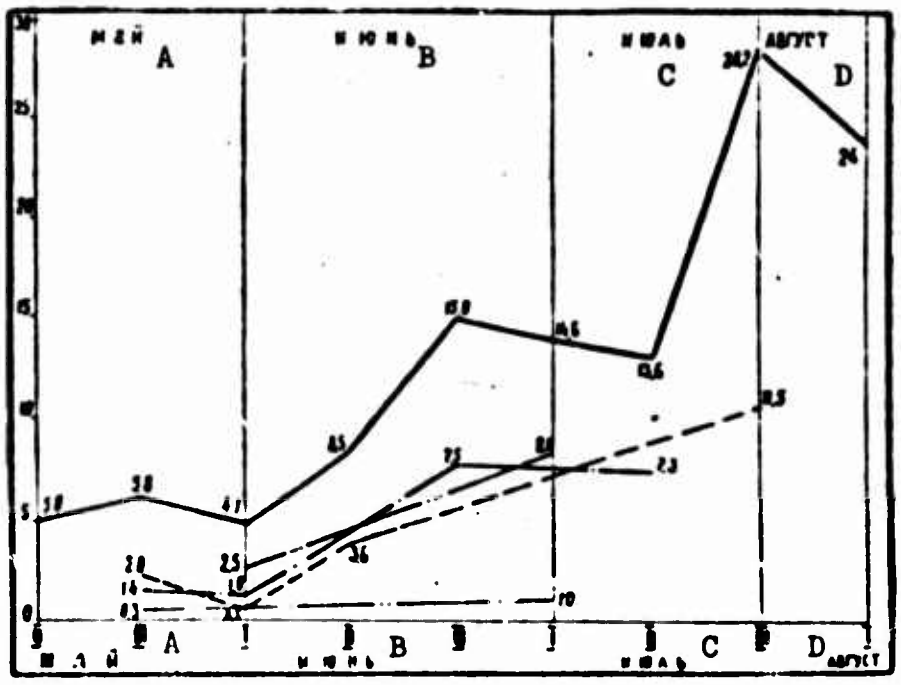

Fig. 2. Change in population of small animals in the vicinity of the settlement of Tokovaya. (Percentage of traps catching mammals during ten-day-period). Conventional symbols: – deciduous taiga; – ·· – sorga (swampy mixed woods); – – – mixed woods with a predominance of broad-leaved trees; – · – aspen woods of moderate age; – ·· – pine woods with undergrowth. A) May; B) June; C) July; D) August.

According to our data on tick abundance and incidence the extent

to which small mammals were infested with these parasites gradually increased (abundance and incidence being 1.0 and 41.1% during the second ten days of May, 1.6 and 27.6% during the third ten days of this month, and 3.4 and 53.6% during the first ten days of June) and reached a maximum during the second (4.4 and 63.1%) and third (5.8 and 60.0%) ten-day-period of June, a sharp decrease (to 0.2 and 9.0%) then occurring until the third ten-day-period of July.

During the first ten days of August tick abundance and incidence stabilized at a low level (0.2 and 11.2%). Comparing the change in the rodent population with the extent to which these animals were infested with ticks, we find that the latter increased until the third ten-day-period of June inclusive, parallel with the increase in the number of mammals. The rise in mammal population continued, but the extent to which they were infested with ticks decreased.

The fact that the increase in the number of small mammals coincided with a rise in tick infestation during the first half of the summer enabled the maximum number of larvae and nymphs to find hosts. This may have been one of the determining factors in the large number of nymphs and adult ticks during the following season. In any case, in the biotopes with large populations of small mammals which are the hosts of the larvae and nymphs (in the dark-conifer taiga), we noted a rather large number of adult ixodid ticks.

Virtually all of the species of mammals which inhabit the sparsely populated forest zone of the nidus are hosts for wood ticks. However, the most feasible hosts for the larvae and nymphs are the numerous wood voles, mice, and shrews, while for the adult stages of the ticks the best hosts are domestic livestock (large and small cattle and horses) and certain wild animals (hares, elk, bears, and roe deer).

In the wooded steppe zone the wood tick was found only on the

red-cheeked suslik.

In addition to mammals, 14 species of birds serve as hosts for the ticks in the wooded zone; these are the song thrush, fieldfare, dark-throated thrush, yellow bunting, white-crested bunting, tree pippet, black grouse, wood hen, starling, magpie, goldfinch, red-necked nightingale, brown-headed titmouse, and capercaillie. Of these species of birds the thrushes and buntings and the tree pippet are the most important as hosts. The majority of the avian hosts inhabit the broad-leaved or mixed woods and only a few of them (the capercaillie, wood hen, and dark-throated thrush) are ordinarily encountered in the dark-conifer taiga. It is obvious that small mammals play a considerably greater role than birds as tick hosts in the biotopes of the dark-conifer taiga, while, in our opinion, in the mixed woods both birds and mammals play substantial parts as tick hosts, especially in carrying the parasites about the area.

The greatest tick infestation of birds occurs during the second half of June and the first half of July. At this time the incidence of ticks on avian hosts varies from 55.7 to 96.4%, their abundance ranging from 1.2 to 6.7 ticks per host.

Wood ticks were not found on birds in the wooded steppe zone, but the tick Ixodes lividus of the group Crenulatus is found on sand-martins and in their nests.

Virological observations were made in order to detect reservoirs of infection, determine the extent to which the hosts were virus carriers, and study the circulation of the causitive agent. We were able to investigate 1174 rodents belonging to 13 species (the bank vole, wood mouse, shrew, matron vole, chipmunk, house mouse, northern mouse, hamster, red-cheeked suslik, field mouse, narrow-headed vole, common vole, and water shrew) and 295 wild birds belonging to 27 species

(the short-eared owl, wood hen, fieldfare, dark-throated thrush, warbler, snipe, sparrow hawk, cuckoo, common kestrel, jay, magpie, carrier sandpiper, great speckled woodpecker, great titmouse, land rail, jackdaw, black woodpecker, starling, goldfinch, red-necked nightingale, crow, yellow bunting, white-crested bunting, tree pippet, chimney swallow, gray wagtail, and tree sparrow).

In addition to the mammals and birds, we investigated 2672 wood ticks collected from the area and from animals and birds, 404 fleas taken from rodents, and fleas and gamasid ticks collected from 9 bank vole nests and three shrew nests in the nidus. From the material subjected to virological investigation we were able to isolate 11 strains of tick-borne encephalitis virus, of which 4 were from wood ticks collected in the area, one was from larvae and nymphs taken from bank voles, one was from the brains of tree pippets and wood hens, one was from the brains of shrews, and one was from the brains of bank voles.

In addition, we were able to isolate a strain of virus from the brains of red-cheeked susliks and from fleas collected from shrews. A total of 174 biological tests were conducted during the working period, 40 of them being to detect tick-borne encephalitis virus in ixodid ticks. Strains of lymphocytic choriomeningitis virus were also isolated from fleas collected from bank voles and from the brains of bank voles inhabiting the nidus (Yu.V. Fedorov, N.I. Igolkin, and M.K. Tyushnyakova, 1959).

Considering the large amount of work which was carried out and the comparatively small number of tick-borne encephalitis virus strains isolated, we are inclined to believe that there was a relatively low incidence of virus both among the small mammals and birds and among the ticks in the nidus during the season when we conducted our investi-

gations.

At the same time, as zooparasitological investigation has shown, all of the necessary conditions for circulation of the causitive agent existed in the nidus: 1) a high wood tick population; 2) a large population of small mammals, which are the hosts for the larvae and nymphs of the wood tick; 3) the presence of large wild mammals (elk, roe deer, bears, hares, etc.) and domestic animals (large and small cattle and horses), which are the hosts of the adult stage of the tick.

The variation in the incidence of tick-borne encephalitis for the nidus as a whole during the 1956-1957 season, by months, was as follows: May - 11.6 and 16.6%, June - 48.9 and 48.2%, July - 32.2 and 35.2%, August (1956) - 7.3%, and September (1956) - 0.2%. Cases of tick-borne encephalitis were recorded in all of the topographic zones: forest (51.5 and 55.5%), wooded steppe (32.8 and 29.6%), and transitional or subsylvan (16 and 15%).

The difference in the natural conditions which obtain in these zones and the extent to which the populace comes into contact with their micronidi were reflected in the variation in encephalitis incidence. Thus, in the micronidi of the wooded steppe zone the incidence for May was 31.2%, that for June 37.5%, and that for July 31.1; while in the forest zone it was 6 6% for May, 60.1% for June, and 33.2% for July. The wood ticks became active earlier in the wooded steppe zone than in the forest zone and intensive contact between the populace and the micronidi of the former zone also began earlier. Despite the fact that there were fewer hosts in the wooded steppe zone than in the forest zone the incidence of infection in the micronidi of the former was considerable, since it is heavily populated. The same phenomenon was also noted in the adjacent Belovskiy Rayon, where

80% of the area of the rayon is composed of the wooded steppe and steppe zones, which are the most densely inhabited. Up to 60-70% of the cases of tick-borne encephalitis were recorded in this area, 75-80% of them occurring during May and June.

During the 1956 season more than 70% of all cases in the Gur'yevsk nidus were recorded among the residents of the cities of Salair and Gur'yevsk, infection occurring in 40 micronidi. The most dangerous epidemiologically was the vicinity of the settlement of Osipovka, where 78 residents of the aforementioned cities were infected. Infection also occurred in the area along the Kubalda River, near Salair (40 patients), and in the environs of this city (34 patients).

A total of 60 micronidi were recorded for the nidus as a whole, infection of residents of 35 populated areas occurring in them.

It must be noted that single cases of tick-borne encephalitis were recorded in 60% of the micronidi. Infection occurred principally: a) while cutting wood for institutional needs (workers of the Gur'yevsk Metallurgical Plant and the Salair Zinc Mine) or while cutting wood and firewood for personal needs; b) during walks in the woods; c) while gathering garlic. These three types of contact were the most widespread in the nidus as a whole.

Our attention is struck by the high incidence of tick-borne encephalitis in children less than 14 years old during the 1956 season, the number of cases in this age group constituting 31% of the total for the nidus. If we take the incidence of the disease among children without considering the urban populace, it amounts to 32.5%. This was the first year in which this situation was observed.

The occupations of the tick-borne encephalitis patients were extremely diverse. In the cities the majority of cases were recorded among the workers of the Gur'yevsk Metallurgical Plant and the Salair

Zinc Mine, housewives, students (in Salair) and preschool children. In the rural areas the disease occurred principally among collective farm workers, students, and preschool children.

Infection usually occurred as a result of being bitten by ticks while the individual in question were in areas inhabited by these parasites; only 5.6% of the patients denied having tick bites and this may be due partially to errors in assembling their anamnesis. Ticks frequently come into farm houses on domestic animals and then come into contact with humans. Cases of tick-borne encephalitis were recorded principally among local residents, visitors constituting only 7% of the total.

The disease frequently occurred in the meningeal (50%) and poliomyelitic (36.1%) forms. There was a considerable decrease (85.8%) in the incidence of tick-borne encephalitis in the Gur'yevsk nidus during the 1957 season, but it was not uniform throughout the nidus. Thus, where aerial dusting had been carried out in May-June in order to combat ticks the decrease in incidence was 98.7%, while outside the treated zone it was 70%.

The territory in the vicinity of Salair and Gur'yevsk and along the Kubalda, Pryamuska, and Tolmova Rivers was treated, since it is the area most frequently visited by the populace.

An area of 13,093 ha (the area of Gur'yevskiy Rayon is 236,000 ha) was dusted continuously between 8 and 22 May and 4 and 13 June with an AN-2 aircraft. The consumption of toxic chemicals was 40-50 kg per ha in the forest zone and 25-30 kg per ha in the wooded steppe zone. The tick population was determined before dusting, in the wooded steppe zone it was 2.5-14 ticks per man-hour in May, while in the forest zone it was 24-87 ticks per man-hour during the same month. No ticks were found in the treated area for 1.5 months after dusting.

In 1957 6 persons were infected in micronidi in the dusted area, 4 of them before the treatment was carried out; in 1956, 98 persons contracted the disease in this area. It must be noted that the decrease in the incidence of tick-borne encephalitis during the 1957 season amounted to 72.7% in micronidi located outside the dusted zone and in which no measures had been taken to combat the carriers.

During the 1957 season not only did the incidence of the disease in the micronidi decrease sharply, but the number of micronidi was reduced by 53%.

The low carrier population during the 1957 season promoted this decrease in the incidence of tick-borne encephalitis. The following natural factors affected the tick population:

1. A reduction in the number of small rodents, which are the hosts of the preimaginal stages of the carriers, during the 1956 season;

2. The cold and not very snowy winter of 1956-1957, which affected the number of ticks which wintered over to a considerable extent. Severe cold set in in November, minimum temperatures reaching 12.4° during the first ten days and 21.4° during the second ten days of the month. The mean monthly temperatures for November and December were below normal. The first snowfall, which was slight, was on 14 November.

We do not have data on the seasonal population of the tick Ixodes persulcatus in the Gur'yevsk during 1956, but data are available for the Tomsk nidus. The comparative data show that the tick population during the 1957 season was only half that of the 1956 season. This is confirmed by data obtained from sporadic tick collections made in various biotopes of the Gur'yevsk nidus during the 1956 season (data gathered by the workers of the Gur'yevskiy Rayon Public Health and Epidemiology Station).

The prolonged cold spring of 1957 not only affected tick activity, but also considerable reduced the extent of contact between the populace and the micronidi of the infection. There was a considerable increase in the small rodent population during June and July of 1957. As may be seen from the data in Table 2 the increase in small rodent population coincided with a rise in the extent to which they were infested by ticks. The abundance and incidence of ticks on rodents increased sharply during the second and third ten-day-periods of June. This fact contributed to enabling the maximum number of larvae and nymphs to find hosts, while the comparatively mild snowy winter of 1957-1958 ensured favorable conditions for the ticks to winter over. This also created conditions for an increase in tick population during the 1958 season and, consequently, for an intensification of the epidemiologically poor conditions in the micronidi.

In comparison with the Tomsk nidus of tick-borne encephalitis, the Gur'yevsk nidus is more heavily populated and has been better studied, having its own topographic and faunistic peculiarities.

The existence of two topographic zones, forest and wooded steppe, in the nidus gives rise to a diversity of sylvan formations and of biocenotic relationships within them.

There is a wealth of diverse birds and mammals in the biotopes of the forest zone. Zooparasitological investigations have established that there are numerous hosts for the preimaginal stages of ticks among the wild animals. The presence of large mammals ensures hosts for the adult ticks. Virological investigations have revealed reservoirs of virus among wood ticks, numerous species of rodents, and wild birds. Circulation of the virus among numerous donors and recipients, following the food relationships of the wood tick is quite widespread and results in the high endemic incidence of tick-borne encephalitis in the

micronidi of the forest zone.

In the presence of favorable environmental conditions this endemic incidence may increase sharply, while the existence of contact between humans and these micronidi may lead to a sharp rise in the incidence of the disease among the populace, as occurred during the 1956 season. Contact between the populace and the micronidi of the forest zone begins later and tick activity also lags in the biotopes of this zone; as a result, the incidence of tick-borne encephalitis differs from that in the wooded steppe zone.

In the wooded steppe zone, where the sylvan formations take the form of stands of birch, wood ticks and their hosts are present in smaller numbers, the principal hosts for the adult ticks being domestic animals, since this zone is the most heavily populated.

Virological investigations have detected tick-borne encephalitis virus only in the red-cheeked suslik. It is necessary to study the role of the ixodid ticks (Ixodes lividus) found on sand martins and in their nests in the circulation of encephalitis virus.

The considerable extent to which the populace is affected by tick-borne encephalitis in the wooded steppe zone, where it remains at approximately the same level during the entire season, results not so much from its endemic incidence in these micronidi as from the extent to which the populace comes into contact with them.

Considering the different natural conditions which obtain in the micronidi of the forest and wooded steppe zones and determine the endemic incidence of tick-borne encephalitis in them, we feel it necessary to recommend the following measures for the prophylaxis of this disease.

In the micronidi of the forest zone: 1) aerial dusting of heavily-used recreation areas and of the environments of settlements of the

timber industry; 2) treatment of domestic livestock with DDT dust from the time when they are turned out to pasture until 15 July; 3) active and passive prophylaxis among the populace.

In the micronidi of the wooded steppe zone: 1) treatment of areas in the vicinity of tourist camps and fixed geological parties; 2) treatment of domestic livestock with DDT dust from the time they are turned out to pasture until July; 3) active immunization among those groups of the populace which have been in the area longest; 4) seroprophylaxis for all persons bitten by ticks.

Broad instruction in public health must be carried out in all micronidi.

MATERIALS TO THE CHARACTERISTIC OF HURYEV NIDUS OF TICK ENCEPHALITIS

Yavya A.P., Igolkin N.I., Feodorov Y.V.

Carried out detailed study of Huryev nidus of tick encephalitis made it possible to find out the presence of wide circle of animals on which the ticks Ixodes persulcatus are feeded both among mammals and birds.

1174 specimens of rodentia, 295 species of birds, 2672 species of ticks Ixodes persulcatus P. Sch., 404 species of fleas and ticks of superfamily of Gamasoidea collected in 12 nests of rodentia were virusologically researched. 11 strains of tick encephalitis virus and 2 strains of lymphocytic choriomeningitis virus were isolated.

The sickness of human beings with tick encephalitis depends upon natural conditions of nidus.

EXPERIENCE IN COMBATING VERNAL-ESTIVAL TICK-BORNE ENCEPHALITIS IN GORNYY ALTAY

V.M. Lyubushkina

As is well known, vernal-estival tick-borne encephalitis is a typical natural-nidus infectious disease with a markedly seasonal character, this resulting from the presence of its vectors (ticks) only in certain localities and from the cycle of their biological development (activity during certain months of the year).

As A.G. Panov, I.A. Minkevich, V.M. Popov, V.A. Nabakov, and others have shown, the vectors of tick-borne encephalitis in the eastern region of the Soviet Union are three species of ticks of the family Ixodidae: Ixodes persulcatus, Dermacentor silvarum, and Haemaphysalis concinna. Ticks of the species Ixodes persulcatus are of special importance as vectors of this disease, while the other species (Dermacentor silvarum and Haemaphysalis concinna) are of far less importance as carriers.

In Gornyy Altay the basic vector of tick-borne encephalitis is the tick Ixodes persulcatus, which is found in all of the aymaks of the oblast, with the exception of Kosh-Agachskiy Aymak. D. silvarun and Haemaphysalis concinna are also encountered in the majority of aymaks.

The first cases of tick-borne encephalitis in Gornyy Altay were recorded in 1950, appearing in certain aymaks more or less continually, year after year, and in others periodically, single cases in isolated

years. No tick-borne encephalitis at all was observed in Kosh-Agachskiy Aymak, high in the mountains, where only ticks of the species D. Nutalli are encountered.

The greatest incidence of tick-borne encephalitis was observed during an eight-year period (1950-1957) among the populace of Mayminskiy Aymak and the city of Gorno-Altaysk, which is located in the center of this aymak (actually, infection of the urban residents occurred outside the city, in Mayminskiy Aymak). Of the total number of cases of tick-borne encephalitis recorded during this period 74% occurred in Mayminskiy Aymak (including residents of Gorno-Altaysk).

Starting from the fact that the only source and carrier of tick-borne encephalitis in Gornyy Altay is the tick, we conducted prophylactic measures in two directions during the preepidemic and epidemic seasons of this year: 1) extermination of the carriers, the ticks; 2) wide dissemination of public health information among the populace to acquaint them with the disease and prevent infection.

The tick-extermination campaign was conducted by treating the area with hexachlorane and DDT dust (manual dusting) and by burning hexachlorane fires. The areas surrounding therapeutic institutions operated during the summer, children's homes located in the country, the "Chemal" health resort, lumbering enterprises, village squares, etc., were treated. A total of 866.46 ha were treated by manual dusting and 398 ha with smoke pots (a total of 1264.46 ha) were treated during 1957.

Of the 1264.46 ha of treated land more than 40% was located in Mayminskiy aymak, since this was the most unfavorable area with respect to tick-borne encephalitis.

In 1956 a total of 34.7 ha was treated by manual dusting and 98 ha with smoke pots. The area treated in 1957 was thus nine times the size

of that treated in 1956.

A spot check of the effectiveness of manual dusting revealed no ticks.

The veterinary service treated farm animals (large and small cattle and horses) with hexachlorane and DDT dust, homogenized creolin, and other insecticides. This was not done as a special antitick measure, but since the insecticides used by the veterinary service have a lethal action on ticks, they undoubtedly played a definite role in combating them.

According to available data, a total of more than 100,000 head of large cattle, 438,000 head of small cattle, and 7850 horses were treated (sheep were treated twice; 89,000 head of large cattle were treated three times). In addition, the veterinary service treated 1 ha of pastureland in the vicinity of Gorno-Altaysk.

In this prophylactic program we paid special attention to disseminating information about tick-borne encephalitis among the populace. A broad campaign was carried out to familiarize the inhabitants of Mayminskiy Aymak with the simplest measures to prevent this disease, since this area was the most unfavorable with respect to tick-borne encephalitis.

The Oblast Public Health and Epidemiological Station prepared, published through a local printer, and distributed among the populace of aymaks unfavorable with respect to tick-borne encephalitis 1500 copies of a booklet entitled "How to Guard Against Tick-Borne Encephalitis" and 500 copies of an illustrated public health bulletin on the subject "Tick-Borne Encephalitis and its Prophylaxis." Problems of encephalitis prevention were explained in the oblast newspapers "Zvezda Altaya" and"Altaydyn Chelmony" (both published in the Altay language) and in a number of aymak (rayon) newspapers. Lectures and seminars were

held (the workers of the Oblast Public Health and Epidemiology Station conducted 33 lectures and 135 seminars alone). One talk on tick-borne encephalitis was given over the radio (on the oblast radio station).

Workers in geological surveying parties and groups of tourists visiting the oblast were acquainted with personal measures for preventing tick-borne encephalitis. A seminar on tick-borne encephalitis was held for the workers Young Pioneer camps located in the oblast before they left for camp. The supervisors of individual enterprises and institutions (lumbering enterprises, children's homes, etc.) were personally instructed in specific measures for preventing tick-borne encephalitis in their institutions.

Medical workers in Gorno-Altaysk and the surrounding countryside were instructed in the diagnosis, treatment, epidemiology, and prophylaxis of tick-borne encephalitis both through lectures, seminars, and individual talks and through literature.

Quinacrine prophylaxis for persons bitten by ticks, workers in geological surveying parties, and tourists was first employed during this year. Unfortunately, not all of the individuals who received quinacrine were followed up through the usual medical channels. According to the incomplete data available to the Oblast Public Health and Epidemiology Station 299 persons received this treatment. Seroprophylaxis was also employed; according to incomplete data, 25 persons bitten by ticks received this treatment.

There were no cases of tick-borne encephalitis among the individuals who received quinacrine or serum prophylaxis.

The incidence of tick-borne encephalitis in the oblast as a whole decreased by a factor of more than three from 1957 to 1956 and by a factor of more than five in Mayminskiy Aymak, the most unfavorable region with respect to this disease.

The wide dissemination among the populace of information on the simplest measures for preventing tick-borne encephalitis and the employment of antitick measures apparently played no small role in the decrease in the incidence of tick-borne encephalitis in the oblast as a whole and especially in the very sharp drop in its incidence in Mayminskiy Aymak, the most unfavorable area with respect to this disease.

CONCLUSIONS

1. Wide dissemination among the populace of information on tick-borne encephalitis and the simplest measures for preventing it must play a leading part in combating this disease, as must antitick measures and quinacrine prophylaxis.

2. Considering the high effectiveness of dusting with DDT and hexachlorane, the area treated in this manner should be systematically expanded.

3. Among antitick measures, extermination of ticks on farm animals must be paid the greatest attention; the animals should be treated by farms of the veterinary service as a special antitick measure in the general complex of such measures being carried out in conjunction with organizations of the public health service.

4. It is necessary to continue the use of quinacrine prophylaxis for tick-bite* victims and those who run the risk of such bites and to study the effectiveness of this treatment further on a larger number of subjects.

Gorno-Altaysk Oblast Public Health and Epidemiology Station

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84	Actually, ticks inflict wounds by sucking rather than by biting (Ed.).

SPECIFIC PROPERTIES OF METHYL-ALCOHOL-PRECIPITATED CEREBRAL DIAGNOSTICUM FOR TICK-BORNE ENCEPHALITIS

M.K. Tyushnyakova, Yu.V. Fedorov, M.S. Zagromova, and F.S. Belova

In studying the antigenic characteristics of the causitive agents of a number of neural infections cerebral diagnosticum was first tested in the complement-fixation reaction by Casals and Palatsios. M. P. Chumakov, A.P. Belyayeva, and many others used this antigen, prepared by Casals' method, in studying tick-borne encephalitis. The specific substance was a 10% thermolyzed suspension of brain cells from white mice inoculated with tick-borne encephalitis virus. The antigen had a high specificity, but was infectious and contained an admixture of lipoid substances which gave it anticomplementary properties.

Despite the fact that A.I. Ivanenko tried to produce this diagnosticum by culturing the virus on ascitic carcinomas from white mice and Ye.N. Levkovich, O.Ye. Rzhakhova, and others used various chick-embryo tissues for this purpose, the cerebral method did not lose its importance, especially when it was taken into account that high virus concentrations may be obtained in this fashion.

Casals, Il'yenko, and others attempted to purify the cerebral antigen of ballast impurities by exposing it to liposolvents. Casals tested acetone, benzine, and ether. V.I. Il'yenko investigated extraction of the cerebral suspension with benzine, toluol, xylol, and chloroform in conjunction with thermolysis. The authors concluded that ether and chloroform are the best solvents and satisfactorily eliminate the anticomplementary properties of the suspension. The virus was inactivated by adding virus and formalin in a ratio of 1:500 to the native cerebral emulsion.

Our observations, which were made on production quantities, showed that the action of ether and chloroform on the formalized suspension does not always satisfactorily eliminate its anticomplementary properties and leads to an extremely low yield of purified diagnosticum.

All of this forced us to seek new modifications of the production method for cerebral antigen, to be used under production conditions. For this purpose we thermolyzed the cerebral suspension by Casals' method and then purified it by precipitating the specific substance

with methyl alcohol by M.I. Tovarnitskiy's method. The "Kargasokskiy" strain of tick-borne encephalitis virus, isolated from the tick Ixodes persulcatus in the Tomsk nidus, was used to inoculate the mice. The titre of virus was 10^{-9}.

A 10% suspension of brain cells from mice killed at the height of the clinical manifestation of the disease was prepared in physiological solution (pH 7.2-7.4). In order to produce the best yield of virus from the cells the suspension obtained was agitated two or three times and kept at a temperature of $+4^{\circ}$ C for 24 hours, then freed of coarse suspended particles by brief centrifuging, and subsequently thermolyzed by being frozen five times with liquid nitrogen or oxygen and then thawed in a water bath at $+37^{\circ}$ C. The flocculate which formed was precipitated by centrifuging at 3500 rpm for 30 minutes and removed.

In order to obtain the specific substrate methyl alcohol cooled to -20° C was added to the centrifugate in a volume of 45 ml per 100 ml of suspension. The mixture was carefully stirred and kept at a temperature of $+4^{\circ}$ C for 18-24 hours. The liquid above the precipitate was discarded and the flocculous residue was centrifuged for 30 minutes at 3500 rpm. The liquid which formed above the precipitate was discarded and the dense residue obtained was dissolved in a phosphate buffer solution (pH 7.2-7.4) to make a volume equal to that of the initial material to which the methyl alcohol was added. The residue dissolved in the buffer was the specific antigen. The latter was a clear, slightly opalescent liquid which retained its infectiousness in titres of $10^{-8} - 10^{-7}$.

The antigenic properties of the diagnosticum thus produced were preliminarily tested in a complement-fixation reaction with hyperimmune and normal horse sera and simultaneously with normal antigen pre-

pared by the same method as the specific antigen.

The virus in the antigen was inactivated by adding formalin in a ratio of 1:1000 and then keeping the mixture at room temperature for 6 days. The absence of active virus in the diagnosticum was subsequently checked by intracerebral inoculation of white mice followed by two blind reinoculations. The negative results obtained for the control animals confirmed that the virus had been completely inactivated. The data obtained are shown in Table 1.

Purification of the antigen freed it of anticomplementary substances. The diagnosticum had good specific properties. Before formalization it yielded clearly positive results in a dilution of 1:20 with hyperimmune horse serum 1/512. The titre of the diagnosticum was approximately halved by formalization, but was still quite sufficient for practical use (the dilution of the antigen was 1:20 and that of the hyperimmune serum 1:256).

TABLE 1

Data Obtained in Control Tests on Formalized Antigen

A	B	C Биоконтроль											
		D Исх. заражение				I пассаж I				II пассаж J			
Титр вируса в исходной суспензии	Титр осажденного вируса	E дата	F к-во мышей	G к-во павших	H к-во живых	E дата	F к-во мышей	G к-во павших	H к-во живых	E дата	F к-во мышей	G к-во павших	H к-во живых
$10^{-8,5}$	10^{-7}	15/III	5	—	5	22/III	5	—	5	27/III	5	—	5

A) Titre of virus in initial suspension; B) titre of precipitated virus; C) control; D) initial inoculation; E) date; F) number of mice; G) number dying; H) number surviving; I) first reinoculation; J) second reinoculation.

The results of the determination of the active properties of the diagnosticum are given in Table 2.

Immediately after the specific activity results were obtained the formalized antigen was diluted with an equal volume of stabilizer

TABLE 2

Antigenic Activity of Diagnosticum With Hyperimmune Horse Serum in Complement-fixation Reaction

Титр антигена в разведении 1:20 в процессе изготовления A			Титр антигена в разведении 1:20 в процессе хранения в месяцах B			
до формализации C	после формализации D	после сушки E	1	2	3	4
1:512	1:256	1:128	1:128	1:128	1:128	1:64

Note: The antigen was prepared on 6 March 1958. Formalization was carried out over a period of 7 days and the preparation was then frozen and dried.

A) Titre of antigen in dilution of 1:20 during preparation; B) titre of antigen in dilution of 1:20 during storage in months; C) before formalization; D) after formalization; E) after one day.

TABLE 3

Complement-fixation Reaction With Blood Sera From Tick-borne Encephalitis Patients

Результаты A	Дни болезни B				
	1–3	4–6	7–10	11–15	16 и более F
C Количество исследований	46	40	25	12	28
D Положительные	28	25	18	9	21
E Отрицательные	18	5	7	3	7

A) Results; B) days of illness; C) number of tests; D) positive; E) negative; F) or more.

(20% sucrose and 2% gelatin, poured into 1 ml ampules, and dried by vacuum freezing. Before drying the antigen was kept frozen at a temperature of -26° C. The diagnosticum was dried in a Dolinov-chamber system for 24 hours.

The first 10 hours of the drying process were at a below-zero temperature (-24° C), while the remaining 14 hours were at temperatures of from +1° to +16°C. Unfortunately, we used ordinary glass am-

pules and this had a detrimental effect on the quality of the preparation.

When a complement-fixation reaction was carried out with the antigen immediately after drying it was found that the titre was halved, amounting to a serum dilution of 1:128 at an antigen dilution of 1:20 (see Table 2).

Observation of the specific activity of the antigen during storage indicated that the titre of the diagnosticum in ampules in which the vacuum was retained to some extent did not change over three months of storage at a temperature of +4° C. After 6 months of storage the antigen began to lose its solubility and its titre amounted to a serum dilution of 1:64 at an antigen dilution of 1:20; in individual ampules sufficient specific activity persisted for up to 1 year. In determining the specific properties of the prepared diagnosticum it was also tested in a complement-fixation reaction with blood sera from patients having clinically-diagnosed tick-borne encephalitis (151 individuals) who were being treated in the Clinic for Infectious Diseases of the Tomsk Medical Institute. The lethargic and meningeal forms of the disease predominated among the patients examined. The blood sera were taken between the 1st and 30th days of illness. Positive results were obtained with the blood of 101 persons (or in 66.89%).

The data in Table 3 indicate that specific complement-fixing antibodies could be detected from the first few days of illness onward.

The titre of complement-fixing antibodies varied from 1:4 to 1:128, a high antibody content being noted in isolated individuals from the 4th-6th day after the onset of clinical symptoms. At our request, the prepared diagnosticum was tested in a number of the laboratories of the public health and epidemiology stations of Krasnoyarskiy Kray and Kemerovskaya Oblast. In examing patients from Krasnoyarskiy Kray

F.V. Krasovsk noted the presence of complement-fixing antibodies from the 4th day of illness on. He examined 72 patients during the acute period of tick-borne encaphalitis and obtained positive results in 47% of these cases. The variation in the titres of antibodies was the same as that which we observed (from 1:4 to 1:128).

Yu.I. Dokuchayeva, T.I. Izrayeleva, and V.I. Tolkuyeva used the complement-fixation reaction to establish that there were immunizing shifts in the blood sera of patients in Kemerovskaya Oblast nidus during the first 10 days of illness.

We offer our sincere thanks to the aforementioned comrades, who tested the antigen which we prepared in the complement-fixation reaction.

CONCLUSIONS

1. Inoculation of white mice with a highly active strain of tick-borne encephalitis ensures production of a cerebral suspension containing large titres of virus.

2. Thermolysis of the cerebral suspension and subsequent precipitation of the centrifugate with methyl alcohol completely purifies the specific antigen of ballast substances and preserves the high titre of virus.

3. Inactivation of the virus in the purified substrate is achieved by adding formalin in a dilution of 1:1000, which leads to a less marked decrease in complement-fixin; activity than when a dilution of 1:500 is used.

4. Antigen dried by vacuum freezing with addition of stabilizer in a dilution of 1:1 (20% sucrose and 2% gelatine, pH 7.4) retains sufficient specific activity when kept in a refrigerator at +4°C for up to 6 months.

Testing the diagnosticum obtained by the complement-fixation reaction with blood sera from tick-borne encephalitis patients showed that it has a high specific activity and enabled us to establish that complement-fixing antibodies are present in titres of from 1:4 to 1:128 during the first few days of illness.

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SPECIFIC PROPERTIES OF CEREBRUM DIAGNOSTICUM OF TICK ENCEPHALITIS PRECIPITATED BY METHYLATED SPIRIT

Tjushnyakova M.K., Feodorov Y.V., Zagromova M.S.

Belova F.S.

Materials on working out the method of preparing antigen for RCC (reaction of connecting the complement) from cerebrum of infected by the virus of tick encephalitis white mice by thermolysis and purifying by methylated spirit were represented.

ZOOPARASITOLOGICAL OBSERVATIONS IN THE ARVICOLAR TULAREMIA NIDUS IN THE OB' RIVER VALLEY

N.I. Igolkin

It is impossible to study the ways in which the causitive agent of tularemia circulates without a thorough investigation of the vertebrate and invertebrate fauna composing the biocenosis of the natural nidi of infection. Nevertheless, the specific composition of the hosts of the causitive agent (mammals) and its carriers (arthropods) in the arvicolar tularemia nidi of Western Siberia has not been sufficiently well studied.

Our observations were made in 1955-1956, in one of the rayons of Tomskaya Oblast, in the Ob' River valley, where an epizootic had previously been observed among rodents.

The rayon which we investigated is an extremely monotonous area with a very low relief. In spring the majority of the local streams rapidly overflow their banks, flood widely, and merge with the Ob' River to form a broad sheet of water.

The valley contains an enormous number of small and cut-off lakes which are frequently marshy or entirely overgrown with sedge. This labyrinth of lakes and bogs is difficult to pass through. The cut-off lakes create favorable conditions for the development of aquatic flora. Duckweed is encountered here, while water lilies, yellow water lilies, and arrowhead are found in deep spots with silty bottoms.

The riparian flora of the lakes consists principally of sedge

mixed with horsetail. Thickets of these plants provide good shelter for divers and certain species of mammals such as the water vole and matron vole.

Grains predominate in the floral associations of the meadows. Broad-leaved grasses such as meadowsweet, cowbane, etc., are also encountered.

The ligneous flora is predominantly willow, which is somewhat stunted and reaches substantial size and age only in groves located along small streams. European birdcherry, viburnum, sharpleaf willow, and red and black current are mixed in with the willow. The stands of grass contain grain, ferns, local nettles, wormwood, etc. Birch, aspen, and conifers are entirely lacking. The spring floods usually do not reach the groves in the highest areas.

During high water a large number of mammals are concentrated in the groves; these stay in the sections free of water and find temporary shelter in the hollows of old trees.

For tens of kilometers along the right bank of the Ob River is a valley which the spring floods fill. The main bank slopes downward and it is only from the character of the plant cover that we can evaluate the area, which is never free of water.

SPECIFIC COMPOSITION AND DISTRIBUTION BY BIOTOPES OF THE MAMMALS OF THE VALLEY

The most common mammals are the water vole, matron vole, bank vole (genus Clethrionomys), and shrew (genus Sorex). The water shrew, harvest mouse, ferret, ermine, and weasel are less frequently encountered, while the field mouse, mole, chipmunk, and (near villages) hooded rat are very rarely found.

The mammals were collected with "Hero" traps, cylinder traps, and bough snares.

It was established that the mammal population in inundated meadows and near ponds is small. Such areas are inhabited primarily by the water vole and matron vole. During the years covered by our observations these rodents were very few in number, there never being more than 16-21 specimens per km of shoreline for lake-like bodies of water.

The rodents also exhibited very little burrowing activity in the meadows. It was only in September 1956 that a few water vole burrows with inhabited nesting chambers were discovered. Matron vole burrows and nests were encountered somewhat more frequently.

In contrast to the data given above, the water vole population in all of the biotopes was immensely greater in 1954. According to an investigation by V.M. Popov, at the end of August "in an area of 250m^2 74 wood voles were collected in 63 traps over a 3-day period. Many tracks traveled by these animals were discovered in the area before the collection was made, numerous mounds of freshly-dug earth were found, and running voles were often seen."

There is a considerable population of small carnivores in the valley. In August and September we observed a great deal of ferret activity along the shorelines of the lakes, the favorite habitats of the water vole and bank vole. Of the 10 ferret stomachs which we studied there were remains of rodents in 4, while the others were empty.

It was noted that the wooded knolls along the banks of small streams and some lakes had a rather high rodent population. The increase in the number of small mammals in this biotope between spring and fall in 1955 was extremely slow. In June the percentage caught in the "Hero" traps was 2.8%, in July 2.6%, in August 2.7%, and in September 3.1%. In our opinion, this resulted from a tularemia epizootic among the mammals. In any case, the bacterialogical investigations

TABLE 1

Specific Composition of Mammalian Fleas

A Виды животных / B Вид блохи	C Собрано блох с млекопитающих											Собрано блох из гнезд O		P Обнаружено блох данного вида
	D водяная полевка	E полевка-экономка	F рыжие полевки (Clethrionomys)	G мышь полевая	H мышь-малютка	I серая крыса	J бурундук	K колонок	L крот	M кутора	N землеройки (Sorex)	водяная полевка	полевка-экономка	
Amphipsylla sibirica Wagn.	3	1	19	1	1			1	2		2		5	35
Ceratophyllus penicilliger Joff.	6	3	73		1	1					4		1	89
Ceratophyllus rectangulatus Wahl.	5	2	17		1	1		1			1		2	30
Ceratophyllus walkeri Roths.	14	7	2			1	9				1	40	23	88
Ceratophyllus tamias Wagn.							2							9
Ctenophthalmus pisticus Joff.														2
Coratopsylla birulai Joff.	1		11		6					9	166		1	191
Leptopsylla bidentata Kol.	6	15	3	1	3			1				2	2	33
" silvatica Mein.	2	8	6											16
Neopsylla acanthina J. et R.							1							1
Определено блох	37	36	131	2	12	3	12	3	2	9	174	42	34	497

A) Species of animal; B) species of flea; C) fleas collected from mammals; D) water vole; E) matron vole; F) bank vole (Clethrionomys); G) field mouse; H) harvest mouse; I) hooded rat; J) chipmunk; K) ferret; L) mole; M) water shrew; N) shrew (Sorex); O) fleas collected from nests; P) fleas of given species detected; Q) fleas detected.

which Ye.I. Kleytman carried out on the small mammals which we collected showed that a high percentage of them were infected with the causitive agent of tularemia. It is obvious that the epizootic which occurred during 1955 had an inhibiting effect on the multiplication of these animals.

During 1956 we were able to determine the small mammal population for June (2.2%) and August (8.7%). The data obtained indicated a marked increase in population over the preceding year, especially among bank voles. It must be noted that the causitive agent of tularemia was isolated in only one case in 1956 (from a bank vole), while in 1955 this infection was detected in water voles, shrews, matron voles, water shrews, and bank voles.

MAMMALIAN ECTOPARASITES

Considering the great importance of ectoparasites in the epizootiology of small mammals, we collected a number of specimens in order to study their specific composition and the extent to which they infest different species of animals.

Fleas, mites, and ticks were found on mammals and in their nests. The specific composition of the fleas (Aphanipthera) which we collected is shown in Table 1.

The most common fleas for bank voles are Amphipsylla sibirica, Ceratophyllus rectangulatus, and C. penicilliger, the incidence of the latter being extremely high. The flea Doratopsylla birulai is specific for shrews and water shrews. The most numerous flea on matron voles is Leptopsylla bidentata. C. walkeri predominate on water voles and in their nests.

Of the 10 species of fleas which we collected Ceratophyllus walkeri is of the greatest epizootiological importance. According to an investigation conducted by V.Ye. Tiflov (1934), this flea carries tularemia from diseased water voles to healthy ones. However, when the fleas which we collected were examined the causitive agent of tularemia was isolated from a batch of parasites taken from bank voles. C. penicilliger predominated among the fleas, while only a few specimens of C. walkeri were encountered.

We noted that the extent to which the mammals of the valley were infested with fleas, as determined from tick abundance and incidence, tended to increase from spring to fall. Shrews were an exception. A sharp decrease in tick infestation was noted among the latter in September (see Table 2).

The ticks (Acarina) which we collected were gamasid (superfamily Gamasoidea), thrombiculid (family Thrombiculidae), ixodid (family

TABLE 2

Extent of Tick Infestation of Most Numerous Mammals in the Valley in 1955

Время обследования A / Вид млекопитающего B	Июнь C					Июль D					Август E					Сентябрь F					G
	Осмотрено млекопит. G	Из них с блохами H	Обнаружено блох I	Встречаемость J	Обилие K	Осмотрено млекопит.	Из них с блохами	Обнаружено блох	Встречаемость	Обилие	Осмотрено млекопит.	Из них с блохами	Обнаружено блох	Встречаемость	Обилие	Осмотрено млекопит.	Из них с блохами	Обнаружено блох	Встречаемость	Обилие	Осмотрено млекопитающих
Водяная полевка L	4	0	0	—	—	19	1	1	5,0	1,05	46	7	7	15,2	0,15	6	2	5	33,3	0,8	75
Полевка-экономка M	5	1	5	20,0	1,0	14	3	12	20,1	0,9	10	4	10	40,0	1,0	10	6	32	60,0	3,2	39
Рыжие полевки (род Clethrionomys) N	49	15	51	30,6	1,0	34	9	42	26,8	1,2	59	17	47	28,8	0,8	32	16	52	50,0	1,6	174
Землеройки (род Sorex) O	38	20	109	52,6	2,9	30	16	98	53,3	3,2	30	17	146	56,6	1,8	54	20	59	37,0	1,1	152

A) Time of investigation; B) species of mammal; C) June; D) July; E) August; F) September; G) mammals examined; H) number with fleas; I) number of fleas detected; J) incidence; K) abundance; L) water vole; M) matron vole; N) bank vole (genus Clethrionomys); O) shrews (genus Sorex).

Ixodoidea), and armoured (subtribe Oribatei). The gamasid ticks contributed the greatest number of species, 13. This group also included ticks of the families Pachylaelaptidae, Ascaidae, Macrochelidae, Parasitidae, and Laelaptidae (genus Hypoaspis) (see Table 3).

The ticks most frequently found on mammals belonged to the families Laelaptidae and Haemogamisidae. Exchange of ticks was observed among certain species of mammals having close biocenotic ties. Thus the tick Laelaps muris, which is specific for water voles, was found on weasels that fed on these animals.

The ticks Haemogamasus ambulans, Hirstionyssus isabellinus and Eulaelaps stabularis are distributed very widely in the floodland and are found on all of the most numerous mammals and in their nests. Cases in which the causitive agent of tularemia was detected among gamasid ticks have been noted by L.M. Khatenever (1930), Ye.I. Martsinovskiy and G.Ya. Sinay (1931), V.P. Dzhanpoladova (1943), and others.

A.A. Zakhvatkin (1948) assigns a great deal of importance in the epizootiology of tularemia to ticks of the species Laelaps, particularly Laelaps muris, a widely occurring specific parasite of the water vole. Ye.N. Nel'zina, V.P. Romanova, G.M. Danilova, and K.S. Sckolova (1957) established that ticks of the genus Hirstionyssus are capable of maintaining and transmitting the causitive agent of tularemia for a long time. Feeding repeatedly, these ticks can support a continuous epizootic among their hosts.

Gamasid ticks obviously play no small role under the conditions which obtain in the nidus which we studied. In one case in 1956 the causitive agent of tularemia was isolated from gamasid ticks (mixed species) taken from bank voles.

Both abundance and incidence in disease show that the most important species of mammals are highly infested with gamasid ticks. Up to 50 specimens were collected from individual water voles. For these mammals gamasid ticks had an incidence of 57.6% and an abundance of 6.0; for bank voles these figures were 28.1% and 0.9 respectively, while for shrews they were 11.8% and 0.4.

As a rule, n. ixodid ticks were encountered in the low lying flooded biotopes of the Ob'River. No ticks were found in massive collections of water voles and other rodents of the valley made by workers of the Tomsk Antitularemia Station in past years.

Taking into account the great importance of ixodid ticks in maintaining the causitive agent of tularemia in nidi of the valley type, a phenomenon which N.G. Olsuf'yev demonstrated for a number of areas in the European portion of Soviet Union, we paid special attention to searching for these tick in the Ob'Valley.

Four specimens of adult ixodid ticks were collected in 1956 from wooded knolls near villages, while ticks were found on only 6 of 439

TABLE 3

Specific Composition of the Ticks of the Valley

A / B	C Количество собранных клещей												
	D с млекопитающих									N с насекомых	P из гнезд		
	E водяная полевка	F полевка-экономка	G рыжая полевка р. Clethrionomys	H мышь-малютка	I мышь полевая	J бурундук	K землеройка (род Sorex)	L козомок	M выдра	O жук мертвоед	Q водяной полевки (6 шт.)	R полевки-экономки (5 шт.)	S Всего
1	2	3	4	5	6	7	8	9	10	11	12	13	14
T Гамазовые клещи													
Laelaps muris Ljungh.	343		1					5					349
. micromydis Zachv.				4									4
. pavlovskyi Zachv.					2			1					3
. clethrionomydis Lang.			3										3
. hilaris Koch.		2										1	3
Hyperlaelaps amphibius Zachv.	50												50
. arvalis Zachv		3	1										4
Eulaelaps stabularis Koch.	11	8	12		1		2				14	18	66
Haemolaelaps glasgowi Ewing.			22				4				5	1	31
U Род Hypoaspis											8		9
Haemogamasus ambulans Thorel.	6	22	34	1		2	6	6			43	93	213
. liponyssoides Ewing.	1		20				6				20	5	52
. nidi Michael												2	2
Hirstionyssus isabellinus Oudem.	8	6	60				6	73	5			2	160
V Семейство Pachylaelaptidae				1							2	1	4
. Ascaidae	1		21		1		1	1				3	28
. Macrochelidae			1							1			2
. Parasitidae	2	4	31				8			23		1	69
W Краснотелковые клещи													
Род Trombicula	7	35	189										231
X Панцирные клещи													
Y подотряд Oribate	9	1	6								5	5	26
Z Иксодовые клещи													
Ixodes persulcatus P. Sch.	1		3				13						17
S Итого	439	81	404	6	4	2	46	86	5	24	92	137	1326

A) Subject of study; B) species of tick; C) number of ticks collected; D) from mammals; E) water vole; F) matron vole; G) bank vole, genus Clethrionomys; H) harvest mouse; I) field mouse; J) chipmunk; K) shrew (genus Sorex); L) ferret; M) water shrew; N) from insects; O) carrion

beetles; P) from nests; Q) water vole (6 specimens); R) matron vole (5 specimens); S) total; T) gamasid ticks; U) genus; V) family; W) thrombiculid ticks; X) armoured ticks; Y) subtribe; Z) ixodid ticks.

mammals examined; three bank voles yielded one nymph each, two shrews 13 larvae, and a water vole 1 larva.

The occurrence of ixodid ticks in the valley has a purely local character and obviously results from the carrying of attached females from the bank of the river by dogs or farm animals. It was established that 100-150 head of cattle were driven yearly (this figure being especially large in recent years) from the left bank of the river into the area where we made our observations, generally in the middle of the summer. The number of ticks on the animals at this time is comparatively small, so that the latter could not have introduced any large quantity of ticks. There cannot be a continual abundance of ixodid ticks in the inundated valley, since the heavy periodic spring floods make it impossible for there to be favorable conditions for all metamorphic stages. The propagation of Ixodes persulcatus in the flooded valley thus has a clearly anthropurgic character.

In our collections the thrombiculid ticks were represented only by the genus Thrombicula. Bank voles are subject to especially intensive infestation with these ticks; matron voles and water voles are affected to a far lesser extent. It was noted that rodents were less infested with thrombiculid ticks during 1956 (a year of heavy precipitation) than during 1955.

Armoured ticks are not hemophages and consequently do not occur widely as ectoparasites among mammals. A few are occasionally encountered in the nesting litter of rodents. It is known from the literature (Ye.Ya. Bashkirova, 1941; V.A. Potemkina, 1941-1944; D.P. Soldatova, 1944) that these arthropods are the intermediate hosts of the anoplocephalids which infest farm animals.

There was comparatively little infestation of mammals with lice during the summer months. Exceptionally high numbers of lice were observed only on certain matron voles, there being 80-100 specimens per animal. The greatest infestation of rodents with lice occurred at the beginning of the summer and then gradually decreased toward fall.

Our two-year study confirmed that there are mammals in the valley which are highly susceptible to tularemia; these include the water vole, matron vole, and bank vole. There were very few of these animals during the years of our investigation.

We also established that the specific composition of the possible and proven carriers of tularemia among the ectoparasites detected in the valley is rather diverse. These factors may explain the severe tularemia epizootics which have occurred in this large river valley of Western Siberia.

Tomsk Scientific Research Institute for Vaccines and Sera

ZOOPARASITOLOGIC OBSERVATIONS IN ARVICOLAR TYPE OF TULAREMIA NIDUS IN THE FLOODED PART OF THE SHORE OF OB RIVER

The observations confirm the presence in conditions of the flooded part the shore of the river highly susceptible to tularemia mammals such as Arvicola terrestris, Microtus oeconomus, gen. Clethrionomys and artropoda possible and proved carriers of tularemia.

There are parasitizing 10 Species of fleas on mammals and in their nests (in collecting there prevail Ceratophyllus penicilliger, C. walkeri and Doratopsylla birulai); about 20 species of ticks of the superfamily of Gamasoidea Reuter; there prevail the ticks of the family of Laelaptidae and Haemogamasidae; the ticks of the family Thrombiculidae Ewing, gen Thrombicula Berl; from superfamily Ixodoidea Banks-Ixodes persulcatus P. Sch. and in small quantity the ticks

from the suborder of Oribatei Dug.

RESERVOIRS AND SOURCES OF LEPTOSPIRAL INFECTION IN TOMSKAYA OBLAST

V.N. Novikova, L.P. Sagaydak and N.I. Igolkin

Leptospiral diseases are encountered among humans and farm animals in Tomskaya Oblast. These diseases occur principally during the summer and autumn months (most frequently among domestic livestock and rural residents) and occasionally have an epizootic or epidemic character.

Leptospirosis has been diagnosed serologically among large cattle in a number of populated areas in Tomskiy, Shegarskiy, and Kozkevnikovskiy Rayons. The investigations which were conducted showed that leptospirosis among large cattle in Tomskaya Oblast is caused by leptospirae of the pomona, tarassovi, and canicola types, principally the first of these.

Among humans leptospirosis of the swamp fever type occurs most widely, while the icterohemorrhagic type is encountered sporadically. The majority of cases were caused by leptospirae of the pomona type, while the remaining cases were produced by leptospirae of the tarassovi, canicola, and hebdomadis types. Having available the aforementioned epidemiological and microbiological data, in 1956 we began research intended to detect reservoirs of the causitive agents of leptospirosis among rodents and insectivores.

Collections of wild mammals were made in areas unfavorable with respect to leptospiral diseases, the specimens obtained being studied

by bacteriological and serological methods to determine whether they carried leptospirae.

Some of the animals were captured in June-August, but the majority were collected in September-October, in the vicinity of the following populated areas: Kozyulino, Gorbunovo, and Vershinino (Tomskiy Rayon), Novo-Il'inka and Batkat (Shegarskiy Rayon), and Tersalgay (Kozkevnikovskiy Rayon).

TABLE 1

Leptospirae-Carriers Among Mammals in Nidi of Leptospirosis

A Вид обследованного животного	B Всего обследовано	C Обнаружены лептоспиры	
		D бактериологически	E бактериоскопически
Сибирская красная полевка F	62	—	—
Красно-серая полевка G	36	—	—
Европейская рыжая полевка H	37	1	3
Полевка-экономка I	11	1	1
Узкочерепная полевка J	125	1	4
Пашенная полевка K	2	—	—
Водяная полевка L	2	—	—
Мышь полевая M	147	—	1
Мышь лесная N	5	—	—
Мышь-малютка O	16	1	1
Серая крыса P	51	—	—
Бурундук Q	2	—	—
Северная мышовка R	3	—	—
Хомяк обыкновенный S	1	—	—
Крот алтайский T	9	1	1
Кутора U	2	—	—
Землеройка (род Sorex) V	127	—	—
W Всего	648	5	11

A) Species of animal examined; B) number examined; C) leptospirae detected; D) bacteriologically; E) bacterioscopically; F) Siberian red vole; G) reddish-gray vole; H) European bank vole; I) matron vole; J) narrow-headed vole; K) field vole; L) water vole; M) field mouse; N) wood mouse; O) harvest mouse; P) hooded rat; Q) chipmunk; R) northern mouse; S) common hamster; T) Altay mole; U) water shrew; V) shrew (genus Sorex); W) total.

The topographic characteristics of the established nidi are very closely related to those of the Tom and Ob' river valleys or of large

livestock farms. The nidi which we investigated were characterized by pastures overgrown with brush, which abutted on swampy areas, alluvial lakes, and mixed woods. These areas were inhabited by a large population of small mouse-like rodents and insectivores, which may be carriers of leptospirae.

Over a two-year period we conducted bacteriological investigations of the kidneys and urine of 648 small mammals belonging to 17 species, as shown in Table 1.

As may be seen from the table, small mouse-like rodents predominated among the animals investigated.

For the bacteriological investigations kidney fragments and urine from the animals were cultured on Terskiy's medium, a solution of phosphate-buffered Zerensen's mixture with rabbit serum added. The cultures were stored for three months. They were checked bacterioscopically for leptospirae every 10-15 days. These microorganisms were detected in 11 cases, in six of them bacterioscopically only.

Pure cultures of leptospirae were obtained in 5 cases and were designated Nos. 2/600, 73, 52, 63, and 22. The strains isolated were carefully identified. For this purpose 2 or 3 young rabbits weighing 200.0-400.0 g, 2 guinea pigs weighing 150.0-250.0 g, and 2 white mice were inoculated with each strain. The rabbits and guinea pigs were given the cultures subcutaneously in a dose of 3-5 ml, while the white mice were injected intraperitoneally in a dose of 0.5-1 ml.

The body temperatures of certain rabbits and guinea pigs rose to 39-40.5° 2-5 days after they were inoculated with the leptospiral cultures and remained at this level for 1-2 days; occasionally they subsequently fell gradually to normal and from time to time they dropped rapidly to 35.5-36°.

The animals suffered a slight loss of weight. After 15-17 days

the inoculated subjects were killed (by ether narcosis). On dissection petechia were detected in the subcutaneous cellular tissue, lungs, and endocardium.

Agglutination and lysis reactions were carried out between blood serum from these animals and standard strains, as well as the strains which we isolated. In addition, the strains obtained from the rodents were identified by lysis and agglutination reactions with typical leptospiral sera.

Pure cultures of leptospirae were isolated from the following species of wild animals: 1) from the urine of the narrow-headed vole - strain No. 2/600 of the pomona type; 2) from the kidneys of the bank vole - No. 73 of the pomona type; 3) from the urine of the Altay mole - No. 22 of the sorex type; 4) from the kidneys of the matron vole - No. 52 of the hebdomadis type; 5) from the kidneys of the harvest mouse - No. 63 of the bataviae type.

Leptospirae were detected bacterioscopically only in kidney cultures from the following species of rodents: narrow-headed voles, bank voles, and field mice.

Serological investigation of dried drops of blood from 615 animals yielded positive results in 28 cases; leptospirae of the grippotiphosa, pomona, and tarassovi types were obtained in titres of from 1:10 to 1:100.

The low percentage of positive lysis and agglutination reactions probably resulted to some extent from the fact that the dried drops of blood were examined later, an average of 2-3 months after blood sampling was begun.

In the nidi which we discovered the total percentage of infected specimens was 1.7%. As is well known, the incidence of the disease among wild mammals in individual natural nidi is usually considerably higher.

Ye.V. Karaseva and V.V. Anan'in (1954) cite a figure of 18.5% for certain species of these animals in the European portion of the Soviet Union.

Definite interest inheres in investigation of certain arthropods which regularly inhabit the nests of rodents. In the nidi which we discovered leptospirosis was detected in more than 20 species of gamasid ticks, among which the families Laelaptidae and Haemogamisidae predominated, and in 18 species of fleas.

We conducted bacteriological investigations and biological tests on animal subjects of 2042 gamasid ticks and 635 fleas collected from captured rodents or from their nests. There was not a single case in which leptospirae were detected either bacteriologically or biologically.

In parallel with the investigations described above, we continued our observations on leptospiral diseases among humans and farm animals.

Of 216 blood serum samples from patients admitted to the Infectious Disease Clinic of the Tomsk Medical Institute and the Shegarskiy Rayon Hospital, 15 yielded positive results, principally for leptospirae of the pomona type.

On examination of 529 blood serum samples from large cattle and pigs from Tomskiy and Shegarskiy Rayons and from the area meat combine, positive results were obtained in 36 cases; 35 of these yielded leptospirae of the hebdomadis type in titres of from 1:400 to 1:4000 and one produced leptospirae of the pomona type.

These investigations make it possible to conclude that wild animals of the following species are reservoirs of leptospiral infection in Tomskaya Oblast: the narrow-headed vole, matron vole, harvest mouse, bank vole, and Altay mole. Large horned cattle also serve as reservoirs.

The Tomsk nidus of leptospirosis is of the mixed type, since both the aforementioned wild animals and domestic farm animals are of great

importance as sources of leptospiral infection.

Tomsk Scientific Research Institute for Vaccines and Sera

Tomsk Medical Institute

RESERVOIRS AND SOURCES OF LEPTOSPIRA INFECTION IN TOMSK REGION

Novikova V.N., Sagaidak L.P., Igolkin N.I.

The investigations on the presence of leptospiras by wild rodentia, insectivorous, cattle, pigs, ticks and fleas were made. It was established that wild animals of following types: stenocranius gregalis Clethrionomys glareolus, Microtus oeconomus, Micromys minutus, Talpa altaica and cattle are reservoirs of leptospira infection.

BIOLOGICAL CHARACTERISTICS OF STRAINS OF LEPTOSPIRAE ISOLATED IN TOMSKAYA OBLAST

V.N. Novikova and L.P. Sagaydak

TABLE 1

Results of Lysis and Agglutination Reactions Between Isolated Leptospirae and Corresponding Typical Leptospiral Immune Sera

Сыворотки A / Штаммы B	Pomona Монаков C	Hebdomadis	Bataviae	Sorex
T-14	1:10.000	--	--	—
2/600	1:10.000	—	—	—
K-22	—	—	—	1:16.000
№ 52	—	1:10.000	—	—
№ 63	—	—	1:80.000	—
№ 73	1:10.000	—	—	—

Note: A dash indicates a negative reaction.
A) Serum; B) strain; C) Monakov.

While studying reservoirs of leptospiral infection in Tomskaya Oblast we isolated 6 strains of leptospirae from various animals. They were obtained primarily from the kidneys or urine of wild mammals collected near populated areas unfavorable with respect to leptospiral diseases; one strain was isolated from the urine of a diseased calf in June 1954 and was designated as leptospirae T-14.

Pure leptospiral cultures were also obtained from the urine of the Altay mole in June 1956 (strain K-22), from the urine of the narrow-headed vole in September 1956 (strain No. 2/600), from the kidneys of

the bank vole in August 1957 (strain No. 73), from the kidneys of the matron vole in September 1957 (strain No. 52), and from the kidneys of the harvest mouse in October 1957 (strain No. 63).

The cultures of leptospirae which we isolated did not differ morphologically from standard strains. They were mobile leptospirae 4-7μ long, considerably shorter than standard strains cultured for extended periods, and had button-like thickenings at their curved ends.

The aforementioned strains of leptospirae were isolated after 20-65 days of culturing on a Terskiy phosphate-serum medium with a pH of 7.2-7.3 at a room temperature of 20-26°.

The strains of leptospirae which we isolated were identified by lysis and agglutination reactions and by biological tests on animals. The lysis and agglutination reactions were carried out with 10 typical leptospiral sera obtained from the Moscow Institute imeni Mechnikov.

Each culture isolated was lyzed and agglutinated with only one typical serum diluted to its titre. The results of the positive lysis and agglutination reactions are shown in Table 1.

According to their serological properties, the strains of leptospirae which we isolated belong to the following 4 types: T-14 and Nos. 2/600 and 73 to the pomona type, K-22 to the sorex type, No. 52 to the hebdomadis (Akiyami B) type, and No. 63 to the bataviae type.

The pathogenic properties of the leptospirae were studied in young rabbits weighing 200-400 g, guinea pigs weighing 150-250 g, and white mice. The animals were inoculated subcutaneously with a 10-15-day-old leptospiral culture in doses of 3-5 ml for the rabbits and 3 ml for the guinea pigs. The white mice were given the culture intraperitoneally in a dose of 0.5-1 ml.

The experimental animals were weighed and had their temperatures taken twice daily.

On the 2nd-5th day after inoculation with the cultures of isolated leptospirae the body temperatures of certain rabbits and guinea pigs rose to 39-40.5° and remained at this level for 1-2 days in some cases and 5-17 days in others; occasionally they subsequently gradually dropped to normal and from time to time fell rapidly to 35.5-36°.

The behavior of the laboratory animals varied in accordance with the strain of leptospira under investigation. Thus, strains T-14 and No. 2/600 and 73, of the pomona type, proved to be slightly pathogenic for guinea pigs and rather pathogenic for young rabbits.

The body temperatures of the rabbits inoculated with culture No.73 rose to 39.5-39.7° on the 2nd day and remained at this level for 13-20 days. All of the rabbits stayed active. Two of them were killed on the 15th day (by ether narcosis). On dissection it was found that the subcutaneous cellular tissue and mucosae were decolorized and there were petechia in the lungs; there were no visible changes in the remaining organs.

TABLE 2

Results of Lysis and Agglutination Reactions

A Вид животного	B Титры реакции лизиса и агглютинации		
	s. № 63	s. bataviae	s. Well-Ratten
C Кролик № 2	1:20.000	1:20.000	1:10.000
D Морская свинка № 1	1: 4.000	1: 4 000	1: 4.000
. . № 2	1: 8.000	1: 4.000	1: 2.000
E Белая мышь № 1	1:400	1:400	1: 200
. . № 2	1:2.000	1:1.000	1: 800

A) Species of animal; B) titres of lysis and agglutination reactions; C) rabbit; D) guinea pig; E) white mouse.

A slight gain in weight and a rise in body temperature to 39.2-39.3° were noted in the guinea pigs on the 3rd-5th day after inoculation.

When the rabbits were inoculated with strains T-14 and 2/600 their

temperatures remained within normal limits, but they lost weight. One rabbit died on the 13th day after inoculation, exhibiting a reduced temperature (36.7°). On dissection small hemorrhages were found in the lungs, but there were no visible changes in the other organs.

Lysis and agglutination reactions were carried out between blood sera from the inoculated animals and 12 strains of leptospirae. All of the sera from the guinea pigs and rabbits yielded positive results with leptospirae of the pomona type and strains T-14 and Nos. 2/600 and 73.

The rabbit sera yielded positive lysis and agglutination reactions with the corresponding leptospirae in higher titres than the guinea pig sera; more precisely, the titres of the rabbit sera ranged from 1:1000 to 1:8000, while those of the guinea pig sera varied from 1:400 to 1:800.

Only rabbits (three) were inoculated with strain K-22. There was not a single case in which an increase in temperature developed, but the animals lost weight. One rabbit died on the 11th day, exhibiting a liquid stool and a temperature reduced to 37.6°. No visible pathological changes were found on dissection.

The rabbit sera yielded positive lysis and agglutination reactions only with strain K-22 in titres of 1:4000, 1:8000, and 1:80,000.

Culture No. 52 was given to 3 rabbits and 2 guinea pigs. Two of the inoculated rabbits died, one on the 4th day and one on the 5th. On dissection it was found that the mucosae and subcutaneous cellular tissue were decolorized, the vessels were conjected, the urinary bladder was distended, and the lungs were of a bright red color and exhibited petechia. There were no visible changes in the other organs.

All of the sera from the inoculated animals (except the rabbits which died) in titres of 1:400 to 1:8000 yielded positive lysis and agglutination reactions with leptospirae of the hebdomadis type and

strain No. 52.

Strain No. 63, which we identified as a leptospira of the bataviae type, proved to be the most pathogenic. Of 6 animals inoculated with this strain 3 died: one rabbit on the 6th day, one guinea pig on the 17th day, and one white mouse on the 14th day.

The temperatures of all of the animals rose to 40.5° on the 2nd-4th day. The rabbits and guinea pigs were sluggish, ate poorly, and sat huddled in a corner of their cage. Their coats were disheveled. Pussy, mucous nasal discharges were observed in the rabbits. The animals lost weight; thus, before inoculation the two guinea pigs weighed 221 and 234 g respectively, while on the 17th day after inoculation they weighed 137 and 173 g.

On dissection severe emaciation and widespread hemorrhaging at the injection sites were noted. The mucosae and subcutaneous cellular tissue were icteric and exhibited multiple petechia, the axillary and inguinal lymph nodes were enlarged, the lungs exhibited petechia and massive hemorrhages, and there were blood clots in the cardiac cavities. The rabbits' livers were greatly enlarged and of a muddy-green color, having tapered margins. The liver of the rabbit which died was mottled in appearance and exhibited numerous nodules, some of which were found to contain puss when opened. The kidneys of the guinea pigs were enlarged and the boundaries between the medullary and cortical layers were effaced.

Blood sera from the inoculated animals yielded positive lysis and agglutination reactions with leptospirae of the bataviae type (bataviae and Weil-Ratten strains) and strain No. 63 in various titres.

The titres for culture No. 63 were somewhat higher than those for standard bataviae and Weil-Ratten strains. Serum from the rabbit which died yielded negative lysis and agglutination reactions. Table 2 gives

the results of the lysis and agglutination reactions between sera from the inoculated animals and leptospirae of strain No. 63 and the bataviae and Weil-Ratten strains.

These investigations enable us to draw the following conclusions:

1. The strains of leptospirae isolated in Tomskaya Oblast belong to four serological types: strains T-14 and Nos. 2/600 and 73 to the pomona type, strain K-22 to the sorex type, strain No. 52 to the hebdomadis type, and strain No. 63 to the bataviae type.

2. The local strains of leptospirae differed in their pathogenicity for laboratory animals; the strains of the pomona type (T-14 and Nos. 2/600 and 73) were least pathogenic, while the strain of the bataviae type (No. 63) was most pathogenic.

Tomsk Scientific Research Institute for Vaccines and Sera

Tomsk Medical Institute

BIOLOGIC CHARACTERISTIC OF STRAINS OF LEPTOSPIRA ISOLATED IN TOMSK REGION

Novikova V.N. and Sagaidak L.P.

Six strains of leptospira isolated from different animals mainly from urine and kidneys of wild mammals were studied. The strains of leptospira belong to four serological types (pomona, hebdomadis, bataviae, sorex) possessirg different pathogen properties for laboratory animals.

Q FEVER IN WESTERN SIBERIA

S.P. Karpov, M.A. Mastenitsa, I.A. Minkevich

A.A. Selezneva and N.I. Igolkin

In recent years a number of scientific collectives (the Ricketsiosis Laboratory of the Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR, the Institute of Virology of the Academy of Medical Sciences USSR, the Tashkent Institute for Vaccines and Sera, etc.) and a number of individual scientific and technical workers in public health in the Soviet Union have published a considerable number of reports on investigations of the epidemiology, diagnosis, symptomatology, treatment, and prophylaxis of Q fever and the characteristics of local strains of Ricketsia burneti. Study of the propagation of this disease by serological investigation of blood sera from humans and animals and the isolation of ricketsiae from sick humans and animals, ticks, milk products, and in some cases from the air of cattle barns indicate that there are natural nidi of Q fever in a number of the republics of the Soviet Union, particularly those in Central Asia. This disease has also been detected in a number of the oblasts of the RSFSR.

Until recently there were no investigations of the existence of Q fever in Siberia. In this connection a group of scientific workers, including various specialists, was organized in Tomsk and in 1955 began to make appropriate observations in Western Siberia.

During the second half of 1956 there appeared an article by N.A.

Zeytlenok and E.R. Pille in which it was shown that Q fever exists in certain rayons of Altayskiy Kray and during the same year one of us (I.A. Minkevich) described a case of this disease in Tomsk. The Tomsk research group presented the first report on their observations at the Interinstitute Scientific Conference on Natural-Nidus Diseases held in 1956 in Tomsk, in connection with the 50th anniversary of the Institute of Vaccines and Sera.

Investigations carried out in 1955 gave us grounds for assuming that this infection occurs in Western Siberia. In June of this year the first case of Q fever was diagnosed in Tomsk.

Simultaneously with the detection of Q fever among patients with indefinite diagnoses, persons involved in the care of domestic animals and the handling of animal products were examined. In order to determine the natural reservoirs and sources of the infection a study was made of domestic and wild animals and ticks.

During 1955-1957 668 patients suspected of having Q fever were examined; the majority of these were admitted for treatment to the hospitals of Tomskaya Oblast, although a few were handled in Altayskiy Kray and Kemerovo. These examinations enabled us to detect 20 cases of the disease (1 in a resident of Kemerovo, 10 in residents of Tomsk, and 9 in residents of the two rayons of Tomskaya Oblast). Of the total of 20 persons found to be suffering from this disease 10 were under the clinical observation of I.A. Minkevich.

In order to characterize its clinical picture, we will give a brief description of the course of the disease in a number of patients.

Patient N-i M.I., 46 years old, various occupations, fell ill on 16 May 1955. The illness was severe at first, being marked by a high two-phase remittent fever (which lasted more than 3 weeks) and chills, the latter being succeeded by a rise in temperature and drenching sweats. There were moderate symptoms of intoxication and an enlargement of the liver and spleen. Roseolus erythema occurred over the entire body and hemorrhagic exanthema over a portion of it, the face was puffy,

and scleritis was present. X-rays showed a small dense infiltration (1 x 1 cm) in the second left intercostal space. The leucocyte count on the 5th day of illness was 2000, 38% of them being lymphocytes; The leucocyte count on the 21st day was 3600 and 51% were lymphocytes. Microbiological examinations for typhoid-paratyphoid diseases, brucellosis, and typhus were negative. On the 27th day of illness the complement-fixation reaction with antigen from Ricketsia burneti was positive in dilutions of 1:80 ++++ and 1:160 ++; the same results were obtained on the 34th and 40th days with dilutions of 1:320 ++++ and 1:640 ++.

Patient A-ch, A.A. 52 years old, groom, fell ill on 6 February 1957. The initial fever lasted 10 days and was followed by subfebrility and a second wave of fever, the total duration of the febrile period being more than one month. Chills were observed several times a day; these were followed by a rise in temperature and drenching sweats. Excrutiating muscle and joint pains were observed, especially in the genual and talocalcaneal joints and the gastrocnemius muscles. The patient suffered from a dry cough, headaches, and vomiting and was delirious at night. The liver and spleen were palpable. X-rays revealed pleuropneumonia in the lower lobe of the left lung.

During the second week of illness the patient exhibited a widespread roseolus erythema accompanied by severe itching, the places affected by the rash merging to cover the entire body and the face; large areas of hemorrhagic exanthema were encountered. The rash recurred during the illness and copious peeling occurred when it disappeared. Leucocytosis with a count reaching 14,000, absolute lymphocytosis, and aneosinophilia were noted. During the period of convalescence the leucocyte count was 6000 and there was a relative lymphocytosis (56%). The urine exhibited toxic albuminuria. The agglutination reactions with various antigens (typhoid-paratyphoid, brucellosis, leptospirae, and listerellae) were negative. The complement-fixation reaction with antigen from Ricketsia burneti was negative on the 10th day of illness and positive with dilutions of 1:20 ++++ and 1:40 + on the 17th and 25th days; blood cultures were negative.

Patient I.v, V.S., 9 years old, student, from a rural nidus, fell ill on 2 March 1957. The disease was severe at first, being marked by headaches, muscle and joint pains, facial hyperemia, and scleritis, while the tongue was covered with white fur and its papillae were enlarged. There was a moderate fever which lasted 9 days. The spleen and liver were enlarged. The leucocyte count on the 20th day of illness was 7000, lymphocytes constituting 42%. The complement-fixation reaction with antigen from Ricketsia burneti yielded a dilution of 1:20 ++++ on the 11th day of illness and dilutions of 1:40 ++++ and 1:80 ++ on the 21st day; blood cultures were negative.

Patient N-y, V.V. 18 years old, tractor operator, from a rural nidus, fell ill on 2 March 1957. The disease was severe at first, being marked by a high temperature and headaches; the patient's tongue was covered with a whitish fur and was thickened. The liver and spleen were palpable. The leucocyte count on the 8th day of illness was 6800, lymphocytes constituting 41%; the leucocyte count on the 19th day was 7000 and on the 33rd day 9100, the leucogram being normal. During the course of the illness there was a second period of fever, which lasted 3 weeks. On the 12th day the complement-fixation reaction with antigen from Ricketsia burneti yielded dilutions of 1:20 ++++ and 1:40 ++, while on the 22nd day the dilutions obtained were 1:80 ++++ and 1:160

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++; the agglutination reactions with typhoid and paratyphoid B diagnosticum were positive in a dilution of 1:1600.

This brief description of the symptoms which occurred in a number of patients indicates that we were dealing with the form of Q fever known as leucopenic atypical pneumonia, severe cases being accompanied by a typhoid-like syndrome and pleuropneumonia, as well as with an influenza-like form. As is well known, these forms of Q fever have been described by many authors. The exanthema of the polymorphic erythematous type, which is unusual in the symptomatology of this disease cannot be included with certainty among its symptoms, since we cannot exclude the possibility that it resulted from the use of antibiotics.

It must be pointed out that in one rural nidus we simultaneously observed patients with typhoid-paratyphoid diseases, Q fever, leptospirosis, and brucellosis.

A more thorough study of the symptomatology of Q fever is necessary and special attention must be paid to the possibility of combined infection in natural nidi.

Blood sera taken from the patients yielded positive complement-fixation reactions with antigen from Ricketsia burneti in titres of from 1:5 to 1:640, the quantity of antibodies increasing by factors of 2-16 on repeated examination. It was retrospectively confirmed that 22 individuals had incurred the disease in the past, the titres obtained being equivalent to serum dilutions of 1:5 to 1:40.

In order to detect the disease and discover individuals who had incurred it a serological examination (with the aid of the complement-fixation reaction) was made of the workers of various shops of the "Zagotskot" Meat Combine, a leather plant, and a boot factory, students at a veterinary school, and others. Blood sera from 622 persons were examined; 23 of these (3.7%) yielded positive complement-fixation

reactions in dilutions of 1:5 to 1:40. Among persons handling cattle positive reactions occurred in 6.2-41.6% of all cases, depending on the group of subjects (thus, the percentage was 6.2% among workers of the collective dairy farm in the village of B. Botkat, Shegarskiy Rayon, Tomskaya Oblast, and 41.6% among those in the village of M. Botkat). Positive complement-fixation reactions were obtained for 10% of the workers in the leather plant, 11.1% of those in the boot factory, and 1.5% of those in the preslaughtering and slaughtering shops of the meat combine.

The results obtained confirm that this disease has occurred in the past and indicate the epidemiological role of animals in spreading it. It must be pointed out that 420 serum samples obtained from relatively healthy persons who served as blood donors at an antimeasles station yielded negative complement-fixation reactions with antigen from Ricketsia burneti.

In order to discover sources and reservoirs of infection an examination was made (also with the aid of the complement-fixation reaction) of 1670 head of large and small cattle in 5 rayons (Tomskiy, Zyryanskiy, Shegarskiy, Tuganskiy, and Kozhevnikovskiy Rayons) of Tomskaya Oblast; a small quantity of serum was from Altayskiy Kray. Of the total number of animals examined 95, or 5.7%, yielded positive complement-fixation reactions with antigen from Ricketsia burneti in dilutions of from 1:50 to 1:40.

This portion of the investigation showed that natural nidi of infection are present in Tomskaya Oblast and demonstrated the necessity for further searches for reservoirs of infection among wild animals and ticks, as well as for more thorough epidemiological observations in individual areas.

Chick embryos, white mice, and guinea pigs were used to detect

Ricketsia burneti, subsequent reinoculations being employed. Blood from 12 humans and 80 head of large and small cattle and organs from 542 wild animals of 10 species collected in two rayons of Tomskaya and Kemerovskaya Oblasts were examined, as were 1260 ticks (including 1200 gamasids) collected in the woods and from the nests of various species of wild animals and 86 fleas collected from wild animals. We were unable to detect Ricketsia burneti. This may have resulted, on the one hand, from an insufficient quantity of material and, on the other hand, from the fact that a considerable portion of the analyses were carried out after the material had been stored in glycerine for an extended period.

Examinations made during 1955-1957 of patients suspected of having Q fever and subsequent epidemiological investigation of the cases of the disease which were discovered led to the detection of nidi of infection in two populated areas (B. Botkat and M. Botkat [sic]) in Shegarskiy Rayon; the disease spread from these nidi to other areas and to the city of Tomsk.

On-the-spot epidemiological examinations conducted by microbiological methods made it possible to detect an outbreak of this disease during January-March. With the aid of the complement-fixation reaction Q fever was diagnosed in 7 patients admitted to the hospital in the rayon center; in addition, it was established retrospectively that 4 persons had had the disease. In order to detect sources of infection 203 head of large cattle and pigs from the dairy farms and individual farms of these two areas were examined with the aid of the complement-fixation reaction. Blood sera from 13 animals (4 cows and 9 ewes) reacted positively with antigen from Ricketsia burneti.

At the same time, farm workers in these areas who had handled or were presently handling animals were examined. Of the total number

of subjects (44 persons) 7, or 16%, yielded positive complement-fixation reactions. The members of the 4 households of the individual farms where animals which reacted positively were detected were also examined. Of the total number of subjects (11 persons) 4 reacted positively.

Further investigation enabled us to gather data on the rather wide occurrence of Q fever in this rayon. Serological examination of blood sera from patients from a number of populated areas who were admitted to the rayon hospital made it possible for us to detect 7 persons in whom the complement-fixation reaction with antigen from Ricketsia burneti proved positive in dilutions of from 1:5 to 1:160.

The data thus gathered gave us grounds for asserting that an epidemic nidus of Q fever had been established in one of the rayons of Tomskaya Oblast.

Detection of specific antibodies to Ricketsia burneti by means of the complement-fixation reaction with blood sera from persons involved in close contact with farm animals and with blood sera from these animals themselves enables us to assume that large and small cattle are the sources and reservoirs of this infection. It was established that a number of Q-fever patients who lived in Tomsk contracted the disease in this epidemic nidus.

The investigations which we conducted make it possible for us to draw the following conclusions:

1. Cases of Q fever among humans in Tomskaya and Kemerovskaya Oblasts and Altayskiy Kray were confirmed with the aid of the complement-fixation reaction with antigen from Ricketsia burneti and took courses typical of this disease.

2. An epidemic nidus of Q fever was detected in one of the rayons of Tomskaya Oblast; current and past cases of the disease were dis-

covered and it was established that the infection occurs among large and small cattle.

3. A study made of the epidemiology of this disease in Tomskaya Oblast enabled us to establish that large and small cattle are the reservoirs and sources of infection. Serological examination of 1670 head of cattle yielded positive complement-fixation reactions with antigen from Ricketsia burneti in 5.7% of the animals. A confirmation is the presence of positive complement-fixation reactions with sera from persons who handle cattle (6.4-41.6%), leather plant workers (10%), and boot factory workers (11.1%).

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Q-FEVER IN WEST SIBERIA

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Materials characterizing the presence of diseases of the people with Q-rickettsiosis on the territory of Tomsk, Kemerovo regions and Altay district were given.

The study of epidemiology of disease in Tomsk region made it possible to state that cattle, horned cattle are the reservoirs and sources of infection.

INVESTIGATION OF LARGE CATTLE IN TOMSKAYA OBLAST FOR THE PURPOSE OF DETECTING Q-FEVER

A.A. Selezneva

Numerous investigations testify to the existence of rural nidi of Q-fever in individual areas of the Soviet Union. T.A. Baktemirov, P.F. Telenkov, L.I. Kislitsina, and A.K. Gritsenko have described such nidi in Chitinskiy Oblast, where sheep and large cattle were the source of infection. According to the data of V.P. Romanova, I.N. Petrovskiy, A.G. Somova, T.A. Nikol'skaya, R.V. Shmatko, A.A. Kosenko, V.I. Balabanova, V.G. Liparskaya, M.A. Kharat'yan, and Ye.M. Kompanets, outbreaks of Q-fever occurred among sheep in Kamenskaya Oblast. N.I. Fedorova, I.V. Tarasevich, A.I. Sergeyeva, Ye.D. Shlyakhturova, and L.M. Popova noted that the source of infection in the Dagestan SSR was apparently large cattle. According to a report by N.A. Zeytlenka and E.R. Pille, outbreaks of Q-fever in Altayskiy Kray were caused by the existence of the disease in cows, yaks, sheep, and horses. In 1955 S.P. Karpov, I.A. Minkevich, and M.A. Mastenitsa were the first to diagnose Q-fever among humans in Tomskaya and Kemerovskaya Oblasts, where the source of infection was large and small cattle. From the data cited it may be seen that Q-fever is rather frequently encountered in conjunction with the presence of the infection in farm animals.

The purpose of our work was to establish the presence of this infection in domestic animals in Tomskaya oblast. This article presents the results of examination of large cattle in Shegarskiy and Tomskiy Rayons.

We tested 1467 serum samples from cows by the complement-fixation reaction with antigen from Ricketsia burneti. The reaction was first carried out with the serum diluted to 1:5. The titre of antibodies was determined when a positive reaction occurred. The complement-fixation was carried out at low temperatures for 18-20 hours. Positive results were obtained with 82 serum samples (5.5%), of which 56 had a reaction titre of 1:5, 8 a titre of 1:10, 4 a titre of 1:20,

and 14 a titre of 1:40.

These investigations enabled us to set ourselves the task of searching for rural nidi of Q-fever in Tomskaya Oblast. Such nidi apparently exist in Shegarskiy Rayon, where 31 of 213 serum samples investigated (14.5%) yielded positive complement-fixation reactions, while only 51 of 1254 serum samples from cows in Tomskiy Rayon (4%) gave positive results.

The detection of blood serum from large cattle in Shegarskiy and Tomskiy Rayons which yielded positive complement-fixation reactions with antigen from Ricketsia burneti indicates the presence of rural nidi of this infection in Tomskaya oblast and necessitates the taking of appropriate prophylactic measures.

CONCLUSIONS

1. Of the 213 blood serum samples from cows in Shegarskiy Rayon which we investigated, 31 (14.5%) exhibited positive complement-fixation reactions with antigen from Ricketsia burneti, while 51 of 1254 (4%) samples from Tomskiy Rayon reacted positively for Q-fever.

2. The positive complement-fixation reactions between the blood sera and antigen from Ricketsia burneti indicated the existence of rural nidi of Q-fever in Tomskaya Oblast.

3. These observations necessitate the organization of further research on Q-fever in Tomskaya Oblast, particularly in Shegarskiy Rayon.

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THE INVESTIGATIONS OF FINDING OUT Q-FEVER BY CATTLE IN TOMSK REGION

Selezneva A.A.

Blood serum of 1467 cows were examined on Q-fever. The positive reaction of connection of complement in one district is 14.5 percent and in the other is 4 percent.

ANALYSIS OF LOCAL DATA ON EPIDEMIC AND SPORADIC POLIOMYELITIS

A.F. Yastebov

Our data are on six cities in the Soviet Union, where we observed massive outbreaks of poliomyelitis during 1949-1955 and took part in eliminating them. In addition, we have included statistical data on the sporadic cases of this disease which occurred in a number of cities and oblasts of Western Siberia between 1950 and 1956. A total of 4750 cases were observed and constitute the basis of this article.

The generalized results of statistical processing and clinical-epidemiological analysis have established that there are certain regularities in the spread of epidemic and sporadic poliomyelitis. The lowest indices occurred in 1953 and 1954, subsequently increasing in 1955-1956.

Tomskaya Oblast exhibits the same general tendency in the spread of the infection, but it has a lower incidence here than in the other oblasts of Western Siberia. The low population density, lack of migration, vast area, distance between populated points, and absence of major railroads or waterways cannot but play some part in this epidemiological favorability.

An especially sharp rise in the incidence of poliomyelitis has occurred in Kemerovskaya Oblast. If we take the incidence in 1950 as 100%, we obtain a figure of 2100% for 1957.

Our attention is struck by the high incidence of this disease among the urban populace, which accounted for 84% of all cases. Over a period of years the highest figures are all for the same cities; five of the 13 cities (Kemerovo, Stalinsk, Prokop'yevsk, Anzhero-Sudzhensk, and Osinniki) furnished the majority of cases (77%), Anzhero-Sudzhensk, Prokop'yevsk, and Stalinsk being the most persistent nidi. It is sufficient to point out that two cities alone (Stalinsk and Prokop'yevsk) accounted for 62.3% of all cases. All of these cities are located on a railroad main line and are growing rapidly; grading is incomplete and there is a certain disproportion between the requirements of the expanding population and the level of community welfare, while intensive migration coupled with the high population density and occasional lacks in medical and sanitary service is one of the probable causes of the unusually high rate of poliomyelitis. In 1955 there was an increase in the incidence of poliomyelitis in a number of cities along the Volga. The Volga being a means of communication, it would be incorrect to discount it as one of the factors involved in the spread of this disease.

It is well known that the role of means of communication in spreading disease appears very clearly in sparsely populated areas with a low level of infection. As an example, we may cite the successive development of poliomyelitis in Tyumen', Tobolsk', and Salekhard in 1953, 1954, and 1955.

The one-year intervals separating these outbreaks resulted from the great distance between these cities, as well as by the fact that the Irtysh River is the only means of communication, which is limited to the period when navigation is possible, travel being on an extremely small scale.

The action of this epidemiological mechanism (communications

links) may also explain the high incidence of poliomyelitis in rural regions lying in close proximity to heavily populated centers; according to our observations, these areas account for 60-75% of all cases of the disease in the rayons of an oblast or kray. Thus, of 88 cases of poliomyelitis in Novosibirskaya Oblast (excluding the city of Novosibirsk) 56 occurred in the Novosibirsk agricultural region (1957).

However, massive outbreaks of the disease are occasionally encountered in remote regions having no contact with cities (Kozhevnikovskiy Rayon of Tomskaya Oblast, Soloneshenskiy Rayon of Altayskiy Kray, Kyshtovskiy Rayon of Novosibirskaya Oblast, etc.); the very remoteness of these areas, the inadequate preparation of local medical personnel, and errors in recognizing and isolating patients enable the epidemic process to take its natural course and when specialists arrive in the area and retrospectively establish the true extent of infection the actual number of cases turns out to far exceed the number recorded.

As for the dynamics of the disease, the months from July to October form a period during which there is a seasonal increase in the number of sporadic cases and during which outbreaks usually develop. An exception to this was the fact that the maximum number of cases in the outbreak in Anzhero-Sudzhensk occurred during September, the incidence curves for the other cities dropping at this time. It is possible that the natural and geographic conditions which obtain in the city are of a certain importance here.

The first cases of poliomyelitis in Stalinsk were noted in April May. This preseasonal onset is due to the role of water in the development of outbreaks. The outbreaks that we observed in certain rural regions began in January-February, although it is impossible to correlate them with water. An inspection of the area convinced us tha

these outbreaks were of the true contact type, since unrecognized cases of poliomyelitis had occurred during the preceding year.

The number of cases recorded during the summer and fall months of an epidemic period occasionally constitute 90-100% of the yearly total, since the beginning of official recording frequently coincides with the onset of the outbreak.

There is no substantial difference between the age-group statistics for sporadic cases and those for epidemics. Children of under 5 years constitute 73-96% of the total. The incidence in pediatric collectives is approximately uniform, varying from 10 to 28% of the total number of sick children; however, detailed statistics on this group show that the incidence of poliomyelitis among its members is three times as high as among the unorganized juvenile population.

The following figures merit some attention; between 1952 and 1954 there were no patients in Novosibirsk older than 10 years of age, while six such cases were recorded in 1955-1956, one patient in 1956 and 9 in 1957 being more than 20 years old. Moreover, while the percentage of kindergarten patients during 1952-1956 was 25.8%, it amounted to 42% in 1957. These figures indicate a certain tendency toward an increase in the incidence of poliomyelitis among older children and adults. If we had detailed statistics on 10,000 individuals of the appropriate age group over a number of years we might be able to speak of this as a markedly regular phenomenon.

Study of the territorial distribution of poliomyelitis has established certain characteristics which are of epidemiological and practical importance:

1) group cases of this infection are most frequently encountered in children in preschool collectives which are overcrowded and do not meet basic hygienic requirements;

2) Nidi with single cases predominate. According to our observations, these constitute from 88 to 96% of the total, while those with two cases account for 2.7-9% and those with 3 or 0.5-3.5%;

3) Despite the wide occurrence of the disease, it does not spread uniformly through populated areas. Its incidence in the administrative regions of cities varies from 0.5 to 18.0 cases per 10,000 inhabitants;

4) Areas with a higher incidence of poliomyelitis may be differentiated within rayons. For the greater part, the sanitary conditions which obtain in these areas are unsatisfactory. In certain cities 40-50% of all cases occur in such areas.

Comparison of the clinical forms of the disease enables us to note certain peculiarities; paralytic forms constitute 84-97% of all sporadic cases, i.e., almost no nonparalytic forms are detected during nonepidemic periods. Of the paralytic forms, spinal (51-69%) and pontine (13-27%) poliomyelitis are encountered most frequently, often with isolated paresis of the facial nerve. Bulbar forms do not exceed 2%.

It is characteristic that forms accompanied by a meningeal syndrome are recognized extremely rarely, these being the most common types of nonparalytic poliomyelitis (constituting 92% of all cases, according to data from Novosibirsk). It is not fortuitous that the majority of poliomyelitis patients admitted to the 4th Novosibirsk Hospital with other diagnoses (1952-1956) were diagnosed as having "serous meningitis." It must also be pointed out that a group case of poliomyelitis (10 cases) in one of the day nurseries in Tomsk was preceded by a series of cases of serous meningitis. This is why the differential diagnostics of diseases accompanied by meningeal syndromes are of great clinical and epidemiological importance, especial-

ly in the presence of indisputable cases of poliomyelitis, not to mention the fact that a diagnosis of meningitis with no indication of its etiology generally cannot be taken as an independent nosological unit.

A large percentage of nonparalytic forms (29-45%) appear during outbreaks under epidemic conditions, the proportion of bulbar (3-8.5%), bulbospinal (4-20%), and pontine (13-24%) forms increasing; the spinal form (21-68%) predominates among paralytic patients. Of patients with nonparalytic poliomyelitis 1-16% have the meningoradicular form and 12-33% the abortive form.

The difficulty of detecting nonparalytic forms is shown by the following data: in Stalinsk 28% of patients with such forms were diagnosed clinically without laboratory examination, in 33.8% the diagnosis was confirmed by examination of the spinal fluid, in 31.0% such examination was the decisive factor in the diagnosis, and in 7% the diagnosis was based on epidemiological data. It must be taken into account that 28% of all nonparalytic cases are not accompanied by neurological changes and this again confirms the difficulty of differentiating nonparalytic poliomyelitis from poliomyelitis-like diseases.

We are consequently obliged to agree that the number of aparalytic cases recognized is directly proportional to the familiarity of the physician with the symptomatology of these forms.

Wide recognition of aparalytic forms is of great practical importance.

In eliminating outbreaks of poliomyelitis the most important antiepidemic measure is active detection and isolation of all dubious (including those with aparalytic forms) and manifest cases.

This was accomplished by house-to-house checks and the organiza-

tion of diagnostic stations to which such patients were referred. The expediency of this method is indicated by the fact that 30-37% of the patients who passed through the diagnostic stations were diagnosed as having poliomyelitis. It has a positive effect on the time of hospitalization, increasing the proportion of patients isolated during the first few days of illness. Thus, in Stalinsk patients with nonparalytic poliomyelitis constituted 27.4% of all cases during the first part of June, while 27.1% of all patients were hospitalized before the 3rd day of illness. As a result of the active detection and hospitalization of persons having or suspected of having poliomyelitis these indices amounted to 64.4% and 50.7% respectively by September.

As a result of the clear predominance of paralytic poliomyelitis the mortality for sporadic cases is usually substantial (5-10-20%), but it is markedly lower during epidemics (3.6-11.3%). Another regular phenomenon is the fact that mortality is higher at the beginning of an outbreak than at the time when it is eliminated; this is easily explained by progress in recognition, earlier detection of paralytic cases, and the larger number of mild nonparalytic forms recognized. Thus, in Anzhero-Sudzhensk the 20% mortality at the beginning of the outbreak dropped to 0 at its end; in Novosibirsk mortality at the beginning of the epidemic was 16%, while the mean for the year was 7.2%. Considerable fluctuations in mortality are also occasionally observed from place to place within a city. In Novosibirsk mortality was 3.3% in the central area of the city and 12.0% in one outlying area.

The available data thus enable us to note certain peculiarities of sporadic poliomyelitis:

1) paralytic forms, particularly the spinal and pontine forms

accompanied by isolated paralysis of the facial nerve, predominate and the range of clinical forms in the cases detected is limited;

2) elevated mortality;

3) there is an almost complete absence of group cases, but there are individual areas with large numbers of single cases;

4) the practical measures taken are frequently incomplete (late and insufficient hospitalization, inadequacies in the sanitation and disinfection of the nidus, defects in observation and quarantine, etc.).

An epidemic outbreak of any infection may be considered as a strikingly clear demonstration of all of the most characteristic clinical and epidemiological peculiarities of the disease in question.

Comparison of the data which we have cited shows that the basic tendencies and regularities of epidemic and sporadic poliomyelitis are similar, despite the difference in incidence and distribution. This is true of the greater incidence among urban than rural inhabitants and among children less than 5-7 years old, the seasonal increase in sporadic cases or the development of epidemic outbreaks in the summer and autumn months, the incidence ratio between organized and unorganized groups of juveniles, etc.

In addition to the elements of similarity between the epidemic and sporadic forms of poliomyelitis, it is no less important that there is a relationship and interaction between them, as in any infection.

Discovery of this relationship first of all means that we will be able to solve the problem of the causes and mechanisms underlying the transformation of single cases of the disease into massive epidemics. Solution of this complex problem is related to the mutability of the circulating strain, its typical specific properties, the imunobiological structure of the collective, its specific and nonspecific

resistance, the role of the environment, etc. Resolution of these problems lies beyond the goals and capabilities of our investigations, since methods of statistical processing and clinical-epidemiological analysis advance more rapidly than problems of this type are solved.

Our aim is more modest; we wish only to confirm that this relationship and sequence exist, to attempt to determine their forms, and to emphasize their practical importance. This relationship may be clear and indisputable when the epidemic rise in the incidence curve is immediately preceded by a certain period of numerous single cases, the epidemic "foreshadowing itself" by means of these cases, to use Pette's words. Observations of a different character also merit attention.

Under epidemiologically favorable conditions, when group cases are almost entirely absent, the distribution of single cases is non-uniform but apparently not fortuitous. In studying the incidence of poliomyelitis in rural areas of Tomskaya and Kemerovskaya Oblasts from 1950 to 1957 we established that single cases were observed predominantly in certain regions.

This unusual finding indicates the existence of certain constant incidence factors in these areas and a tendency toward the formation of nidi of prolonged effectiveness. Analogous phenomena were also observed for certain cities. During the preepidemic period of 1956 in Novosibirsk (using the word preepidemic in its true sense, to indicate a complete absence of group cases) a high concentration of cases in certain areas was noted; these areas were the 7th and 52nd blocks, the tin plant, and Parkhomenko Street in Kirovskiy Rayon and the 22nd block in Dzerzhinskiy Rayon. Group cases (2-3 patients) were observed in certain locales and institutions (the 12th, 18th, and 52nd day nurseries) in these areas. During the following year, when a wide-

spread epidemic outbreak developed, these two rayons had the highest incidences (10 and 7.6 cases per 10,000 inhabitants).

The regularity of this pheno enon is evaluated in general form in the following statement: "An epidemic is the highest point in a series of events which follow one another in definite sequence to an inevitable conclusion" (Dzhil).

As applied to our data, the "series of events" is the series of individual cases, the territorial distribution of which is not fortuitous and the increasing concentration of which logically leads to the development of outbreaks and selective dislocation of the most massive nidi.

In conclusion we may make certain recommendations on the measures which must be taken when sporadic or epidemic poliomyelitis occurs.

First of all, it is necessary to increase our requirements for antiepidemic work with respect to single cases. In practice the following measures are necessary: 1) correct and prompt recognition and unfailing hospitalization of all cases of paralytic poliomyelitis; 2) prompt recognition and isolation of single aparalytic cases in which sufficiently marked neurological changes are present, particularly forms accompanied by a meningeal syndrome; 3) broader investigation of the spinal fluid; 4) exhaustive employment of all measures to be used in treating the nidus, including disinfection, isolation, quarantine, seroprophylaxis, and observation of persons who have come into contact with the disease.

There is no need of proving the great practicability and effectiveness of our measures for sporadic cases.

When an outbreak develops especially great importance attaches to:

1) Intensive training of general practitioners, especially physicians in the affected areas, in problems of poliomyelitis;

2) early and complete hospitalization of all patients detected;

3) employment of local public health measures in accordance with epidemiological indications;

4) availability of a sufficient number of beds for diagnostic purposes;

5) virological confirmation of detected cases of paralytic and nonparalytic poliomyelitis, isolation of virus strains, and determination of their immunological type;

6) broad dissemination of public health information, especially among mothers;

7) employment of active immunization measures (on authorization of the Ministry of Public Health);

8) since, as experienced in certain cities as shown, 61% of paralytic patients and 42% of all patients recorded during an epidemic require prolonged therapy, early orthopedic prophylaxis plays an exceptionally important role as an inseparable element of the treatment of acute poliomyelitis.

In recent years active immunization against poliomyelitis has become a possible mass measure.

We assume that a conjunction of active specific prophylactic measures and improved antiepidemic work based on a study of local conditions will foster an increase in the practical success enjoyed in preventing this infection.

Tomsk Scientific Research Institute for Vaccines and Sera

ANALYSIS OF LOCAL MATERIALS OF EPIDEMIC AND SPORADIC POLIOMYELITIS

Yastrebov A.F.

Comparative study of epidemic and sporadic poliomyelitis shows

that at quantitative difference they have identical main clinical-epidemiologic principles typical for this infection. The connection and succession observed between them influences upon the scale of distribution of disease and should be taken into consideration at organizing the measures of struggle and prophylaxis.

PROPHYLAXIS OF DYSENTERY IN COLLECTIVES COMPOSED OF ADULTS

N.Z. Yakobson

The prevention of illness, including infectious diseases, is the most important task of Soviet public health. The importance of prophylaxis as the basis of disease prevention has frequently been pointed out by eminent representatives of Russian medical science (M.Ya. Mudrov, N.I. Pirogov, S.P. Botkin, and many others). N.I. Pirogov's brief phrase "the future belongs to preventive medicine" defines tasks of today and tomorrow. It has become the Soviet trend in medicine. The most outstanding worker in Soviet public health, Z.P. Solov'yev, has written "The combination of therapeutic and prophylactic activity into a single system is a specific and inseparable characteristic of the Soviet regime."

The prophylactic trend in Soviet public health appeared in the first plan for dispensary service. In a resolution of the 1954 All-Union Conference of Therapists it was pointed out that "dispensary service is one of the most important measures taken by public health organizations and is in complete accord with the prophylactic trend in Soviet medicine...

Dispensary examination of the populace is accompanied by the employment of public health measures directed at improving labor and living conditions and therapeutic-prophylactic measures intended to maintain the working capacity of patients and to prevent progression of diseases and their transition to chronic forms. Dispensary service for the populace is thus a synthesis of prophylactic and therapeutic measures."

Prophylaxis, in all its numerous and diverse manifestations, methods, and forms, has been, is, and will continue to be the basis of Soviet public health.

Dispensary service has come into wide use in Soviet public health. This method has completely justified itself in the cases of tuberculosis, hypertonia, malaria, and a number of other diseases.

On the basis of available experience in dispensary service we have recently come to grips more widely and completely with the problems involved in the use of this method for infections such as bacterial dysentery, which are still rather widespread.

The substantial distribution and prolonged course of dysentery, the difficulty of treating patients, the frequent transition from an acute to a chronic form, the difficulty in certain cases in detecting the source of infection and thus in employing complete anti-

epidemic measures, and many other factors present the practical public health service with the problem of finding more radical ways of preventing this infection and a rational system of special prophylactic measures.

Good prospects in this direction have been opened up by a progressive method which has already proved itself in practice, a combination of dispensary service intended to prevent the further spread of dysentery and employment of radical sanitary measures for the populace.

This work is of especially great significance under the conditions of large organized collectives composed of adults, in which maintenance of the health and working capacity of each individual and the collective as a whole is exceptionally important.

In solving the problem of prophylaxis as applied to intestinal infections, particularly bacterial dysentery, it must always be kept in mind that only a complex approach, i.e., treatment of both the persons involved and their environment, will yield the desired result. Treatment of only the sick or healthy individual (the source of infection and the susceptible organism) or, conversely, only of the environment will not have the requisite effect. Our many years of experience have convinced us that it is only a medically wise combination of the two, radical, timely, and complete treatment of both sick and healthy individuals and their environment which will prove fruitful and lead to the desired results.

We shall not dwell on the third link in the epidemic chain, the healthy individual; since there are as yet no radical methods of specific prophylaxis for bacterial dysentery, we shall consider the other aspects of this problem. We are speaking of the general sanitary measures and special antiepidemic measures which have been taken and are being taken in the Soviet Union for the prophylaxis of this "most important intestinal infection," bacterial dysentery.

Prime among these problems are the establishment of favorable living and working conditions, the improvement of the level of personal and collective sanitation and hygiene, and the employment of the proper therapeutic-prophylactic and antiepidemic measures. The latter, i.e., antiepidemic and therapeutic-prophylactic measures, must be directed not only at the individual, but also at the collective as a whole.

Sanitary and hygienic problems precede and form the basis for all special medical measures to prevent dysentery in collectives. We are speaking primarily of improving living conditions, creating favorable conditions in the home and the area surrounding it, ameliorating problems of nutrition, and the collection and disposal of sewerage, rubbish, and garbage, and the maintenance of sanitation and personal hygiene at the proper level. It is only on this basis that we can expect our medical antiepidemic and therapeutic-prophylactic measures to be successful.

We have not set ourselves the task of giving a detailed description of the general sanitary measures which have been carried out over a period of years in the collectives which we investigated; we

reed point out only that from year to year the situation of the staffs of the collectives has improved, as have their living, working, and studying conditions, individual public health knowledge has increased, and personal hygiene has improved immeasurably. It is only on this basis that it is possible to employ special measures with the desired success.

The principal of dispensary service formed the basis for the special prophylactic and antiepidemic measures taken against dysentery in the collectives which we investigated.

This work, dispensary service for the prophylaxis of dysentery in collectives, began among military collectives during the Second World War. About 1943 the works of S.V. Viskovskiy, who proposed the initiation of this type of service, made it possible to develop a system of assanation for organized collectives composed of adults, active detection of all persons suffering from dysentery, and organization of massive treatment of patients followed by observation of the success of therapy.

Complex clinical and laboratory examination of persons suffering from intestinal infections and subsequent dispensary service for individuals in areas of military operations have been rather widely employed for a number of years in the Soviet Army. The work conducted i this direction proved to be effective in the general system of measures for preventing dysentery. Reports by N.A. Sinel'nikov, I.A. Shi frina, S.S. Kurkuzova, and many others indicate the success of these measures.

In 1951 bureaus of intestinal infections were introduced into the system run by the Ministry of Public Health USSR; these were a new organizational format for the prophylaxis and irradication of dysentery. The bureaus of intestinal infections organized at hospital

nd polyclinic institutions paid special attention to two problems, he detection of dysentery patients and dispensary service for individuals who had recovered from this disease. The experience of many hysicians (I.A. Chernova, N.G. Shcherbak, K.G. Fedotova, P.A. Zagaine, E.N. Shlyakhova, A.L. Popik, and others) has confirmed the expediency of this new organizational format for the prophylaxis and rradication of dysentery.

The available data on the military-medical service of the Soviet rmy and the Ministry of Public Health thus indicate the wisdom of aking measures for the complex active detection of dysentery patients nd subsequent dispensary observation of individuals who have recovered from this disease.

The experience amassed in this area enables us to state that dispensary observation of persons who have recovered from dysentery, in conjunction with the other general-public-health prophylactic measures and special antiepidemic measures employed in connection with dysentery, has some effect in reducing the incidence of this infection.

All of the material cited above served as our basis for performing this work widely among organized collectives composed of adults, while analysis of the work done has confirmed it.

This work was carried out during 1952-1957 in a whole series of organized collectives of adults. It began with an epidemiological study of the collectives. It was necessary to determine the general epidemiological conditions which obtained in each collective and its environs, the incidence of dysentery, and the basic factors which affected the rate of intestinal infection under the specific conditions in question.

In order to discover who fell within the scope of our study and

subsequent treatment we first carried out individual interrogations of all persons in the collective for the purpose of determining which of them had had dysentery and which had suffered from intestinal dysfunctions within the two years prior to questioning. All persons who indicated that they had suffered from such infections were selected for special study.

The "anamnestic patients" thus turned up were carefully examined (studied) epidemiologically and received their first clinical, bacteriological, and instrumental examinations (the latter by rectoromanoscopy).

All persons in whom the clinical, bacteriological, or instrumental examination showed a pathological condition to exist were moved to the infectious-diseases department. Here they received a complete clinical examination and were treated.

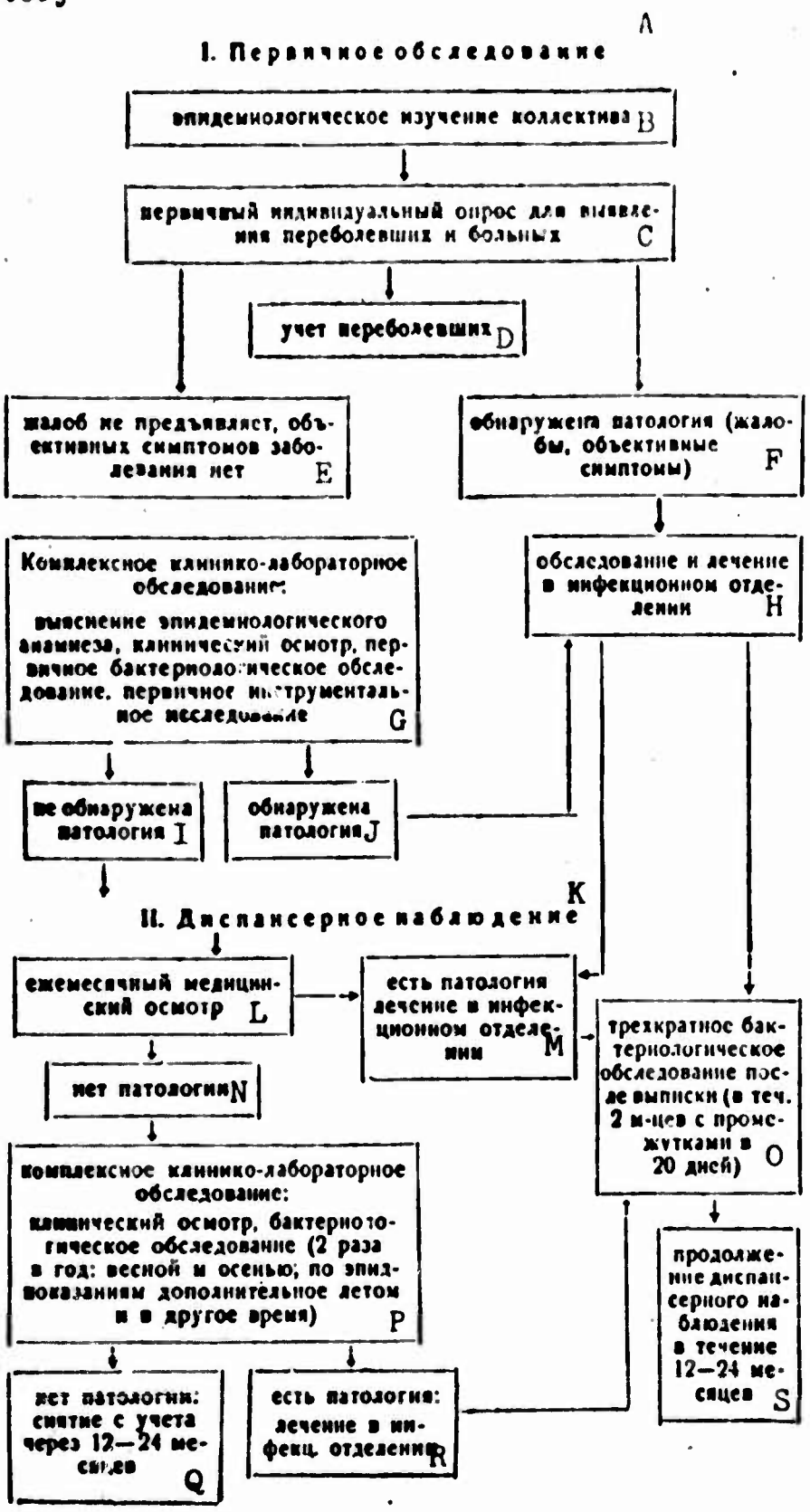
The remaining persons, in whom no pathological conditions were found (by the first clinical, bacteriological, and rectoromanoscopic examinations) were placed under dispensary observation by medical personnel. This dispensary observation consisted of a medical examination once a month and a complex clinical-laboratory examination (clinical, bacterial, and instrumental) twice a year, spring and fall.

All individuals who had been discharged from the hospital after having recovered from dysentery and all those who had been admitted for examination and treatment after the initial complex examination were also placed under dispensary observation. At the same time, each of them were given bacteriological checkups after discharge (three examinations, at intervals of 20 days for two months).

In addition, during the summer the recovered patients were subjected to special complex examinations in accordance with epidemiological indications for the specific conditions which obtained; this

DIAGRAM 1

Diagram Showing Complex Examination and Dispensary Service for Persons Having Had Dysentery



A) Initial examination; B) epidemiological study of collective; C) initial individual interrogation to detect persons having or having had the disease; D) study of persons having had the disease; E) no complaints and no objective symptoms; F) pathological conditions (complaints, objective symptoms) detected; G) complex clinical-laboratory

examination: determination of epidemiological anamnesis, clinical examination, initial bacteriological examination, and initial instrumental examination; H) examination and treatment in infectious-diseases department; I) no pathological condition detected; J) pathological condition detected; K) dispensary observation; L) monthly medical examination; M) pathological condition present, treatment in infectious-diseases department; N) no pathological condition; O) three bacteriological examinations after discharge (over two-month period, at intervals of 20 days); P) complex clinical-laboratory examination: clinical examination, bacteriological examination (twice yearly, spring and fall, and also during summer and at other times when warranted by epidemiological indications); Q) no pathological condition, kept under study for 12-24 months; R) pathological condition present, treatment in infectious-diseases department; S) continued dispensary observation for 12-24 months.

was done in order to facilitate early detection of sources of infection, prompt treatment, and elimination of the disease from the collective. Complex examinations were also carried out for this purpose at other times of the year, in accordance with epidemiological indications (taking into account epidemiological conditions in the collective and in the area around it).

The duration of dispensary observation was and is dependent on the specific epidemiological conditions, but is never less than 12-24 months. As has already been noted, during this period the recovered patients undergo repeated medical examination and complex clinical-laboratory examination. Subsequently, when their health is normal, such individuals are not subjected to complex examination, but they remain under medical observation during their entire stay in the collective.

The entire scheme of complex examination and dispensary service for persons who have suffered dysentery or intestinal dysfunctions may thus be represented in the manner shown in Diagram 1. Since collectives are frequently formed and staffed by the organized arrival of large groups rather than all at once, it is necessary to indicate the principles of the organization of new arrivals into groups.

Such contingents are handled in accordance with the scheme of

initial examination described above, but with certain additions. These latter are determined by the epidemiological conditions with respect to intestinal infections which obtain in the area from which the contingents have come or to which they are going. If these conditions are unfavorable at the time of arrival at or departure from the assembly point all of the individuals concerned receive a bacteriological examination for dysentery in addition to the initial examination. We naturally must take into account the epidemiological conditions and incidence of intestinal infections among the new arrivals on route to their destination. In accordance with indications, the entire contingent may be subjected to bacterial examination, or individual groups may receive a complete clinical-laboratory examination including clinical observation, bacteriological examination, and rectoromanoscopy.

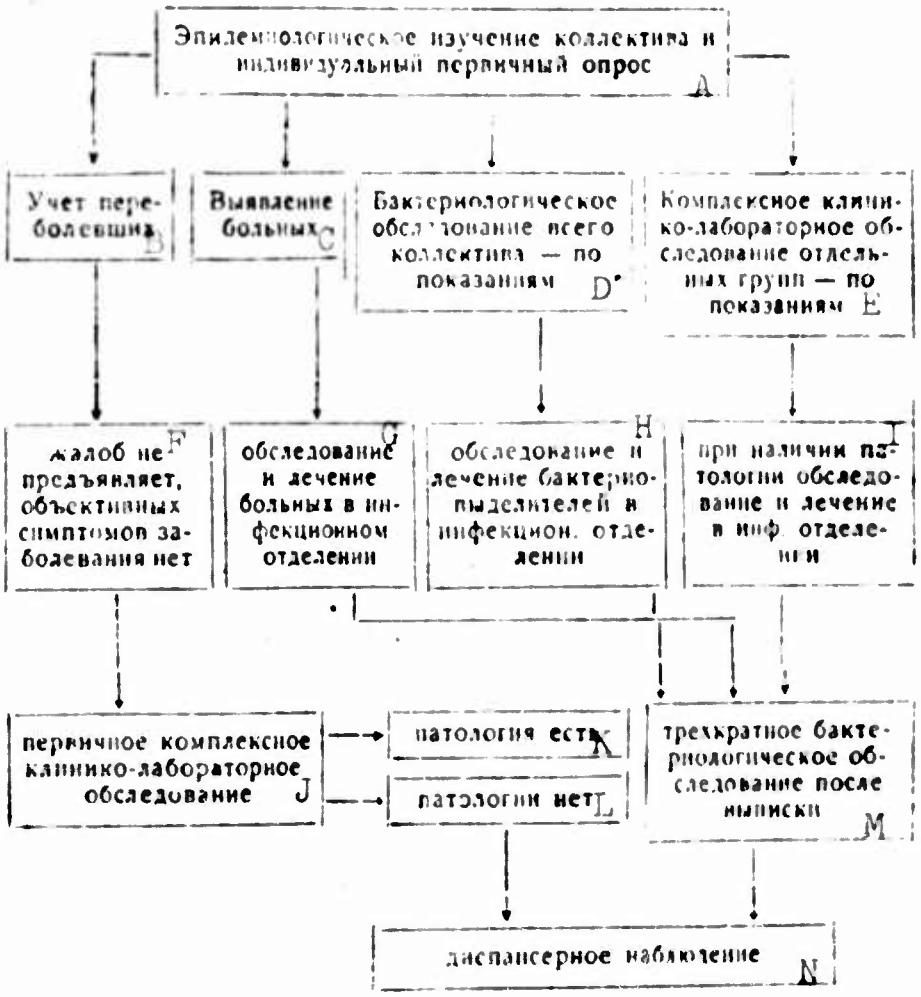
Diagram 2, which is based on the material presented above, shows the scheme employed for the reception, study, examination, and further observation of newly-arrived contingents.

One of the most important factors determining the success of dispensary service for individuals who have had dysentery is continuity. By this we mean continuity both in therapy and prophylaxis and in medical observation; for persons who have had dysentery these should be handled by medical workers during the period of dispensary observation.

An indispensable condition for and guarantee of the success of the work conducted is that the medical workers of the general medical system and of therapeutic and prophylactic institutions have a common outlook on these problems. This may be facilitated by having a single form of documentation. It may also be achieved by having uniform methodological instructions on a given problem and by holding joint methodological conferences involving the medical workers of the

DIAGRAM 2

Diagram Showing Reception of New Arrivals in a Collective



A) Epidemiological study of collective and initial individual interrogation; B) study of persons having had dysentery; C) detection of patients; D) bacteriological examination of entire collective, as indicated; E) complex clinical-laboratory examination of individual groups, as indicated; F) no complaints or objective symptoms; G) examination and treatment of patients in infectious-diseases department; H) examination and treatment of bacteriologically-detected patients in infectious-diseases department; I) examination and treatment of persons exhibiting pathological conditions, in infectious-diseases department; J) initial complex clinical-laboratory examination; K) pathological condition present; L) no pathological condition; M) three bacteriological examinations after discharge; N) dispensary observation.

general medical system and of therapeutic and prophylactic institutions. Reviews of the work done in given areas are also helpful.

As for documentation, a standard form should be worked out for keeping track of persons who have had dysentery; it should describe the results of all examinations and treatment, as well as of subsequent dispensary observation.

If an individual under study as one who has had dysentery leaves the collective the results of dispensary observation should be entered in his personal medical record book.

This information is necessary for the medical worker under whose observation the individual in question subsequently comes. It ensures continuity in therapeutic and prophylactic measures.

The system described above, i.e., active detection of sources of dysentery, prompt treatment of manifest and latent cases, and active dispensary observation on recovery, leads to assanation of the collective. All this, in conjunction with other antiepidemic and general-public-health measures taken in connection with bacterial dysentery, leads to a sharp decrease in the incidence of dysentery in organized collectives of adults. As a result of the measures which have been taken, over a period of years loss of work in collectives as a result of all forms of acute gastrointestinal disease (dysentery, enteritis, colitis, gastroenterocolitis, etc.) has decreased sharply and these diseases have come to play a minor part among other infectious pathological conditions.

According to our data, this complex system for the prophylaxis and irradication of bacterial dysentery has made it possible to reduce the incidence of this disease by a factor of 2-4, has ensured rapid and sufficiently complete detection of sources of infection, has reduced the number of cases in which the acute form passes into the chronic form, and, in last analysis, has created stably favorable conditions with respect to intestinal infections in the collectives.

Wide dissemination of the available information on active detection of sources of dysentery and the dispensary observation of persons who have had this disease has begun. The people's democracies, particularly Czechoslovakia, have begun to make use of the advanced

experience of Soviet public health in this field.

Epidemiological and Public Health Section

DYSENTERY PROPHYLAXIS IN THE COLLECTIVE BODIES OF GROWN-UPS

Yakobson N.Z.

The author showed that complex prophylaxis system and the struggle against bacterial dysentery in the collective bodies of grown-ups had allowed to lower the morbidity twice or four times.

STUDY OF THE MUTABILITY OF THE TYPHOID BACTERIOPHAGE

K.N. Kondrat'yev

A number of methods for altering the hereditary properties of bacteriophages are based on replacement of a homologous by a heterologous culture. In this case the latter plays the part of changed environmental conditions. During the interaction of the phagic population with the live heterologous culture individual altered phagic particles develop; these have a far greater ability to lyze the culture than the initial phages. Other properties of the phages besides their lyzing ability may be altered. The investigator's task reduces to detecting and reproducing these altered microorganisms.

This article describes a series of experiments on the interspecific mutability of the typhoid bacteriophage. The experiments were carried out with standard bacterial strains and "pure lines" of standard typhoid phages. Ten inoculations were made in each experiment. The method employed, its variants, and the preparation of the initial material have been described in detail in an earlier article.*

Experiments Nos. 1-6. Phages L_1, D_2, and D_4 did not lyze strain 519/183 of Gertner's bacillus. Two experiments were carried out with each type of phage; in one 0.1 ml of filtrate was used for the reinoculations, while in the other 1 ml was used. The experiments were repeated in order to determine the influence of the quantity of material introduced in the reinoculations. These experiments yielded no results.

Experiments Nos. 7-14. Adaptation of phages D_2 and D_4 to strains 130 and 8/38 of Bacterium paratyphosum A, phages D_2 and F_1 to strain 675 of Bacterium coli, and phages D_2 and F_2 to strain 116 of Bacterium paratyphosum B yielded negative results.

Experiments Nos. 15-26. Adaptation of phages M and C to strain 130 of Bacterium paratyphosum A and strain 675 of Bacterium coli and phages R and Vi-I to strain 8/38 of Bacterium paratyphosum. Two methodological variants were used for these experiments; in the first a heterologous culture was added in the reinoculations, while in the second homologous and heterologous cultures were added simultaneously (in a ratio of 2:1).

Experiments Nos. 27-28. Phage O_1 was adapted to strain Vi-I by two methods; in one the material for reinoculation was filtered and in the other filtration was replaced by heating in a water bath at 58-60°C for 1 hour. Adaptation was not successful in these experiments.

Experiment No. 29. Phage R_2, which is a variant of phage R obtained from R by intrastrain mutation, was adapted to strain 130 of Bacterium paratyphosum A. The phage multiplied weakly on this strain. After 10 preinoculations it had a high titre in comparison with strain R and formed isolated sterile spots on the dishes containing strain 130.

Experiment No. 30. Phage R_2 lyzed strain 0-901, forming isolated sterile spots on the dishes. By carrying out a number of reinoculations on a solid nutritive medium we were able to adapt this phage to strain 0-901. The newly-obtained phage R_2O had a wide sphere of action (Table 1) and, in contrast to the initial phage, was able to lyze strain 116 of Bacterium paratyphosum B and strain 675 of Bacterium coli. From this we got the idea of adapting the new phage to these strains.

After 15 months of storage at +2-5°C phage R_2O lyzed 0-901 in a titre of 10^{-10} and R in a titre of 10^{-5}.

Experiment 31. Strain 116 of Bacterium paratyphosum B was inoculated with phage R_2O on a solid nutritive medium. 22 inoculations were carried out on meat-infusion bouillon. The last 15 reinoculations were performed with material taken from a single isolated sterile spot, in order to obtain a pure strain of the phage. The sphere of action and titre of the phage are shown in Table 1.

After 15 months of storage in a refrigerator, the phage lyzed strains R and 0-901 to 10^{-8} and strain 116 to 10^{-4}.

Experiment 32. Strain 675 of Bacterium coli was inoculated with phage R_2O on Martin's bouillon. A total of 73 inoculations were performed. After each inoculation titration was carried out on liquid and solid media. Over a space of 53 inoculations the titre of the phage with respect to strain 0-901 on the liquid medium gradually decreased from 10^{-10} to 10^{-1}. However, the phage was detected in all of the dishes.

No lysis of strain 675 was noted on the bouillon, but a mass of sterile spots was detected in the dishes after each inoculation. These spots varied in diameter from 1 mm to scarcely-visible size. The secondary growth differed in extent, so that the spots continuously overgrown with secondary growths appeared "dark," while those with only a small quantity of secondary growth appeared "light." Certain of the sterile spots were surrounded by areas of intensified culture reproduction, while others had zones of incomplete lysis.

Attempts to culture the phage on a solid medium yielded no results; the phage disappeared after several inoculations.

It must be noted that other strains of Bacterium coli were not lyzed by this phage, either on solid or liquid nutritive media.

TABLE 1

Spheres of Action and Titres of Phages on Liquid Medium

A / Штаммы / B / Фаги	A	H	D_2	L_1	91 858	91 501	R	V_1-I	T_{y_2}	O-901	99	5702	8 38	519 183	116	675
R_2	10^{-8}	10^{-2}	10^{-9}	10^{-5}	10^{-10}	10^{-9}	10^{-9}	10^{-1}		–	10^{-1}	10^{-5}			–	–
R_2O	10^{-10}	10^{-3}	10^{-10}	10^{-6}	10^{-11}	10^{-10}	10^{-7}	10^{-6}	10^{-10}	10^{-11}	10^{-6}	10^{-6}	10^{-8}	–	10^{-1}	10^{-2}
R_2B	10^{-10}	10^{-2}	10^{-8}	10^{-2}	10^{-11}	10^{-10}	10^{-11}	10^{-10}	10^{-10}	10^{-11}	10^{-11}	10^{-11}	10^{-10}	10^{-2}	10^{-8}	--

Notes: A, H, D_2, L_1, 91/858, 91/501, R, V_1-I, T_{y_2}, and O-901 are strains of Bacterium typhi; 99, 5702, and 8/38 are strains of Bacterium paratyphosum A; 116 is a strain of Bacterium paratyphosum B; 519/183 is a strain of Gertner's bacillus; 675 is a strain of Bacterium coli; R_2O is the phage R_2 adapted to strain O-901; R_2B is the phage R_2 adapted to strain 116; a dash indicates no lysis. A) Strains; B) phages.

Strains 116 and 675 were tested for lysogenicity. A day-old bouillon culture of strain 116 was centrifuged and the liquid above the precipitate was tested on strains 91/858, O-901, A, and Vi-I in dishes prepared by Gracia's two-layer method. No sterile spots were detected at any time.

A control experiment was performed with strain 675. A day-old bouillon culture of this strain was centrifuged, the liquid above the precipitate was heated in a water bath at 58-60°C for 30 minutes, and 0.5 ml of strain 675 was added. 20 inoculations were carried out. The heated residual liquid from each inoculation was tested in dishes containing strains 675, 4, and 5 of Bacterium coli. The experiment yielded a negative result, no spontaneous phage appearing.

Conclusions. 1. We were unsuccessful in obtaining direct adaptation of typical typhoid phages to strains of bacteria of different species (Bacterium paratyphosum A and Bacterium coli).

2. We were able to adapt phage R_2 to strains of Bacterium paratyphosum B and Bacterium coli after preliminary adaptation to strain

-901, which was an intermediate strain in this case.

Department of Microbiology, Tomsk Medical Institute

THE INVESTIGATION OF VARIABILITY OF S. TYPHI PHAGE

Kondratyev K.N.

The phage R_2 of S. Typhi (variation of phage R) was adapted to he strain of S. Paratyphi B. and to the strain of Escherichia coli fter preliminary adaptation to the strain O-901.

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150 K.N. Kondrat'yev, Trudy TomNIIVS [Trans. Tomsk Scientific Research Institute for Vaccines and Sera], 1959, Vol. X, pages 263 and 271.

THEORY AND PRACTICE OF

IMMUNOLOGY

IMMUNOLOGICAL CHARACTERISTICS OF POLYTOXOIDS AND MORE COMPLEX ASSOCIATED MULTIANTIGEN VACCINES*

B.G. Trukhmanov

The complex study of methods for preventing various infectious diseases is more and more often culminating in the creation of new bacterial preparations intended for active prophylaxis. The recently developed vaccines against influenza, brucellosis, tick-borne encephalitis, pertussis [7], and, particularly, poliomyelitis have now come into wide use. Vaccines against Q-fever [20] and leptospirosis are gradually beginning to be employed. Next in line are vaccines against parotitis (Smorodintsev, Klyachko, etal. [18]), scarletina (P.V. Pavlov [11]), and, in the near future, measles [19].

Unfortunately, in the overwhelming majority of cases these preparations are designed for the prophylaxis of one given infection. This abundance of individual monopreparations not only greatly complicates vaccination prophylaxis, but has now led to the permanent immunization of many groups of children and this, in addition to everything else, excludes the possibility of introducing new and very important preparations.

All of this urgently necessitates a certain reorganization of vaccination; this primarily involves the pressing need for combining individual monoantigens into complex grouped preparations. In 1948 we proposed the combination of a number of the most harmless and highly concentrated toxoids into a single preparation, a polytoxoid, and

studied certain of its properties.*

However, it is now apparently necessary to develop other bacterial complexes, in the form of enteric polyvaccines, viral polyvaccines, complexes of live vaccines, and, possibly, other more complex associated preparations.

We must note that after the well-known articles by G. Ramon [40], I.I. Rogozina [15], A.V. Ponomarev [12], N.I. Aleksandrov and N.Ye. Gefen [1, 5] (1941-1943), N.G. Klyuyeva and E.M. Gintsburg [8] (1940-1945), A.I. Gorokhovnikova [6], and others appeared in the literature, there was a long period, more than ten years, during which almost no articles devoted to the properties of new complexes of preparations were published. The only article which we can name was that by L.K. Arzhelas and M.M. Mayevskiy [3], who studied a complex of antigens prepared from triple vaccine, tetanus toxoid, and typhus vaccine.

Important data on the use of polytoxoids (Vygodchikov [4], Ponomarev [13], Alymov [2], Cherkas [38], and others), as well as on other more complex associated preparations (Krestovnikova [10] and Kondrat'yev [9]) have been presented in recent years, particularly at the Conference on Anaerobic Infections (Khar'kov, 1956). Rogozina and Siroko [16] published some data in 1957. At the Special Conference on Associated Vaccination held in Moscow in April 1958** many research workers of the Moscow Institute imeni Mechnikov, the Institute of Epidemiology and Microbiology imeni Gamaleya and the Leningrad Scientific Research Institute for Vaccines and Sera presented extremely interesting data which indicated a considerable broadening of interest in this important problem.

However, despite their substantial value, these investigations almost entirely failed to deal with the influence of the new preparations on the vaccinated organism, the allergic activity of these as-

sociations of antigens, their immunological effectiveness when the sensitivity of the organism is altered, and other problems which we feel must be solved before administration of these preparations as a part of standard epidemiological practice can be recommended.

In addition, the preparations are studied separately from one another and no attempt is made to determine how they affect previously or subsequently administered preparations, or how bacterial preparations and preparations of the sulfanilamide and antibiotic types, which have come into wide use, interact.

Having been occupied in the solution of similar problems for almost 10 years [22, 24-36], we have amassed a great deal of experimental data, so that we are able in this article to report on them only schematically, giving definite results and certain illustrations of individual situations.

Essentially, all of our investigations may be divided into experiments intended to determine the reactogenicity of preparations and experiments intended to study their immunological effectiveness.

We studied preparations of varying complexity: a polytoxoid consisting of diphtheria, tetanus, botulin A and B, edematiens, and staphylococcus toxoids (dysentery and perfringens toxoids were employed in certain experiments), a polyvaccine consisting of ordinary quadruple vaccine and pertussis, listerellosis, encephalitis, and rabies monovaccines, and finally a multiantigen, a very complex mixture containing 15 or more individual monopreparations in certain experiments.

It must be noted here that we did not regard this collection of antigens as some new preparation which would soon be recommended for introduction into vaccination practice; we created it in order to elucidate the problems of how an organism would react to administration of a multicomponent association of antigens, what the remote consequences

of such a vaccination would be, and whether it would in principal be possible to obtain sufficient immunity to the individual components of a preparation as complex as the multiantigen. We investigated all of these associations of antigens in comparison with individual monoantigens, the latter being diphtheria and tetanus toxoids, listerellosis vaccine, and, in certain cases, sheep corpuscles so that the antibodies obtained as a result of immunization with these monopreparations could be rather precisely and rapidly titrated.

In the first group of experiments, which were performed on guinea pigs, white rats, rabbits, white mice, dogs, and horses, we studied the reactogenicity of the complex associated preparations [29]. The reaction to administration of various doses of preparation was studied (we determined the limit of transmissivity in a number of cases), as was the influence of different modes of administration – subcutaneous, intracutaneous, intramuscular, intravenous, etc.

The extent of the reaction was determined from the general condition of the animals and their survival rate; in the larger animals, body temperature, change in weight, and local reactions in the form of erythema, infiltrations, and other changes were recorded.

In addition, we determined the changes in hematological indices and, in a number of cases, studied the changes in serum proteins in the animals under investigation. It must be noted that changes in hemoglobincontent were observed only on the 7th day in the rabbits which received the polyantigen and multiantigen. It is possible that a delayed reaction occurred because of the complexity of the preparations and we must consequently assume it to take place somewhat later than is customary when ordinary monopreparations are used.

We very soon became convinced of the practical safety of complex associated preparations and our hypothesis of the possible summation of

the reactogenicities of the individual preparations was not confirmed in practice. Quite the contrary, in certain cases we noted a slightly less marked reaction to the polypreparation than to the monoantigens. This equilibration, or interference, of the reactions undoubtedly indicates that the individual preparations affect the organism differently, involving antagonistic systems, so that the ultimate effect on the organism is even less than usual. In experiments involving administration of massive doses of the preparations (20-40-60 ml for a single rabbit) the polytoxoid proved to be the most areactogenic and the polyvaccine the least areactogenic, while the multiantigen occupied an intermediate position. We did not observe any intensified or abnormal reaction in checking the primary reactogenicity of the complex bacterial preparations against a background of simultaneous administration of antibiotics and sulfanilamides, particularly norsulfazol, penicillin, and streptomycin.

Observation of the reactions of the animals during prolonged immunization also failed to reveal any marked difference among the monopreparation, dipreparation, polypreparation and multipreparation. Investigation of blood from the rabbits, guinea pigs, and horses [32] showed that there were no serious hematological shifts, much less pathological changes in the blood.

According to the data of Ye. N. Rodyukova [14], prolonged immunization of production horses with diphtheria-tetanus ditoxoid did not cause any marked changes in the immunized animals.

The results of all these observations led us to conclude that the primary reactogenicity (direct reaction to vaccination) of the various associations of antigens, including the most complex, is essentially no different from that of the bacterial preparations ordinarily employed.

The next series of experiments was devoted to determination of the allergic activity of the complex associated vaccines. It was necessary to find out what the total allergic potential of a preparation consisting of 6-8 or even 15-16 individual antigens was and whether all of the properties inherent in its separate components were summed. This hypothesized intensification of allergic activity might easily exceed the permissible limit and make it impossible to use such undoubtedly valuable preparations as the polytoxoid or the more complex multiantigen in practice. In addition, little research has been done on the allergic properties of viral preparations and still less on mixtures of these preparations with preparations of microbial origin.

Experiments on active anaphylaxis performed on a large group (more than 300) of guinea pigs showed that the allergic reactions caused by complex preparations of the polytoxoid or multiantigen types generally do not differ from those induced by sensitization of the animals with monotoxoids.

We checked this with different combinations of extremely diverse preparations [29].

In addition, working in conjunction with L.Ya. Tikhonova [30], we made comparative observations on quite similar animals, in one case after a single sensitization with diphtheria toxoid and in the other after repeated treatment with various preparations of increasing complexity - monotoxoid, ditoxoid, tetratoxoid, pentatoxoid, and other toxoids and multiantigen.

In investigating the dependence of the intensity of the reaction on the size of the dose employed we found that administration of different quantities of preparation produced the same result. Guinea pigs which received shock-inducing (reacting) injections in doses of 0.25, 0.5, and 1.0 ml reacted in absolutely the same manner with respect to

appearance of anaphylaxis.

The experiments were conducted in different directions. Since we are not able to dwell in detail on them here, we may say in summation that after induction of shock the animals which received tetanus toxoid exhibited the most mild reactions. The guinea pigs in this group manifested no general reaction to administration of comparatively large doses of preparation (up to 2 ml) and showed almost no symptoms of anaphylaxis. The animals which received diphtheria-tetanus ditoxoid and polytoxoid for shock-induction purposes also scarcely reacted at all to administration of the preparation and, just as those which received the tetanus toxoid, exhibited no signs of severe anaphylaxis leading to death.

It is interesting to note that in a number of cases purified diphtheria toxoids caused a more severe allergic reaction than ordinary diphtheria toxoid. The weak anaphylactic reaction to the same quantity of diphtheria toxoid used as a component of a more complex associated preparation is also very interesting.

We must place special emphasis on the observed fact that the anaphylactic reaction to a polytoxoid is somewhat weaker than that to a single monotoxoid forming one of its components (see p. 177).

In this case we are apparently dealing with some unusual interference among the reactions, the individual allergens making up the complex associated preparation having opposing effects on the organism and thus causing anaphylaxis to fail to appear.

We encountered a similar phenomenon in studying the allergic properties of meningococcal toxin [21] and concluded that the shock which it causes apparently occurs in temporally-separated components, as a result of the presence of several individual fractions, and consequently does not always develop into a hyperergic attack which threatens the

life of the animal. It is occasionally possible for the individual shocks to interfere if the allergens involve organ systems which differ in physiological action.

We must also mention the experiments which we performed in order to determine the possibility of reducing or entirely suppressing the allergic activity of certain bacterial preparations by direct addition of certain antihistamines of the dimedrol (Trudy TomNIIVS [Transactions of the Tomsk Scientific Research Institute for Vaccines and Sera], 1956, Vol. 8, p. 251) and other types; working in conjunction with L.V. Sapozhnikova [27], we were able to show that dimedrol has no detrimental effect on diphtheria toxoid, even when the two have been in contact for a year.

Not having obtained any marked reaction in a model of the ordinary anaphylactic reaction, we decided to compare the allergic activities of the complex preparations in Shwartzman's hyperergic-reaction model [31]. Analysis of all the data obtained in this area of the investigation (the experiments were conducted on 80 rabbits) indicates that, just as their components, complex preparations of the polytoxoid or even the multiantigen type exhibit little activity in Shwartzman's phenomenon. Special notice must be taken of the fact that the rabbits which had previously received a single immunization exhibited a rather severe hyperergic reaction; a considerable number of the animals in this group died after intravenous injection of the preparation, although the control animals, which received the same or a considerably larger quantity of preparation, especially the polytoxoid, survived. The single immunization apparently led to an increase in the animals' sensitivity. This must be kept in mind, since in vaccination practice one frequently encounters cases in which vaccination has been discontinued after the first injection for one reason or another. Such incomplete

immunization is undoubtedly harmful.

Experiments conducted to obtain a comparative characterization of the simple and complex antigens from Arthus' phenomenon also revealed no essential differences among these preparations.

Summing up the first group of investigations, it must be noted that the associated vaccines do not differ particularly in reactogenicity from the ordinary monopreparations. The polytoxoid is an absolutely safe preparation, while the polyvaccines and multiantigen are more reactogenic, but are essentially no different from the analogous vaccines if the monopreparations which compose them are taken in their native state. This reactogenicity may be further reduced by purifying and concentrating the active fractions, as we showed in the work which we conducted jointly with A.A. Tri[illegible]olitova and A.I. Vasilenko on diphtheria toxoid [23]; in addition, it will be possible to accomplish this by adding special antiallergic substances.

The second group of experiments was devoted to a study of the immunological response of animals immunized with preparations of increasing complexity, containing from 1 to 15-18 individual monoantigens. The well-known theory of antigen concurrency did not permit us to expect to obtain a complete immunizing effect in response to such a large quantity of antigens. In any case, it was assumed that the intensity of the immunity produced to a single monoantigen in the mixture would naturally be considerably less than would be obtained if this antigen were administered separately.

Our first experiments, which were conducted jointly with G.V. Pafnut'yeva and V.G. Krasnova [25, 26] on diphtheria-tetanus ditoxoid and a tritoxoid consisting of diphtheria, tetanus, and dysentery toxoids, showed that it is quite possible to use double and triple antigens with no loss of effectiveness over the individual monoantigens. Quite the

contrary, we noted a synergism, the titres of certain antibodies being higher on administration of the tritoxoid than after separate immunization with one of the monotoxoids.

In the experiments which Meislowa, Ryzewska, and Spovzinska [39] carried out to determine the quantitative relationship among the antigens in a triple vaccine (typhoid, tetanus, and diphtheria toxoids) administration of the antigens in different quantities by volume proved to be most successful, affecting all three antigens. Working in conjunction with Ye.N. Rodyukova [28], we also failed to observe antigen concurrency on immunization of production horses with diphtheria-tetanus ditoxoid. However, it was necessary to elucidate this phenomenon for considerably more complex associations of antigens.

The experiments were performed on guinea pigs, rabbits, white mice, and horses. Various groups of guinea pigs were immunized with preparations of increasing complexity, ranging from diphtheria monotoxoid to a polycomponent multiantigen. The increase in titres was determined from the accumulation of diphtheria and tetanus toxoids and agglutanins to all of the ingredients of the tetravaccine and to the listerellosis vaccine and from the titre of virus-neutralizing antibodies to spring-fall tick-borne encephalitis virus.*

The data obtained indicated a rather intensive formation of antibodies to the antigens of all groups tested.

The difference in titres occasionally observed after revaccination particularly remote revaccination 7 months after the beginning of immunization, disappeared completely. Basic to this problem is selection of the correct antigen proportions, taking into account their activities (in our experiments the tetanus toxoid was the most active immunologically). If this fact is ignored it is easy to employ a weakly active antigen under disadvantageous conditions and thus artificially

eate antigen concurrency. This is an extremely serious problem and e very difficult to study. By adding different quantities of a new tigen to that already tested it is easy to find the required optim amount for the former; however, careful checking of the influence the new antigen on the degree of immunity produced by the previously-sted antigen complex is obligatory.

Since the antibody levels were not the same in the different groups f guinea pigs and since we took into account the fact that antibody ndices do not identify immunity as a whole, we investigated the ext of general protection by administering toxin in very substantial oses in a number of cases [33].

Guinea pigs immunized with diphtheria monotoxoid alone withstood 000 MLD of diphtheria toxin well, while those immunized with tetanus oxoid withstood 3000-6000 MLD of tetanus toxin. The guinea pigs which eceived the diphtheria-tetanus ditoxoid, the polytoxoid, and the multintigen survived administration of the same doses of diphtheria and etanus toxin. In order to demonstrate that the animals were sufficiently well protected against these two infections we investigated he survival rate among guinea pigs after double intoxication with both oxins.

Completely immunized animals (those which received remote revaccination) survived after being given quantities of toxins as large as 000 MLD of diphtheria toxin and 3000 MLD of tetanus toxin simultaneously.

The results of this experiment completely convinced us of the ossibility of obtaining the requisite high degree of immunity by mploying sufficiently large doses of preparation, taking into account he optimum proportions for the individual antigens.

In experiments on antitetanus immunity in white mice we found no

differences among groups immunized with tetanus monotoxoid, diphtheria-tetanus ditoxoid, the polytoxoid, and the multiantigen.

Immunization of rabbits yielded similar data for both titre of antibodies and dermal reactions induced by administration of diphtheria toxin and by intoxication with a large quantity of native toxin.

The extent of immunity was substantial both in the rabbits immunized with the monopreparations and in those immunized with the polytoxoids or multiantigens. By way of illustration, in one paper we cited data on the content of certain antibodies in rabbits immunized with preparations of varying complexity, as well as material on the importance of revaccination in immunizing rabbits, using the titre of antitoxins to diphtheria and tetanus as an example. It was shown that the titres of these antibodies are independent of one another.

In certain cases we followed the change in the titres of certain antibodies in rabbits, varying the proportions of the monoantigens composing the associated preparation in individual cycles.

In immunizing horses we first tested the diphtheria-tetanus ditoxoid, obtaining good results; the titres achieved with respect to both antigens were completely satisfactory for serum production. The data which we obtained enable us to recommend double diphtheria-tetanus immunization as a method for the rapid selection of horses for serum production [28].

The possibility of regulating as desired the titres of the antibodies obtained on administration of double, triple, and more complex preparations by altering the proportions of the individual monoantigens enabled us to obtain a number of antibodies in titres sufficient for serum production from a single producer.

Thus, for example, on 9 May 1957 serum from horse No. 147 contained 900 units of diphtheria antitoxin and 400 units of tetanus anti-

toxin per ml. In addition, it contained antibodies to spring-fall tick-borne encephalitis in titres considerably exceeding the minimum established for this type of serum.

Our serum department used this horse as a producer of antiencephalitis serum, the other antibodies not being utilized at all, for obvious reasons. At the same time, if we could separate the individual antibodies when we purified and concentrated the multiserum, the same horse might serve as a producer of several antitoxic sera.

Preliminary experiments on electrophoresis of the multiserum showed the presence of a whole series of individual peaks (up to 7 or 8) in the globulin fraction. We began these experiments quite recently, but they have opened up prospects for the use of more precise fractionation methods in separating various groups of immune proteins and obtaining at least two or three antitoxins in good titres from a single horse. It must be mentioned that I.Ya. Sil'd (Leningrad Scientific Research Institute for Vaccines and Sera) has reported that the individual pseudoglobulin fractions of hyperimmune horse serum are saturated to differing degrees by diphtheria and tetanus antitoxins.[17].

In analyzing the literature on associated vaccination investigators have been found to be interested primarily in determining the immunological effectiveness of various new associations of antigens. The problem of the influence of these new vaccines on the degree of immunity achievable when preparations already in use in immunization practice have been administered has not been studied at all, despite its great practical importance.

In order to elucidate the question of what happens when a polytoxoid or multiantigen is administered in the presence of immunity to individual infections, we conducted experiments involving supplemental immunization with smallpox vaccine of animals repeatedly immunized with

complex multicomponent associated vaccines [34].

We conducted similar investigations in conjunction with Ye.I. Kleytman [35] on supplemental vaccination against tularemia. Tests of immunity to smallpox by a second inoculation with smallpox vaccine and to tularemia with the aid of an allergy test with tularin and by direct inoculation with a tularemia culture yielded positive results; this showed that preliminary administration of a very complex associated vaccine does not exhaust the immunological capacities of the organism.

It is interesting to note the slight observed lag between the rate of increase in resistance to tularemia in animals immunized with complex polyantigens and that in animals which received tularemia vaccine alone.

It is very important that these supplemental vaccinations did not have any negative effect on the titres of the antibodies produced in response to administration of the polytoxoid or multiantigen; this again speaks against the concept of antigen concurrency.

We must state that in carrying out this work we encountered certain phenomena which we were not able to explain; for example, the titres of antibodies to individual monoantigens dropped suddenly after immunization with polyantigens, in the rabbits the titres of antibodies were minimal after administration of massive doses of the preparations, etc. An insufficient number of experiments were conducted to check the duration of immunity in the animals which received antigen associations as complex as the multiantigen.

In conclusion, we wish to emphasize that multicomponent associated preparations of the polytoxoid and, particularly, multiantigen types are qualitatively new preparations which obey their own special rules; measuring them by old standards, comparing their properties with those

of ordinary monoantigens, is consequently quite invalid.

We must obtain effective protection against many infections and whether or not this is accompanied by especially high antibody levels is of no practical importance. In solving the fundamental problems involved in evaluating complex associated vaccines it is apparently necessary in general to pay more heed to the survival rate among animals tested with massive doses of the appropriate toxin or live culture than to the antibody titres.

In using very complex associated vaccines to create a stable immunity to 18-20 or more infectious diseases 3 or 4 double vaccinations may possibly be required; however, this is undoubtedly justified and we must certainly work in this direction. It is necessary to take measures to regulate vaccination and the production of associated vaccines, carrying out all of this work, which is of manifest theoretical interest and vast practical importance, in an extremely well-planned fashion.

It is with satisfaction that we emphasize the increase in the amount of attention which our immunologists are paying to the problem of associated vaccination and note that the Tomsk group of immunologists, who have obtained a number of new data, has made a definite contribution toward the solution of this problem.

As a result of all of the observations which we have made we may draw the following general conclusions:

1. The new antigen associations – a polytoxoid consisting of six individual monotoxoids and a still more complex preparation, a multiantigen, comprising toxoids and bacterial and viral antigens (15 or more components) – are immunologically effective preparations.

Animals treated with the multiantigen retain their ability to develop an effective immunity to smallpox, tularemia, and certain other infections.

2. Further careful study of the allergic reactions which occur in the presence of various combinations of bacterial preparations is necessary, but substantial corrections are already needed in our theory that certain of the sensitizing properties of polypreparations are particularly intensified.

3. Selection of correct doses and observation of the requisite vaccine proportions makes it possible to entirely avoid so-called "antigen concurrency," which is produced by inadequate knowledge of the immunological activities of the individual antigens, and to use their observed synergism with great effectiveness.

4. A very considerable degree of immunity may be achieved by using complex associations of antigens, especially when remote revaccination is employed. In testing this by direct intoxication, animals have survived thousands of lethal doses of individual toxins and combinations of toxins.

5. It is also necessary to study as carefully as possible the feasibility of separating individual antibodies from complex multisera and obtaining several antitoxins in commercially feasible titres from a single producer.

6. Thorough analysis of the data obtained reveals certain unusual mechanisms inherent in very complex associated preparations; these include the unusual interference and equilibration of the reactions caused by individual monopreparations, a slight lag in the rates at which the resistance of the organism increases, a certain noncorrespondence between the antigen level and the generally high resistance of the organism, etal.

All this indicates that very complex associated vaccines of the multiantigen type are a new type of preparation which requires a somewhat different approach and, possibly, new tests to determine their

immunological effectiveness.

7. The data which we amassed, which have convinced us of the complete safety and immunological effectiveness of complex associations of antigens, and the material presented at the Special Conference on Associated Vaccination have made it possible for us to set ourselves the task of employing every means to broaden work on the production and introduction of new associated preparations; this will considerably facilitate vaccination and make it possible to expand the arsenal of methods used for the active prophylaxis of various infections.

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IMMUNOLOGIC CHARACTERISTICS OF POLYANATOXINS AND MORE COMPLICATED ASSOCIATED VACCINES-MULTIANTIGENS

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This article represents a review of author's works made after studying complicated associated vaccines of 6-7 component polyanatoxin type and still more complicated association of antigens - multiantigen consisting of some anatoxins, microbe and virus vaccines. Reactogenicity of these new preparations and their immunologic full value was examined by different tests. The tests were made on a large quantity of small laboratory animals (guinea pigs, white mice, white rats, rabbits, dogs) and on horses - producers of medical antiserums.

This article cannot be summarized briefly.

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[Footnotes]

156 Prepared paper (without tables) read 30 May 1958 at the joint scientific conference of the IEM AMN SSSR [Institute of Experimental Medicine, Acad. Med. Sci. USSR] and TomNIIVS [Tomsk Scientific Research Institute for Vaccines and Sera].

157 *The first reports on polytoxoids were published in the Trudy TomNIIVS (1955, Vol. VI) and in ZhEMI [J. Epidemiology, Microbiology and Immunology] (1955, No. 5).

157 **At this conference, we reported on our research on a multiantigen-anassociation of antigens from toxoids and bacterial and viral vaccines [36].

165 The titres of the listerellosis antibodies were determined by A.A. Tripolitova, and those of the encephalitis antibodies by M.K. Tyushnyakova, to both of whom we express our comradely gratitude.

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ANAPHYLACTIC PROPERTIES OF SIMPLE AND COMPLEX ASSOCIATED VACCINES

B.G. Trukhmanov

The active prophylaxis of infectious diseases is more and more often involving the vaccination of persons who have already received certain preparations.

Experimental elucidation of the problem of the influence of toxoids, particularly the extremely complex polytoxoids, and other associated vaccines on persons who have previously been repeatedly sensitized with various preparations is of interest both for immunological theory and for the practice of vaccination.

The problem of toxin, and particularly toxoid allergy has not been sufficiently well studied. The allergic properties of individual bacterial toxins, toxoids, have been investigated. Diphtheria toxoids (Nil, Sag, and Richardson; Nikonov, Morgunov, Vagner-Sakharova, Kudryavtseva, Trukhmanov, Tripolitova, etal.), dysentery and scarletina toxoids (Nikonov), meningococcal preparations, particularly scarletina toxoid (Trukhmanov) tetanus, staphylococcal, and botulin toxoids (Morgunov), and others have been studied from this point of view. The anaphylactogenic properties of antiencephalitis preparations have been investigated (Trukhmanov, Yegorshina, and Zel'tina).

Summing up all of these observations, we may draw the general conclusion that a state of elevated sensitivity is created on administration of individual preparations to the organism under investigation;

this appears in the form of an anaphylactic reaction of varying intensity, ranging up to lethal anaphylactic shock, when injection of the antigen is repeated. It was thus very important to determine what happens to the allergenic properties of individual monoantigens when they are associated in a complex preparation of the 6-component polytoxoid or multiantigen type, the latter including 6-8 different bacterial and viral vaccines in addition to the polytoxoid.

In order to compare the allergenic properties of preparations of varying complexity it was necessary to use previously worked-out doses. For this purpose we conducted special investigations on 120 guinea pigs; as a result we became convinced that the quantity of preparation administered was of greater importance in sensitization of the animal than in the reacting injection. The interval between the first and second injections of the allergens was very important.

A dose of 5 ml proved to be completely satisfactory for sensitization and we also chose this dose because it enabled us to use guinea pigs which had been injected with diphtheria toxoid to check its safety for certain observations. The shock-induction dose was fixed at 0.5 ml for intracardiac injection.

After establishing the optimum doses, we conducted experiments involving active anaphylaxis with preparations of varying complexity on 300 guinea pigs.

For the experiments we used diphtheria and tetanus monotoxoids, a mixture of the two (diphtheria-tetanus ditoxoid), and the 6-component polytoxoid consisting of diphtheria, tetanus, botulin A and B, edematiens, and staphylococcus toxoids. We ran a parallel test on another complex associated preparation, a polyvaccine consisting of the four components of enteric tetravaccine (pertussis, listerellosis, encephalitis, and rabies vaccines).

Finally, we checked the allergenic properties of a complex 14-component multiantigen preparation containing all of the antigens present in the aforementioned polytoxoid and polyvaccine.

Since we were trying to find differences in the allergenic properties of the simple and complex preparations and were little interested in the intensities of the anaphylactic reactions which they produced, we tested the toxoids in their native state in the majority of the experiments. It must be assumed that appropriate purification of the preparations would make their allergenic properties less marked.

The preparations were tested to determine their sensitizing ability; the guinea pigs first received preparations of varying complexity and shock was then induced with some one preparation. We simultaneously investigated the intensity of the shock-inducing effect; the guinea pigs were given one toxoid or the polytoxoid and shock was then induced by preparations of varying complexity.

Each major experiment was accompanied by control experiments on shock induction; untreated guinea pigs were given intracardiac injections of equal doses of the preparations used for the reacting injections.

On analysis of these data it was found that, with the exception of certain cases in which the guinea pigs sensitized with the polyvaccine and multiantigen exhibited severe reactions, the allergic manifestations in all groups were generally almost identical in intensity. The mildest reactions were observed in the group which received tetanus toxoids. When autopsies were performed on the guinea pigs which died of anaphylactic shock we noted, in addition to the expected symptoms, a severe engorgement of the vessels of the cervical portion of the spinal cord with venous blood, a phenomenon which we had previously observed but not described. On transection of the cervical portion of

e spinal cord dark oily blood was withdrawn in large quantities from e cerebrospinal canal. We observed this phenomenon without exception der various experimental conditions.

We subsequently tested the allergenic properties of the various eparations in an experiment involving shock induction in completely iform groups of guinea pigs sensitized with native diphtheria toxoid. is experimental setup is the closest to natural conditions, since is preparation was at the time the only one used for the prophylaxis f diphtheria and a considerable portion of the Soviet populace had een vaccinated with it.

Experiments were carried out in various directions.

For ease of comparison Table 1 shows almost all of the data obained on the allergenic properties of the various preparations, as etermined from their shock-inducing effects.

Different series of observations are entered in the Table. The uinea pigs in groups I and II were subjected to a single sensitiza- ion with diphtheria toxoid and no further treatment, those in group III were sensitized repeatedly, those in group IV were also given hyper- mmune diphtheria serum, those in group V received sulfanilamides, ntibiotics and cortisone, and those in group VI were subjected to a econd shock induction after a considerable time interval.

However, no matter what the characteristics of the individual groups, the guinea pigs in all of them were subjected to shock induction with monopreparations or the polypreparation under absolutely identical conditions. Careful analysis of the data given in Table 1 shows quite clearly that there were no essential differences between the anaphylactic reactions caused by the diphtheria monotoxoid and those induced by associated preparations as complex as the 6-component polytoxoid or the multiantigen.

TABLE 1

Comparative Data on Determination of the Allergenic Properties of Simple and Associated Preparations

A Серия опытов	B № групп свинок	C Сенсибилизация (препарат)	D Шокизация (препарат)	E Кол. свинок	F Симптомы анафилаксии: G Отсутствие явлений	H Легкие явления	I Тяжелые явления	J Смертельный шок
I	1	Дифанатоксин K	Дифанатоксин	10	—	8	2	1
	2	Полианатоксин L	—.—	23	—	20	3	1
II	3	Дифанатоксин	Дифанатоксин	36	18	10	8	7
	4	—.—	Столбанатоксин M	11	11	--	—	--
	5	—.—	Бианатоксин N	12	6	5	1	—
	6	—.—	Полианатоксин	16	5	9	2	—
III	7	Дифанатоксин	Дифанатоксин	23	6	14	3	--
	8	—.—	Полианатоксин	5	2	3	—	—
IV	9	Дифанатоксин	Дифанатоксин	27	10	10	7	2
	10	—.—	Полианатоксин	15	7	7	1	1
V	11	Дифанатоксин	Дифанатоксин	25	13	4	8	4
	12	—.—	Полианатоксин	25	14	5	6	2
VI	13	Дифанатоксин	Дифанатоксин	8	3	3	2	2
	14	—.—	Полианатоксин	21	6	9	6	2
	15	—.—	Мультиантиген O	6	2	3	1	1
Итого P			Моноанатоксин Q	129	51	49	30	16
			Полианатоксин	120	37	72	20	8

A) Series of experiments; B) number of group of guinea pigs; C) sensitization (preparation); D) shock induction (preparation); E) number of guinea pigs; F) symptoms of anaphylaxis; G) no symptoms; H) mild symptoms; I) severe symptoms; J) lethal shock; K) diphtheria toxoid; L) polytoxoid; M) tetanus toxoid; N) ditoxoid; O) multiantigen; P) total; Q) monotoxoid.

The insignificance of these reactions shows that there was no summation of the allergic properties of the individual antigens when they were associated into the complex preparation. If we carefully examine the allergenicity of the preparations by the most serious test, the number of severe manifestations of anaphylaxis and of extremely severe reactions in the form of lethal anaphylactic shock, we find that the allergenic activity of the polytoxoid is comparatively less than that of the monotoxoids. This is noticeable in groups I and V and may

be seen especially clearly in group II, where 7 of the 36 guinea pigs died.

This also follows rather clearly from a comparison of the reactions expressed as totals by preparation; this is done at the end of the table, where we show the sums and include the previously cited data. These figures speak for themselves. This fact becomes still more paradoxical if we recall that the polytoxoid included diphtheria monotoxoid, in quantities equal to that in the monopreparation (calculated in units) in a number of cases. We can explain this phenomenon only by the fact that certain of the antigens present in the polytoxoid apparently had opposing effects on different systems of the organism. This results in an unusual interference and equilibration of reactions and the symptoms of anaphylaxis are consequently less severe on the whole.

In any case, all of these facts, which we tested on a large group of animals, undoubtedly indicate that complex associated preparations of the polytoxoid and multiantigen types have no special sensitizing properties; this will enable us to use them in practice.

On the basis of the data obtained in all of the experiments on active anaphylaxis we may draw the following conclusions:

1. Bacterial preparations, including toxoids of various degrees of complexity, are able to cause allergic manifestations in animals sensitized to them, these ranging up to lethal anaphylactic shock.

On dissection of the animals which died of shock we regularly observed severe engorgement of the cervical portion of the spinal cord with venous blood, in addition to the expected symptoms of death from anaphylaxis.

2. The allergic properties of the various preparations may be studied both by the sensitizing test and by comparison of their shock-

inducing effects. In the first case the dose administered and the number of times the animal has been sensitized are of great importance.

3. The complex associated preparations, the polytoxoid and multiantigen, which we studied under various experimental conditions have allergenic properties which do not differ essentially from those of ordinary monopreparations of the diphtheria-toxoid type; this indicates that allergic properties do not sum when preparations are associated.

4. The rather insignificant reaction observed in response to complex preparations in many cases may be explained only by a certain interference among the reactions caused by the individual monoantigens; it is possible that this results from their having different effects on the organism, simultaneously involving antagonistic regulators of the vital activity of the organism.

Tomsk Scientific Research Institute for Vaccines and Sera

ANAPHYLACTIC PROPERTIES OF SIMPLE AND COMPLICATED ASSOCIATED VACCINES

Trukhmanov B.G.

On experiments of active anaphylaxis on guinea-pigs, on large quantities of animals (above 300) the properties of various complicated associations of antigens were studied. At associations of separate monopreparations the summation of their allergenic properties does not happen. On the contrary, weaker anaphylaxis manifestations are noted, the author explaining this by an original interference, by different action of separate monopreparations on regulators of organism activity.

ACTIVE ANAPHYLAXIS PRODUCED BY ADMINISTRATION OF TOXOIDS IN THE PRESENCE OF ALTERED SENSITIVITY

B.G. Trukhmanov and L.Ya. Tikhonova

In a prior work* we described an investigation of the allergenic properties of various combinations of monotoxoids and polytoxoids administered to experimental animals which had been subjected to a single sensitization.

However, it is no less important to elucidate the problem of how the allergenic properties of these preparations manifest themselves under the complicated conditions which obtain when the sensitivity of the organism is altered.

Our observations were made on 140 guinea pigs which had been used to test the safety of production diphtheria toxoid.

In the first series of experiments we evaluated the importance of multiple sensitization. All of the groups of guinea pigs were sensitized with a single injection of 5 ml of diphtheria toxoid. The first subgroup contained 14 guinea pigs which were subjected to two additional sensitizations with diphtheria toxoid, while the animals in the second subgroup (17 guinea pigs) were immunized six times. Ordinary native diphtheria toxoid was employed for sensitization. Shock was then induced in all of the subjects by intracardiac reacting injections of various preparations. For this purpose we used native diphtheria toxoid and a previously-studied 6-component polytoxoid consisting of diphtheria, tetanus, botulin A and B, edematiens, and staphylococcus toxoids.

Table 1 shows the results of the tests on this group of guinea pigs.

TABLE 1

Results of Induction of Shock in Guinea Pigs Repeatedly Treated With Diphtheria Toxoid

A № № групп	B Кратность сенсибилизации	C Препарат для шокизации	D Кол. животных	E Общая реакция на введение	F Проявления анафилаксии: G Отсутствие явлений	H Легкие явления	I Тяжелые явления	J Гибель от шока
1	3	Дифанатоксин K	14	13	6	5	3	—
3	6	—"—	9	5	—	9	—	—
4	6	Полизнатоксин L	5	3	2	3	—	—
5	К	Дифанатоксин	2	1	2	—	—	—
6	К	Полизнатоксин	2	2	2	—	—	—

A) Number of group; B) number of sensitizations; C) preparation used for shock induction; D) number of animals; E) general reaction to injection; F) symptoms of anaphylaxis; G) no symptoms; H) mild symptoms; I) severe symptoms; J) death from shock; K) diphtheria toxoid; L) polytoxoid.

TABLE 2

Anaphylactic Symptoms in Guinea Pigs With Various Antibody Contents

A №№ групп	B Введение антидифтерийной сыворотки	C Кол-во животных	D Симптомы анафилаксии: E Отсутствие явлений	F Слабые проявления	G Тяжелые явления	H Смертельный шок	I Изменения в весе, в %
1	За 5 часов до опыта J	20	10	9	1	—	+2.5
2	За 1 час до опыта K	20	8	7	5	—	−2.4
3	Не вводилась L	14	6	3	5	4	−4.7
4	Контрольные M	4	4	—	—	—	—

A) Number of group; B) injection of antidiphtheria serum; C) number of animals; D) symptoms of anaphylaxis; E) no symptoms; F) weak symptoms; G) severe symptoms; H) lethal shock; I) change in weight, in %; J) 5 hours before experiment; K) 1 hour before experiment; L) not administered; M) control.

Our attention is struck by the rather weak reaction of the guinea pigs to the reacting injection. Only three of the animals in the group

which received three immunizations exhibited severe symptoms of anaphylaxis. The reactions were considerably milder than those observed among the animals which were sensitized only once.

This appeared even more markedly among the guinea pigs which were treated six times. The only symptoms which the subjects in this group exhibited were those of a very weak anaphylaxis, taking the form of great restlessness, frequent scratching, and biting of the front paws. The insignificance of the reactions noted in this group led us to wonder whether this phenomenon was not a strong immunity caused by the repeated immunization of the animals and whether it was not associated with a large content of the appropriate antibodies.

In order to check this hypothesis we employed artificial addition of diphtheria antibodies in the second series of experiments. We used an equivalent group of guinea pigs which preliminarily received 5 ml of native diphtheria toxoid.

The animals were divided into three groups. The guinea pigs in the first group (20 subjects) were given 3000units of antidiphtheria serum purified by the method developed by Diaferm III of the Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR 5 hours before the basic experiment. The second group of 20 guinea pigs were given the same dose (3000 units) of diphtheria serum one hour before the experiment. The third group (a control group containing 14 subjects) did not receive serum.

Shock was then induced in all of the guinea pigs by intracardiac reacting injections. Some of the subjects received 0.5 ml (in the experiment in question we used only this dose) of concentrated diphtheria toxoid, some received the 6-component polytoxoid, and others were given a mixture of the two preparations; in the latter case the volume of mixture injected was increased to 1 ml.

The symptoms of anaphylaxis recorded for all 54 guinea pigs on induction of shock are shown in Table 2.

In this case the mildest symptoms of anaphylaxis were observed among the guinea pigs of the first group, in whose blood the largest quantity of antibodies circulated. The most severe symptoms were noted in the third group of animals, which did not receive serum and consequently had the lowest titre of antibodies. The second group occupied an intermediate position, having a moderate antibody content in comparison with the first and third groups of guinea pigs.

The data obtained in this experiment indicate that the anaphylactic reactions of animals become less intense as their antibody levels become higher.

As is well known, in M. Roshkovskiy's experiments sensitization became safe when 1/30 unit or more was present in the blood. In our experiments* with purified diphtheria toxoid we did not find any definite relationship between the titres of antibodies and the extent of the anaphylactic reaction.

It is interesting to note that the change in weight observed in the guinea pigs 48 hours after the experiment also reflected their general reaction to administration of the preparation rather indicatively, corresponding fairly precisely to the intensity of the reactions noted. The guinea pigs in the first group exhibited the least loss in weight, actually gaining.

In this experiment the subjects in which shock was induced with the polytoxoid exhibited somewhat milder symptoms than those which received the diphtheria monotoxoid. It is especially important that severe reactions were almost entirely lacking in this group.

In the following, third series of experiments we made a comparative study of the effects of certain chemotherapeutic drugs now in

TABLE 3

Symptoms of Anaphylaxis in Guinea Pigs After Treatment With Various Preparations

A №№ групп	Дополнительное воздействие B		E Кол-во животных	F Общая реакция при шокизации	Признаки анафилаксии G			
	C Препарат	D Доза			H Отсутствие явлений	I Легкие явления	J Тяжелые явления	K Смертельный шок
1	L Норсульфазол	0,3 г Q	10	2	8	2	—	—
2	M Пенициллин	20000 ед. R	10	10	8	—	2	2
3	—.—	40000 ед.	10	5	5	3	2	—
4	N Стрептомицин	10000 ед.	10	1	5	4	1	—
5	—.—	15000 ед.	10	5	5	—	5	1
6	O Кортизон	5 мг S	10	7	4	2	4	3
7	P Контроль на шокизацию		4	2	4	—	—	—

A) Number of group; B) additional treatment; C) preparation; D) dose; E) number of animals; F) general reaction on induction of shock; G) symptoms of anaphylaxis; H) no symptoms; I) mild symptoms; J) severe symptoms; K) lethal shock; L) norsulfazol; M) penicillin; N) streptomycin; O) cortisone; P) shock-induction control; Q) g; R) units; S) mg.

rather wide use on the organism; administration of these drugs may either accompany or precede a course of immunization with certain vaccines.

Various groups of guinea pigs were given norsulfazol, penicillin in two doses (20 and 40 thousand units), and streptomycin in two doses (10 and 15 thousand units). In addition, we administered cortisone, a hormonal preparation having specific characteristics and known to be a drug which reduces anaphylactic symptoms, to the same group.

The observations were made on 64 guinea pigs.

The experiment was conducted in the following fashion. All of the guinea pigs received 5 ml of diphtheria toxoid 40 days before the experiment in order to sensitize them. 4 hours before the experiment the guinea pigs were given the aforementioned preparations. At the time of the experiment shock was induced in all of the guinea pigs by intra-

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cardiac injection of the toxoids, the same dose being used for all of the animals (0.5 ml). Table 3 gives the results obtained.

As may be seen from Table 3, the change produced in the sensitivity of the organism by administration of various chemotherapeutic drugs had a marked effect on the anaphylactic symptoms of the animals which had been sensitized.

While 8 of the guinea pigs in the first group, which received norsulfazol, exhibited no symptoms and only two displayed mild symptoms, the animals in the second, fifth, and sixth groups manifested severe anaphylactic symptoms, extending as far as convulsions, and in some cases the anaphylactic shock terminated in death.

Despite our assumptions, the number of animals in the sixth group which exhibited signs of anaphylaxis was not reduced by the cortisone. Quite the contrary, this group contained the greatest number of reacting subjects and of cases of lethal anaphylactic shock. It is quite probable that this resulted from administration of an insufficient dose of the hormone, a total of 5 mg per guinea pig.

We might incidentally note that when autopsies were performed on the guinea pigs which died of anaphylactic shock we regularly observed a severe hyperemia of the spinal cord in the vicinity of the cervical vertebrae, a phenomenon previously described by one of us (B.G. Trukhmanov*), in addition to the usually recorded pathologoanatomical data: distended white (exsanguinated) lungs and hyperemia of the liver and the large vessels of the abdominal cavity.

In comparing the reactions observed in the third series of experiments as a whole with those noted in the first two series, in animals repeatedly immunized or given hyperimmune serum, we are struck by the considerably greater severity of the anaphylactic symptoms in the animals which were treated with chemotherapeutic drugs, antibiotics, and

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the hormonal preparation.

This draws our attention and is undoubtedly of interest from standpoints other than the study of the allergic properties of certain bacterial preparations. As for the allergic activity of the various toxoids, in these experiments we did not detect any differences between the monotoxoids and the complex polytoxoid used for the reacting injection.

The observations described above enable us to draw the following conclusions:

1. The anaphylactic symptoms produced by administration of toxoids are considerably weaker in organisms which have been repeatedly sensitized (3-6 times).

2. A similar phenomenon is observed when the titre of antibodies in the circulating blood of the experimental animals is artificially increased by a supplementary injection of hyperimmune serum.

3. There is a marked change in the intensity of the allergic symptoms under the influence of various chemotherapeutic drugs (the sulfanilamide norsulfazol, the antibiotics penicillin and streptomycin, and the hormonal preparation cortisone).

4. The monotoxoid and the considerably more complex 6-component polytoxoid do not differ in allergenic properties.

The allergenic properties of the complex bacterial preparations which we studied are thus not intensified when the sensitivity of the organism is altered.

Tomsk Scientific Research Institute for Vaccines and Sera

ACTIVE ANAPHYLAXIS TO ANATOXINS IN CONDITIONS OF CHANGEABLE SENSIBILITY OF THE ORGANISM

Trukhmanov B.G., Tikhonova L.Y.

Anaphylactic properties of mono - and polyanatoxin on guinea

pigs subjected to different influences – repeatedly made sensibility (reduction of intensiveness of anaphylactic phenomena), passive immunization (reduction) and various chemicotherapeutic matters were studied. Diphtheritic monoanatoxin and six-component polyanatoxin did not differ by their allergenic properties and these properties were not intensified in conditions of change of reactivity of experimented animals.

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[Footnotes]

180 B.G. Trukhmanov and L.Ya. Tikhonova, Trudy TomNIIVS [Trans. Tomsk Scientific Research Institute for Vaccines and Sera], 1959, Vol. X, page 136.

183 Trudy TomNIIVS, 1955, Vol. VI, page 332.

185 See page 175 of the present volume.

ARTHUS' PHENOMENON FOR POLYTOXOIDS AND OTHER COMPLEX ASSOCIATED PREPARATIONS

B.G. Trukhmanov

Determination of the degree of safety of bacterial preparations is of undoubtedly great practical interest, particularly when dealing with complex associated vaccines of the polytoxoid type or the even more complex associations of antigens known as multiantigens, which consist of a whole series of toxoids and bacterial and viral vaccines.

In studying these preparations on models of active anaphylaxis [1, 2] and in Shwartzman's hyperergic-reaction phenomenon [3] we demonstrated that there is no essential difference between simple monovalent and complex polyvalent preparations.

We decided to make a comparative characterization of monopreparations, polypreparations, and multipreparations in Arthus' phenomenon, since, as Nikolya recognized, this phenomenon is closely related to the symptoms of general anaphylaxis [4]. The majority of leading immunologists (Zdrodovskiy [5] and Zil'ber [6]) and pathologists (Sirotinin [7], Skvortsov [8], and Ado [9]) now consider it to be a local symptom of an anaphylactic allergy which occurs in the presence of general sensitization of the organism.

It seemed to us that we might obtain a positive Arthus' phenomenon as a result of very prolonged treatment with complex associated vaccines, since we had obtained this on repeated (7-8 times) injection of a single preparation, antiencephalitis vaccine, in work conducted jointly with Yegorshina and Zel'tina [10]. There apparently must also be a definite difference in this respect among preparations of varying complexity as regards the number of individual antigens composing them.

We used 54 rabbits of the same strain in the experiment.

Experimental method. Each rabbit was given an injection of the preparation in a depilated area of the skin every 5 days. As many as

15 individual injections were given. Preparations of varying complexity were tested; these included a monoantigen - diphtheria toxoid, a polyantigen - a six-component polytoxoid consisting of diphtheria, staphylococcus, tetanus, botulin A, perfringens, and edematiens toxoids, and a multiantigen - a 14-component preparation including, in addition to the polytoxoid, enteric tetravaccine (typhoid, paratyphoid B, and Flexner and Sonne dysentery vaccines), listerellosis, pertussis, cerebral encephalitis, and rabies vaccines.

The doses varied from 1 to 3 ml per injection and from 15 to 50 ml over the course of the experiment. In certain cases the preparations were administered intramuscularly and in others subcutaneously. The reaction was evaluated from the extent of dermal hyperemia and infiltration of the subcutaneous cellular tissue. When a positive Arthus' reaction was obtained the inflamed area was measured in millimeters. The rabbits were divided into equal groups by weight.

The experiments were carried out with an increasing degree of preparation complexity. We first checked the possibility of obtaining Arthus' phenomenon in its classic form. For this purpose 12 rabbits were given three intramuscular injections of the aforementioned preparations at 5-day intervals.

Even though we gave as many as 13 injections neither symptoms typical of Arthus' phenomenon nor changes of the ordinary type occurred. Clarification of the difference in the reactogenicities of the complex preparations and the monopreparation, diphtheria toxoid, seemed to merit our attention, especially since the multiantigen contained two preservatives (formalin and carbolic acid). However, during the prolonged sensitization the reactions of the rabbits under investigation, as evaluated from the changes in their weights, did not exceed normal limits. Thus, in the first experiment no special difference was noted

between the weights of the animals which received injections of the monopreparation (A) and those of the animals which received the multipreparation (B). During the day after the first injection the animals in group A lost an average of 133 g, while those in group B lost 117 g. These losses in weight equaled 4.5 and 4% respectively of their mean weights.

During the period between the first and fifth injections the rabbits in group A gained 4 g and those in group B gained 73 g. In the second experiment the gain in weight between the 1st and 13th injections was 380 g for group A and 890 g for group B. This appreciable difference in weight indicates that the multicomponent multiantigen apparently had a somewhat lower reactogenicity than the diphtheria monotoxoid, despite the fact that it included the same quantity of the same diphtheria toxoid.

This fact is related to the previously-observed [11] signs of an unusual interference among the individual reactions caused by the monopreparations composing the complex associated vaccine.

We somewhat complicated the experimental setup for the following group, carrying out preliminary sensitization. When we ran Shwartzman's phenomenon we obtained positive reactions from a large number of the rabbits which had first been subjected to a single administration of the preparations. We were interested in using this phenomenon in the series of investigations in question.

Despite this complication of the experiment, not a single rabbit developed a positive Arthus' phenomenon. Having become convinced that our preparations did not of themselves cause a specific inflammatory reaction, we decided to check the possibility that such a reaction would develop in rabbits subjected to repeated administration of various preparations, including an extremely complex multiantigen.

Arthus' phenomenon was induced in a group of rabbits which had first been sensitized with preparations of varying complexity; these included the four-component tetravaccine, the six-component polytoxoid, and the 14-component multiantigen. All of the rabbits then received a series of intramuscular injections, of the monopreparation (diphtheria toxoid) in some cases and of the multiantigen in others.

After this preparation we attempted to produce a positive Arthus' phenomenon by repeated subcutaneous injections of serum, having in mind the possibility of giving repeated injections of a therapeutic serum to persons first subjected to multiple immunization with various antigens.

Two types of sera were used in the experiment: 1) normal horse serum, series 111, from horses which had never been subjected to any type of immunization; 2) serum from horse No. 147, which had been hyperimmunized with our multiantigen. This multiserum contained various antibodies in rather high titres; more precisely, it contained 600 units of diphtheria antitoxin and 400 units of tetanus antitoxin and had agglutination titres of 1:240 for listerellosis antitoxin, 1:3000 for typhoid antitoxin, 1:3000 for paratyphoid B antitoxin, 1:400 for Flexner dysentery antitoxin, 1:400 for Sonne dysentery antitoxin, etc. Table 1 shows the experimental conditions and the results obtained.

As may be seen from the table, a positive Arthus' phenomenon was recorded for 18 of the 23 rabbits. It must be noted that the number of inflammatory reactions which developed in the group preliminarily sensitized with preparations of varying complexity and in the group which did not receive any initial sensitization was identical. Approximately the same number of reactions occurred in the group which received 5 injections and in the group which received 13 injections of the monopreparation and multipreparation.

TABLE 1

Arthus' Phenomenon in Rabbits Given Various Associated Preparations

A Группы опыта	B № кроликов	C Предварительная сенсибилизация: D К-во компонентов	C Предварительная сенсибилизация: E Доза препарата	F Феномен Артюса с ассоциированными вакцинами: G Препарат	F: H Доза (мл)	F: I Кратность введения	F: J Результат	K Феномен Артюса с сывороткой: L Вид	K: M После какого введения
	91	—	—	Дифанатоксин N	3	13	Гладко Q	147	÷ 2
I	92	—	—	•	•	13	•	•	— —
	93	—	—	•	•	13	•	•	+ 6
		—	—	•	•	13	•	•	+ 2
	93	—	—	Мультиантиген O	3	13	Гладко	147	— —
II	100	—	—	•	•	13	•	•	+ 9
	102	—	—	•	•	13	•	•	+ 2
	96	4	0,2	Дифанатоксин	3	5	Гладко	147	+ 6
	88	4	5,0	•	•	5	•	•	— —
III	90	4	5,0	•	•	5	•	111	— —
	109	6	0,2	•	•	5	•	•	+ 3
	110	6	2,0	•	•	5	•	•	+ 8
	112	6	10,0	•	•	5	•	•	± 12
	95	14	10,0	•	•	5	•	•	+ 3
IV	103	4	2,0	Мультиантиген	3	5	Гладко	147	+ 4
	89	4	5,0	•	•	5	•	•	÷ 13
	92	6	0,2	•	•	5	•	•	+ 2
	97	6	10,0	•	•	5	•	•	+ 2
V	105	14	0,2	•	3	5	Гладко	111	+ 4
	107	14	2,0	•	•	5	•	•	+ 8
	108	14	10,0	•	•	5	•	•	+ 8
	111	6	2,0	•	•	5	•	•	+ 6
	113	4	0,2	•	•	5	•	•	— —
VI	114	—	—	Контрольный P	—	—	—	147	— —
	115	—	—	•	—	—	—	•	— —
	116	—	—	•	—	—	—	111	+ 9
	117	—	—	•	—	—	—	•	— —

Note: Both sera (147 and 111) were administered in a dose of 1 ml.

A) Experimental group; B) number of rabbits; C) preliminary sensitization; D) number of components; E) dosage of preparation; F) Arthus' phenomenon in response to associated vaccines; G) preparation; H) dose (ml); I) number of injections; J) result; K) Arthu. phenomenon in response to serum; L) type; M) after which injection; N) diphtheria toxoid; O) multiantigen; P) control; Q) smooth.

Thus, no special differences among the preparations of varying complexity were detected in this case.

Our attention is struck by the fact that the different sera had

TABLE 2

Antibody Titres and Arthus' Phenomenon in Repeatedly Sensitized Rabbits

A Группа	B Препарат, взятый для сенсибилизации	Титр антител (в АЕ) C		Феномен Артюса F	
		дифтерийных D	столбнячных E	–	+
I	Дифтерийный анатоксин G	2	–	1	3
II	Мультиантиген H	1	1	1	2
III	Дифтерийный анатоксин	2	–	2	5
IV	Мультиантиген	1	1	0	4
V	Мультиантиген	1,5	1,5	1	4

A) Group; B) preparation used for sensitization; C) titre of antibodies (in units); D) diphtheria; E) tetanus; F) Arthus' phenomenon; G) diphtheria toxoid; H) multiantigen.

somewhat different effects. Thus, if we count up all of the cases in which Arthus' phenomenon developed in all groups we find that there were no special differences between the rabbits which received polyvalent immune multiserum and those which were given serum from series 111, which did not contain any antibodies.

However, we are struck by the fact that when the multiserum was used a number (5 groups) of the rabbits began to develop hyperergic inflammation as a result of the second injection. Arthus' phenomenon was recorded no earlier than the third injection on administration of normal horse serum. In general, inflammation of the Arthus' type develops in an unsensitized animal only after repeated (6-7) injections of serum [12]. Our attention is also drawn by the fact that only one of the four control rabbits (No. 116) developed a positive Arthus' phenomenon (the experiments were discontinued after the 14th injection of serum).

It is well known that Arthus' phenomenon is a complex process and is a local manifestation of a sharp change in general reactivity. It is based on antigen-antibody interaction.

The data shown in Table 2 were obtained by comparing the titres of diphtheria and tetanus antitoxins determined for the various groups of experimental rabbits with the number of positive Arthus' reactions.

The data shown in Table 2 do not indicate any relationship between the high titre of antibodies in the animals investigated and the positive Arthus' phenomenon. However, we must take into account the fact that the rabbits did not exhibit large variations in antibody content and titres of 1, 1.5, and 2 units may be considered to be of approximately the same order. The sharp decrease in antibody titres in these rabbits after Arthus' phenomenon developed is very interesting.

CONCLUSIONS

1. Arthus' phenomenon did not develop in experiments involving repeated administration (as many as 13 injections) of various vaccines of the multiantigen type, including very complex preparations, to rabbits.

2. We were able to obtain Arthus' phenomenon under complicated conditions, against a background of preliminary sensitization by supplementary injection of horse serum.

3. Arthus' phenomenon began to develop somewhat earlier when multivalent hyperimmune serum was used in this experiment than when serum lacking any antibodies was used.

4. The complex of various preparations (multiantigen) did not differ in reactogenicity, as determined from the subjects' change in weight on repeated administration, from ordinary bacterial monopreparations of the diphtheria-toxoid type.

5. There was no essential difference between the frequency with which the Arthus reaction developed when simple monoantigens were used and when very complex associated vaccines were administered; this again

demonstrates the ordinary reactogenicity of the latter.

Tomsk Scientific Research Institute for Vaccines and Sera

ARTJUS'S PHENOMENON WITH POLYANATOXINS AND OTHER COMPLICATED ASSOCIATED VACCINES

Trukhmanov B.G.

By studying new complicated associated vaccines of multicomponent polyanatoxin type and still more complicated preparation – multiantigen consisting of 15 and more separate monoantigens the author tried to get by their usage Artjus's phenomenon. One succeeded in obtaining positive reduction only at very complicated test conditions by additional introducing of horse serum. Two serums-normal horse's and multiserum containing above eight various antibodies behaved themselves differently.

Principle difference between monopreparations and complicated complexes of their type of multiantigen in various test conditions was not defined.

MENINGOCOCCAL TOXIN AND ITS DERIVATIVES IN SHWARTZMAN'S PHENOMENON

B.G. Trukhmanov

In studying thermolabile meningococcal toxin [1, 2] we were able to show that it contains two toxic substances with different properties. One, the more labile, resembled a true exotoxin and the second had the characteristics of an endotoxic substance. The clearest difference between them was found to relate to the temperature factor.

The presence of these two toxic fractions in very different proportions in the filtrate from a bouillon culture of meningococcus determined the properties of the meningococcal toxin and the meningococcal toxoid obtained from it [3].

We were forced to reckon with the existence of two fractions in producing an experimental model of cerebrospinal meningitis in rabbits [4] and in evaluating the properties of the meningococcal toxin from the changes in the dermal reactions of animals during immunization [5].

We were interested in using Shwartzman's model of the hyperergic inflammatory reaction to determine whether there were any differences between the individual fractions of the meningococcal toxin. At the same time, we decided to employ this phenomenon to clarify the difference between the toxins of the two basic types of meningococcus, A and B (according to Nikolya's classification).

Our observations were conducted on rabbits, principally of the chinchilla strain, with average weights of 2 kg. The first experiments

(which were conducted in 1938) showed that it is quite possible to obtain a positive Shwartzman's phenomenon with meningococcal toxin and toxoid.

Since we had set ourselves the task of making a comparative characterization of meningococcal toxin and its derivatives, we had to obtain a positive Shwartzman's phenomenon to at least 3-4 preparations administered simultaneously to the same animal. We found no appropriate information in the literature. However, after some work with the dosages, we were able to obtain Shwartzman's phenomenon unfailingly at four (see Fig. 1) or more points on intracutaneous injection of the preparations, provided that the latter had the necessary activity in preparing the skin for the development of hyperergic inflammation.

Fig. 1

The following preparations were tested on 50 rabbits in the basic experiments:

1) meningococcal toxin consisting of the native filtrate from a 7-day-old culture of meningococcus strain A 74 cultured on a special MMT (Martin's meningococcal toxin) medium containing 1% glucose and 0.5% sodium acetate;

2) meningococcal toxoid consisting of the formalized toxoid obtained from meningococcal toxin by treating it with formalin (0.4-0.6%) at 40°C;

3) the thermolabile fraction of meningococcal toxin, obtained from the first preparation by purification and concentration by Ando-Verzhikovskiy's method, which is used to purify Streptococcus scarletinae toxin [6];

4) the thermostable fraction of meningococcal toxin, obtained by simple heating of the first preparation at 80° for 30 minutes;

5) similar preparations obtained from meningococcal toxin type B (meningococcus strain B 641).

In certain experiments we tested scarletina toxin which we obtained for the production of antiscarletina therapeutic serum and standard scarletina toxi.. series 165a, produced by the All-Union Institute of Epidemiology and Microbiology as original controls.

In order to illustrate the experimental setup, the materials employed, and the results obtained, Table 1 represents almost the whole of one of the experiments, which was conducted on 5 rabbits.

TABLE 1

Shwartzman's Phenomenon With Meningococcal Toxin and its Fractions

A Препарат / B Учет	C Кролик 524		Кролик 531		Кролик 528		Кролик 532	
	D через 24 часа	E через 48 часов	через 24 часа	через 48 часов	через 24 часа	через 48 часов	через 24 часа	через 48 часов
	F кожная реакция	G феномен Шварцмана	кожная реакция	феномен Шварцмана	кожная реакция	феномен Шварцмана	кожная реакция	феномен Шварцмана
H а) внутрикожно								
I Очищенный токсин серия 14 — 15 л.	эритема O	—	эритема	—	эритема	++ 25	эритема	—
J Токсин, серия 21 А-74	эритема P + инф.	++ 12	•	—	•	# 40	•	# 25
K Анатоксин, серия 21А-74	•	+ 5	•	—	эритема + инф.	± 15[3])	эритема + инф.	±[3])
L Токсин 21 А-74 инактивированный	•	+ 5	•	—	•	# 35	эритема	+ 10
M Токсин, серия 21 В-641	•	—	•	—	•	# 20	эритема + инф.	+ 5
N Токсин, серия 24 А-787	•	+++ 20	•	—	эритема	# 35	•	# 35
б) внутривенно Q	Менингококковый токсин 21 А-74 R				Менингококковый токсин 21 А-74 инактивированный		S	

Notes: 1) The intensity of the Shwartzman's phenomenon is represented by crosses and ranges from a slight reaction of the ecchymotic type (+) to a very severe necrotic hemorrhagia (#); 2) a not fully developed reaction, an icteric papule; 3) the figures in millimeters represent the mean of the two diameters (length by width); 4) rabbit No. 531 was not ill when Shwartzman's phenomenon was investigated and exhibited no reaction whatsoever.

A) Preparation; B) investigation; C) rabbit; D) after 24 hours; E) after 48 hours; F) dermal reaction; G) Shwartzman's phenomenon; H) intracutaneously; I) purified toxin, series 14-15 l; J) toxin, series 21 A-74; K) toxoid, series 21 A-74; L) inactivated toxin, series 21 A-74; M) toxin, series 21 B-641; N) toxin, series 24 A-787; O)erythema; P) erythema and infiltration; Q) intravenously; R) meningococcal toxin 21 A-74; S) inactivated meningococcal toxin 21 A-74.

As may be seen from Table 1, a positive Shwartzman's phenomenon was obtained in response to both types of meningococcal toxin, both in homotypal (type A intracutaneously and type A intravenously) and heterotypal (A intracutaneously and B intravenously or vice versa) combination. It is possible that this resulted from the fact that, according to V.L. Troitskiy's data, meningococcus type B contains a certain quantity of antigen A [7, p. 49].

The meningococcal toxoid also yielded a positive reaction. Toxin purified by Ando-Verzhikovskiy's method did not cause a hyperergic reaction.

The most marked Shwartzman's phenomenon was noted when toxin inactivated by heating was used for the reacting injection. The thermostable fraction apparently is better preparation for and acts more decisively in the inflammatory reaction.

We must point out that in a whole series of cases the true Shwartzman's phenomenon, and ecchymosis of an almost black color, did not develop; however, there was an undoubted tissue (skin and subcutaneous cellular tissue) reaction in the form of a limited dense infiltration of the papular type, which was yellowish in color. We got the impression that somewhat different tissue elements, which could not cause a true hyperergic reaction to develop, participated in this formation.

When the rabbit died dissection showed that the same unusual inflammation characteristic of a Shwartzman's phenomenon fully developed at the dermal surface always occurred beneath these areas of the skin. In our laboratory we designated this type of underdeveloped reaction by $\pm$ and noted it as an "icteric phenomenon." A positive Shwartzman's phenomenon was indicated by plus signs, while a severe reaction with subsequent necrosis was designated by four crosses (++++).

Our attention is struck by the fact that Shwartzman's phenomenon

did not develop and the reaction took a somewhat different course when the rabbit became sick, particularly when it subsequently died.

We must emphasize the frequently observed potentiating action of the toxin injected intracutaneously on the toxin administered intravenously. In a number of cases, while setting up Shwartzman's phenomenon, intravenous injection of a dose of inactivated meningococcal toxin known to be nonlethal after intracutaneous administration of small quantities of toxin killed the rabbit. The considerable attenuation of Shwartzman's reaction in preliminarily immunized animals also merits further study.

TABLE 2

Composite Data on Cross-Administration of Meningococcal Preparations in Shwartzman's Phenomenon

A Группы	B Подготавливающая инъекция (внутрикожно) \ C Разрешающая инъекция (внутривенно)	D Токсин типа A						E Токсин типа B					
		нативный F			инактивированный G			нативный			инактивированный		
		+	±	−	+	±	−	+	±	−	+	±	−
I	Препараты типа A H												
	1. Нативный токсин I	13	0	4	9	1	1	4	2	4	1	0	0
	2. Инактивированный G	9	1	1	4	1	0	1	1	2	1	0	0
	3. Очищенный по Андо J	0	1	11	2	0	3	1	0	4	0	1	0
	4. Анатоксин K	1	2	1	5	2	1	1	2	2	1	0	0
II	Препараты типа B L												
	1. Нативный токсин I	2	0	3	4	0	1	6	3	2	1	0	0
	2. Инактивированный G	1	0	0	1	1	0	0	0	0	1	0	0
	3. Анатоксин K	0	0	0	0	0	0	0	0	0	0	0	0
III	Скарлатинозный токсин M	1	2	4	0	0	3	0	0	5	0	0	2

A) Group; B) sensitizing injection (intracutaneous); C) reacting injection (intravenous); D) toxin type A; E) toxin type B; F) native; G) inactivated; H) preparations type A; I) native toxin; J) purified by Ando's method; K) toxoid; L) preparations type B; M) scarletina toxin.

Table 2 presents data on the Shwartzman's phenomenon obtained with toxins of various types used in different states: unaltered native toxin and toxin inactivated by heating at 80° for 30 minutes. It must be noted

that the table does not reflect the absence of positive reactions to all of the preparations when meningococcal toxins purified by Ando-Verzhikovskiy's method was used for the reacting injection.

At the same time, it follows from the data in Table 2 that the inactivated toxin, which we believe to be identical to its thermostable fraction, proved to be very active. Thus, for example, using this toxin we twice obtained a positive reaction to the purified toxin and got positive results with meningococcal toxoid 5 times.

It is interesting that we obtained a positive Shwartzman's phenomenon with Streptococcus scarletinae when it was used for sensitization to meningococcal toxin by intracutaneous injection.

We were unable to obtain the phenomenon with scarletina toxin, using it for both the sensitizing and reacting injections.

CONCLUSIONS

1. A marked Shwartzman's phenomenon (fully-developed necrotic hemorrhagia) was obtained with meningococcal toxin and its derivatives.

2. Shwartzman's phenomenon was obtained with toxins of various types in both homotypal and heterotypal combinations.

3. This model also showed that there is a difference between the two fractions of meningococcal toxin; its thermostable fraction has a greater activity in Shwartzman's phenomenon.

4. We were able to obtain a marked hyperergic reaction at 4 or more points on the skin of a single rabbit.

5. It is necessary to keep records and make observations until the end of the development of the reaction, when the inflammatory process persists as an unusual infiltration with a strongly yellowish color.

6. In a number of cases intravenous injection of a dose of heat-inactivated meningococcal toxin known to be nonlethal after intracutaneous injection of small quantities of the toxin caused the animal

to die.

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MENINGOCOCCUS TOXIN AND ITS DERIVATIVES IN SHWARTZMAN'S PHENOMENON

Trukhmanov B.G.

In Shwartzman's phenomenon received in some points on one and the same rabbit a maningococcidal toxin was tested its thermostabile and thermolabile (received by purifying after Ando-Vergikovskiy) fractions and meningococcus anatoxin as well, the most active being thermostabile fraction. Shwartzman's phenomenon was received in cross-experiments independently upon the type of meningococcus.

Interesting facts of potentiation of toxins and weakening of reaction by sick persons and immunized rabbits were noted.

SHWARTZMAN'S PHENOMENON WITH MENINGOCOCCAL PREPARATIONS IN THE PRESENCE OF ALTERED SENSITIVITY

B.G. Trukhmanov

In experiments on the induction of Shwartzman's phenomenon with meningococcal toxin and its fractions we noticed that the hyperergic reaction developed somewhat differently when the sensitivity of the experimental animal was altered.

The goals of the work described herein were to induce Shwartzman's phenomenon in the presence of preliminary active or passive immunization and to verify the possibility of repeatedly obtaining a marked phenomenon in the same animal.

Our observations were made on 48 rabbits* and 15 guinea pigs. In the first experiment we checked the importance of preliminary immunization in 9 rabbits which had first received meningococcal toxin and 10 rabbits used as controls and not immunized in any way. Shwartzman's phenomenon developed in the rabbits of both groups.

Both types of toxin proved to be active when administered intracutaneously, although the heat-inactivated toxin yielded a somewhat more intense reaction. Meningococcal toxoid was found to be the least active, especially when it was used for the reacting injection.

We noted a definite difference in the reactions of the immune and nonimmune rabbits; thus, of the 36 points at which the immunized animals received intracutaneous sensitizing injections Schwartzman's phenomenon developed at 11 (less than 1/3), while of the 40 corresponding points

in the control animals the reaction was positive in 23 cases (somewhat more than 1/2).

TABLE 1

Shwartzman's Phenomenon in Rabbits Preliminarily Given Meningococcal Serum

A №№ кроликов	B Предварит. введение сыворотки в мл	C Феномен Шварцмана на местах внутрикожного введения: D токсин: F нативный	D токсин: G инактивированный	E анатоксин: нативный	E анатоксин: инактивированный	Примечание H
3911	10	+++ 35	## 40	+++ 25	—	
4033	"	## 50	## 30	+++ 25	## 15	
4044	"	—	—	—	—	болен I
4047	"	—	—	—	—	болен
4065	"	—	## 25	—	± 15	
4075	"	## 55	## 60	## 30	## 40	
3292	—	± 20	+ 27	± 20	++ 22	
3999	—	## 15	+++ 20	++ 25	++ 20	
4068	—	++ 15	## 15	++ 15	++ 10	болен

Explanation of Table: 1) The intensity of the Shwartzman's phenomenon is expressed by plus signs and ranged from a slight ecchymosis (+) to a severe hemorrhagic necrosis (##); 2) the numerals indicate the mean diameter of the hyperergic inflammatory reaction, expressed in millimeters.

A) No. of rabbits; B) preliminary administration of serum, in ml; C) Shwartzman's phenomenon at points of intracutaneous injection; D) toxin; E) toxoid; F) native; G) inactivated; H) notes; I) became ill.

It is interesting to note that in many of the immunized rabbits intracutaneous injection of the preparations did not cause the ordinary reaction, erythema and infiltrations, as well as that there was no positive Shwartzman's reaction in the five rabbits which received intracutaneous doses of the toxin so large that they soon died.

In the second series of investigations the animals were given 10 ml of meningococcal serum before Shwartzman's phenomenon was induced. After two days they were given 0.5 ml of the following preparations intracutaneously at four points: 1) native meningococcal toxin; 2) the same

toxin inactivated by heating at 80° for 30 minutes; 3) meningococcal toxoid; 4) the same toxoid heated at 80° for 30 minutes. All four preparations were of the same type (A).

One day later all of the rabbits were given 1.5 ml of heat-inactivated meningococcal toxin intravenously, since it had proved to be the most active in our prior experiments. In the first experiment none of the 9 rabbits had been treated previously in any way, while in the second experiment five of the 10 rabbits had been the subjects of a similar investigation. By way of illustration we may cite data on the development of Shwartzman's phenomenon in the first group of rabbits (Table 1).

In this case a hyperergic reaction developed at almost all of the points at which the preparations were injected intracutaneously. The reaction was quite intense, the hyperergic inflammation extending for as much as 60 mm in a number of cases (covering an area 6 cm long and 6 cm wide). It must be noted that in this series of observations Shwartzman's phenomenon developed more intensively in those areas where the skin had been prepared by injection of inactivated toxin. Rabbits Nos. 4044 and 4047, which became sick after intravenous injection of the preparation, did not yield any reactions whatsoever.

The severe hyperergic reaction which occurred in the rabbits after induction of passive immunity apparently resulted from the artificial increase in the quantity of antibodies in their circulating blood.

We attempted to utilize this to obtain Shwartzman's phenomenon in the guinea pigs, which under ordinary experimental conditions never reacted to administration of the same preparations which always caused a positive phenomenon in the rabbits.

The experiment was performed on 15 guinea pigs, 5 of which preliminarily received meningococcal toxin, 5 inactivated meningococcal

toxin, and 5 antitoxic meningococcal serum series 43. However, Shwartzman's phenomenon was obtained in only one guinea pig of the latter group, in a very small area of the skin.

In the third experiment Shwartzman's reaction was induced in 16 rabbits 4 times, each of the intracutaneous injections being given in the same area of skin used previously.

Table 2 shows the results of the first and fourth inductions of Shwartzman's phenomenon. As may be seen from the table, the intensity of the reaction obtained decreased markedly in the fourth experimental setup, the phenomenon developing in only two of the rabbits and then not in all of the intracutaneously-prepared areas; where Shwartzman's phenomenon did develop the area involved in the reaction was small.

Even the heat-inactivated meningococcal toxin, which is the most active in Shwartzman's phenomenon, did not cause a true hyperergic reaction passing into necrosis.

This is a very interesting fact and indicates that some sort of reorganization apparently occurred in this area of the animal's skin, leading to an attenuation of the reaction. We must suppose that the general immunity and the quantity of antibodies in these rabbits was undoubtedly sufficient, since they received a considerable amount of the preparations during the observation period and were sufficiently immunized.

On the basis of all the experiments conducted we may draw the following conclusions:

1. Preliminary immunization reduces the number of rabbits which react positively in Shwartzman's phenomenon with meningococcal preparations.

2. A sharp intensification of Shwartzman's phenomenon, extending both to the number of areas involved in the process and to the strength

TABLE 2

Shwartzman's Phenomenon Induced Four Times With Meningococcal Preparations

A	Реакция на местах внутрикожных инъекций B																	
	В первом опыте C									В четвертом опыте J								
№ кролика	Токсин A D		Анатоксин A E		Инакт. токсин A F		Токсин B G		№ кролика	Токсин A		Анатоксин A		Инакт. токсин A		Токсин B	
	H ф. Шв.	I некроз	ф. Шв.	некр.	ф. Шв.	некроз	ф. Шв.	некроз		ф. Шв.	некроз	ф. Шв.	некроз	ф. Шв.	некроз	ф. Шв.	некроз
102	+++ 15	+	++ 18	+	++ 18	+	++ 25	+	102	—	—	—	—	—	—	—	—
104	—	—	—	—	—	—	—	—	104	—	—	—	—	—	—	—	—
107	++ 20	+	+++ 20	—	++ 27	+	++ 27	+	107	+++ 9	—	±	—	± 18	—	+++ 25	—
135	—	—	—	—	—	—	—	—	137	++ 15	—	±	—	++ 10	+	++ 15	—
137	++ 17	+	++ 25	+	++ 8	+	++ 13	+	138	±	—	—	—	±	—	—	—
138	++ 27	+	++ 24	+	++ 17	+	++ 30	+	140	—	—	—	—	—	—	—	—
140	++ 25	+	+++ 25	—	+++ 25	—	++ 27	+	148	—	—	—	—	—	—	+++ 6	—
148	++ 15	+	+++ 20	—	+++ 15	+	++ 20	+	133	—	—	—	—	—	—	—	—
134	++ 30	0	++ 26	0	++ 40	0	++ 37	0	163	—	—	—	—	±	—	±	—
136	++ 20	0	++ 10	0	++ 10	0	++ 20	0	179	++ 12	+	—	—	++ 15	+	+++ 10	—

Notes: 1) Rabbits Nos. 104 and 135 died after intravenous injection of inactivated toxin; 2) Shwartzman's phenomenon was induced three times in rabbit No. 133 and two times in rabbit No. 163.

A) Number of rabbit; B) reaction at site of intracutaneous injection; C) in first experiment; D) toxin A; E) toxoid A; F) inactivated toxin A; G) toxin B; H) Shwartzman's phenomenon; I) necrosis; J) in fourth experiment.

of the inflammatory reaction, is observed in the presence of passive immunity.

3. On repeated induction of Shwartzman's reaction a marked decrease in the intensity of the reaction occurs in the fourth experiment.

4. In these experiments we also noted that toxins administered intracutaneously and intravenously have a potentiating action, this leading in a number of cases to death from doses absolutely nonlethal for unsensitized animals.

5. The rabbits which became sick after intravenous injection of the toxin generally exhibited no positive Shwartzman's phenomenon,

lthough they occasionally displayed a more intense local reaction to ntracutaneous administration of the preparations.

6. The various meningococcal preparations caused the same reaction hen the sensitivity of the organism was altered as under ordinary ex- erimental conditions.

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SHWARTZMAN'S PHENOMENON WITH MENINGOCOCCI PREPARATIONS IN CONDITIONS OF CHANGEABLE SENSIBILITY OF ORGANISM

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In conditions of changeable animals' reacitivity the intensiveness of Shwartzman's phenomenon was changed. Its sharp intensification was observed at passive immunization due to introducing anti-meningococcus serum (meningococcus antiserum); weakening – at active immunization of animals and at reproduction of phenomenon for the fourth time at one and the same parts of the skin. Various meningococci preparations produced the same reactions as in general conditions of the experiment.

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[Footnote]

203 For a description of the method, see page 197 of the present volume.

CHANGE IN THE TITRE OF COMPLEMENT DURING STORAGE*

N.O. Sukhova

The complement-fixation reaction is widely used for diagnosing diseases such as syphilis, gonorrhea, glanders, ricketsiosis, brucellosis, influenza, tick-borne encephalitis and a number of other virus infections. This is explained by the increasing demand for the specific and nonspecific ingredients necessary to produce a reaction.

The complement which contains the greatest volume of nonspecific components is that which laboratories obtain from institutes of venerology, those for vaccines and sera, or those of epidemiology and microbiology.

One difficulty in the normal supplying of this preparation to consumers is the limited useful life (2 months) established by the working instructions.

On the one hand, this short useful life creates a difficult situation for the producing institutes, especially in Siberia, where it is impossible to make regular shipments of drugs because of the winter frosts; it consequently becomes necessary to replace series which have been produced but not shipped in time, so that the complement reaches the consumers with the requisite amount of useful life to spare. On the other hand, this makes it impossible for the consuming institutions to store complement during the winter.

In order to eliminate these abnormalities the majority of complement-producing institutes now manufacture it in dry form, by vacuum

freezing.

According to an investigation carried out by T.S. Sil'chenko (1955), dry complement retains its activity for up to a year and a half.

However, not all of the complement-producing institutes have the necessary equipment for drying; we consequently deemed it important to make observations on the maintenance of activity in native complement during storage. The fact that different opinions had been expressed in the literature on the change in the activity of complement during storage was an additional spur. While A.P. Nevodov (1927) showed that it is possible to store preserved complement for up to 1-1.5 months, V.S. Kalinin (1942) increased this time to 3 months, provided that the preparation is refrigerated.

We preserved the complement (guinea pig blood serum) with 4% twice-recrystallized boric acid and 5% sodium sulfate. Between the time when it was bottled and the second check the preparation was kept in a refrigerator at +4°. The observations were made on 35 series of complement, 21 of which were checked after different storage periods (from 8 to 18 months).

The data obtained in these investigations showed that of the 21 series of complement stored for from 8 to 18 months the titre of complement remained unchanged in 11 series, decreased slightly (by 0.01-0.02) in 5 series, and dropped considerably (by 0.04-0.05) in the remaining series.

Three series of complement were checked monthly for a year and a half. These observations showed that a slight change in the titre of complement was noted after 7 months of storage in one series, after one year in a second series, and toward the middle of the second year in a third series. 11 series of complement were tested twice over a one-year period. At the end of the year 7 series exhibited no change in

titre, but 4 displayed a slight decrease in titre after 6 months of storage, although they remained suitable for the complement-fixation reaction.

The data cited show that the initial titre of correctly stored complement usually does not change before 6 months have elapsed.

THE CHANGE OF COMPLEMENT TITRE DURING ITS KEEPING

Suchova N.O.

The activity of liquid complement at keeping it in refrigerator (4-5°C) was examined, the titre being kept no less than six months.

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[Footnote]

209 Senior laboratory technician T.P. Andreyeva took part in the work.

REPEATED IMMUNIZATION OF RABBITS IN PRODUCTION OF STANDARD FLEXNER DYSENTERY AGGLUTINATIVE SERA

N.O. Sukhova

In accordance with the current instructions for the storage of the intermediate products for agglutinative sera single-cycle immunization of rabbits followed by total exsanguination is now employed. Despite the unprofitableness of the monocyclic regime of animal exploitation, this method of serum production has been retained because of the existing opinion that animals have an extremely low production of group antibodies during the first cycle of immunization.

This report describes certain results obtained in the repeated immunization of rabbits with standard strains of Flexner dysentery bacteria.

The experiments on multicyclic immunization were carried out on rabbits, which, according to the observations of G.V. Vygodchikov (1943), S.A. Zelevinskaya (1935), M.I. Fedorovich (1949), and others, yield a more specific serum than other species of animals (sheep, goats, and horses).

18 rabbits (Vienna blue) were immunized, their weight varying from 3.1 to 3.68 kg. The animals were divided into six equal groups. The first group was immunized with strain 266 of Flexner dysentery bacteria type a, the second with strain 1964 of type b, the third with strain 538 of type c, the fourth with strain 414 of type d, the fifth with strain 224 of type e, and the sixth with strain 324 of type f.

The morphological, biochemical, and serological properties of the strains were determined before each immunization cycle. Bacteria killed by heating in a water bath (at 56° for one hour) were used as the antigen. Three intravenous injections were given at intervals of 5 or 6 days, the doses being 0.2, 0.4, and 0.8 ml.

Production phlebotomies were performed on the 6th and 7th days after the final injection of each cycle. The two phlebotomies yielded from 95 to 120 ml of blood, or 50-65 ml of serum, from each rabbit.

It must be noted that the data available in the literature (F. Z. Azarginova, L.Ye. Khudanov, and Ye. D. Shkuro 1956) on the quantity of serum which may be obtained from a single rabbit (15-20 ml) are extremely low and do not correspond to the actual situation. This small serum output may be attributed to the use of an insufficiently correct phlebotomy technique.

The experimental animals were again immunized after an interval of one month. All of the rabbits were subjected to six immunization cycles.

Analysis of the data obtained shows that immunization of animals with killed bacteria, the appropriate standard strains being employed in all immunization cycles (including the first), causes the formation both of antibodies both to the serological type of Flexner dysentery bacteria used for immunization and of grouped antibodies to strains of other serological types.

In the subsequent immunization cycles we were able to obtain serum with a higher titre than that produced after the first cycle. However, the titres of specific and group antibodies do not increase uniformly during the subsequent immunization cycles.

Thus, immunization of rabbits with strain 266 of Flexner dysentery bacteria of serological type a, enabled us to obtain a serum which was

sufficiently specific from the first to the sixth immunization cycles (the titre of antibodies to strains of the serological type used for immunization was no less than 20 times the titre of group antibodies).

A different picture is presented by the increase in antibody content on repeated immunization of rabbits with antigen prepared from strain 1964 of Flexner dysentery bacteria of serological type b. While the titre of antibodies to strains of serological type b was higher than that of group antibodies to strain 538 of serological type c after the first immunization cycle, the titres of antibodies to a strain of serological type c in the serum obtained from the subsequent cycles were just as high as those to a strain of type b and the titres of antibodies to other serological types of Flexner dysentery bacteria were slightly higher.

The lowest titre of antibodies in this immunization was to strains of Flexner dysentery bacteria of serological type f (1:200 after the third immunization cycle).

On immunization of rabbits with strain 538 of serological type c there was an increase in the content of group antibodies during the subsequent immunization cycles. While the increase in antibodies to strains of serological types d and e corresponded to the increase in the titre of specific antibodies, the rise in the content of antibodies to strains of serological types b and a was excessive, especially with respect to type b.

After the first and subsequent immunization cycles carried out with strain 414 of serological type d, the greatest formation of group antibodies was to serological type e (strain 224).

The blood serum of the animals immunized with strain 224 of serological type e contained group antibodies to strains of Flexner dysentery bacteria of all serological types, but the highest antibody con-

tent was to the strain of serological type d. The serum obtained from immunization with strain 324 of type f contained rather high titres of group antibodies to strains of all serological types, but the highest were to the strains of types d and e; this relationship was maintained throughout the course of the prolonged multiple immunization.

If we appraise the ability of a culture of a certain serological type to cause the formation of large numbers of group antibodies to another serological type as a consequence of their antigenic affinity, we must consider types b and c and types d and e to be the most similar serological types of Flexner dysentery bacteria.

The affinity between the aforementioned types of Flexner dysentery bacteria, detected in the experiments which we performed, may be taken into account in preparing antigens for the immunization of animals in producing standard agglutinative sera for this bacterium.

CONCLUSIONS

1. Repeated immunization of rabbits with standard strains of Flexner dysentery bacteria makes it possible to obtain a serum with a high antibody content, in contrast to monocyclic immunization.

2. Repeated immunization may be used as a method of producing intermediate products for the manufacture of standard specific agglutinative sera for Flexner dysentery bacteria with those antigens which do not cause a considerable increase in the formation of group antibodies when administered in successive immunization cycles.

Tomsk Scientific Research Institute for Vaccines and Sera

ABOUT REPEATED IMMUNIZATION OF RABBITS AT PREPARING STANDARD DYSENTERIC FLEXNER'S AGGLUTINATING SERUMS

Suchova N.O.

One cycle and multicycle immunization of rabbits at getting ag-

glutinating dysenteric standard serums was compared. Repeated immunization by some standard strains is recommended.

ANTIMICROBIAL PROPERTIES OF NORMAL HUMAN AND ANIMAL SERA

(Report 4)

N.V. Vasil'yev

The problem of the nature of the antimicrobial properties of normal human and animal sera, one which has a considerable history, has recently begun to attract the attention of Soviet and foreign immunologists once more. Louis Pillemer's discovery of the properdine system forced a reconsideration of our customary, seemingly secure concepts of the mechanism by which complement and normal antibodies act. In this connection it is regrettable that the thermostable antimicrobial elements of normal sera such as leukin, betalysin, lysozym, and a number of others, which were discovered long ago but on which little research has been done, are still only vaguely known. Now, just as several years ago, the statement which L.A. Zil'ber made in 1947 to the effect that the true role of these substances in immunity has not yet been determined is still true. It must be noted that in the 3rd edition of Principles of Immunology, which appeared in 1958, Zil'ber was more reserved in speaking of these substances and even generally questioned the role of certain of them in the processes of immunity.

Four years ago, attempting to fill in this gap to some extent, we began to study certain properties of the thermostable elements of normal sera.

In order to determine whether or not the role of a certain biological characteristic is important it is first of all necessary to study its occurrence in nature. From the evolutionary standpoint we may state a priori that if a certain biological property is encountered in a considerable number of animal or plant species (particularly if these are far apart on the evolutionary scale) it is certainly an essential adaptive characteristic, since it is otherwise inconceiveable that it should be stably maintained during the evolution of species. Hence we may conclude that if the presence of thermostable antimicro-

bial elements in blood serum is a common phenomenon in nature their role in immunity must undoubtedly be considerable.

In our prior reports we were able to show that, according to the data in the literature, the serum of rabbits, sheep, and man contains thermostable substances which are apparently similar to Peterson's betalysin.

We set ourselves two goals in the work described herein: first, to study the extent to which these elements occur in various species of animals and, second, to check their action spectra for various types of microbes.

The method used in this work is described in our article in Vol. X of the "Transactions of the Tomsk Scientific Research Institute for Vaccines and Sera."

We studied sera from 39 horses, 3 cows, 3 goats, and 20 humans, the latter being blood donors. The tests were performed with day-old cultures of Bacillus subtilis, M. lysodeicticus, Bacterium coli, Proteus morgani, staphylococcus albus, Sarcina aurea, B. brevis, Saccharomyces rubrum, Bacillus pseudoanthricus, Pseudomonas aeruginosa, Bacterium prodigiosum, B. faecalis alcaligenes, Vibrio metchnikovii, and Vibrio phosphorescens. A total of 65 experiments comprising 410 individual observations were conducted.

In studying the activity of unheated horse sera we established that, under experimental conditions, they are usually active in dilutions of 1:4 or more with respect to Bacillus subtilis, Saccharomyces rubrum, Bacillus pseudoanthracis, M. lysodeicticus, Sarcina, and B. faecalis alcaligenes. The sera acted most regularly on Bacillus subtilis and Saccharomyces rubrum. Inactivation of these sera by heating at 56° C for 30 minutes reduced their activity with respect to certain microorganisms (Bacillus subtilis, Bacillus pseudoanthracis, Saccharo-

myces rubrum, and M. lysodeicticus) and completely eliminated their activity with respect to others (Staphylococcus albus and B. faecalis alcaligenes). The extent to which the activity of the sera decreased on heating differed, frequently amounting to 50% or more.

More or less similar results were obtained in studying the antimicrobial properties of the sera from the cows and goats. Just as the horse sera, those from the cows and goats had their most marked effect on Bacillus subtilis, M. lysodeicticus, and V. Metchnikovii. In addition, they were active in a dilution of 1/10 with respect to B. morgani, Sarcina lutea, and certain others. Destruction of the complement considerably reduces the effect of the sera on Bacillus subtilis, Bacillus pseudoanthracis, and Saccharomyces rubrum and, under experimental conditions, greatly attenuates their action on M. lysodeicticus (Fleming strain). It is interesting to note that in certain cases the sera not only fail to suppress the growth of the microbes but, quite the contrary, stimulate their vital activity. Thus, Pseudomonas aeruginosa did not form pigment on ordinary meat-extract bouillon, but copious pigment formation was observed in the presence of both heated and unheated bovine sera.

The canine sera were tested only for their action on Bacillus subtilis and M. lysodeicticus. It was found that both unheated and heated sera markedly suppress the growth of these microbes, while heating the sera causes a drop in their antimicrobial titres, just as in other species of animals.

As a supplement to our previously published data we expanded our observations on the antimicrobial properties of human serum. The data obtained in these experiments confirms the material which we published earlier. Unheated donor sera have a marked effect on Bacillus subtilis, Bacillus pseudoanthracis, M. Lysodeicticus, Saccharomyces rubrum, B.

rgani, V. metchnikovii, Sarcina lutea, etal. The inactivated sera e active with respect to Bacillus subtilis, Bacillus pseudoanthracis, ccharomyces rubrum, and M. lysodeicticus, although to a far lesser tent than the heated sera.

In analyzing the data obtained we first came across the following gularity: unheated and inactivated sera from various species of ani- als, including species rather far apart on the evolutionary scale, ave marked antimicrobial properties with respect to a number of micro- rganisms. Both the intensity and range of this effect may vary in ifferent species and individuals, but it is most constant with respect o Bacillus subtilis, Saccharomyces rubrum, and M. lysodeicticus.

Under experimental conditions this effect for the most part had bacteriostatic character; the microbes were observed to grow in tests nvolving high serum concentrations, but this growth began later and as less marked than in the control tests. We were unable to find any pecial selective specificity of this effect with respect to M. ly- odeicticus; although heated and unheated sera attenuated its growth n a number of cases, they also affected certain other microbes such s Saccharomyces rubrum in the same dilutions. In this connection we re doubtful that serum lysozym plays an exclusive role in the action f thermostable serum elements. Unfortunately, the intensive coagula- tion of the sera studied on heating (in dilute bouillon form) in the eighborhood of 70°C made it impossible for us to determine precisely the temperature at which the elements under investigation are completely nactivated. However, the decrease in antimicrobial activity with res- pect to M. lysodeicticus which occurs even on inactivation at 56-60° C forces us to conclude that the action of animal sera on this microbe ay result from the presence of both lysozym and other, more labile substances.

In the later articles of Pillemer and his colleagues one evermore frequently detects a tendency to explain the antimicrobial activity of serum as being due exclusively to the action of properdine. While we do not doubt in the least that this compound plays an important role we do not believe it possible to overlook completely all of the substances not related to the complement-antibody system which have now been described. Our data, in conjunction with that in the literature, makes it possible to assert that the sera of various species of animals (rabbits, sheep, dogs, horses, cows, goats, and man) contain thermostable antimicrobial substances, which are similar to betalysin in a number of cases.

In this connection it must be noted that there are a large number of observations by various authors which speak in favor of the existence of these elements and that there is no basis to doubt them. Thus, thermostable substances active with respect to Bacillus subtilis were detected in horse sera by Savchenko. In 1934 Terskikh concluded that the blood of certain species of animals contains thermostable leptospirolysins which withstand heating to 70° C and do not require the presence of complement in order to exert their action. We will not dwell on the well-known works of I.I. Mechnikov and his colleagues on leukins, Peterson on betalysin, etc.

Finally, still another thermostable antimicrobial substance, bactericidin, which does not require the presence of complement has very recently been recorded in rabbit serum.

All of the material presented above gives us grounds for concluding that the presence of active thermostable elements in the sera of various species of animals is a quite common phenomenon in nature; this is an important argument in favor of recognizing that they play an essential adaptive role in infection and immunity.

CONCLUSIONS

1. The sera of man, rabbits, sheep, dogs, horses, goats, and cows contain thermostable antimicrobial elements.

2. The properties of these elements are similar to those of betalysin in a number of cases.

3. These elements are capable of acting in the absence of complement.

4. It is impossible to explain the antimicrobial properties of unheated normal sera solely by the fact that they contain the properdine-complement-antibody system.

5. The antimicrobial properties of inactivated sera cannot be explained solely by the fact that they contain lysozym.

6. The presence of active thermostable antimicrobial elements in animal sera is a biological phenomenon which occurs widely in nature and speaks in favor of recognition of the importance of these mechanisms as factors in immunity.

Tomsk Scientific Research Institute for Vaccines and Sera

Department of Microbiology, Tomsk Medical Institute

ABOUT THE ANTIMICROBE PROPERTIES OF NORMAL SERUMS OF THE HUMAN BEING AND ANIMALS

Information 4

Vasilyev N.V.

The question about the degree of distribution in nature thermostable acting beginnings of animals' serums is considered. It was shown that the blood of a horse, ram, rabbit, cow, goat and also of a human being contains thermostable antimicrobe substances close to betalysin. It is stated that it is impossible to explain the bacteriostatic of serums only by the presence of properdin in them. Thermostable acting

agents at least in the number of cases differ from lysozym. On the basis of wide distribution named substances in organic nature the author makes his conclusion about their important adaptable meaning.

THERMAL STABILITY OF NORMAL ANTIBODIES

(Report 1)

N.V. Vasil'yev

The problem of the relationships between factors of inherent and acquired immunity in general and normal and immune antibodies in particular has a history of more than half a century, but we cannot as yet consider it to be conclusively solved. Its practical and theoretical importance are so obvious that the insufficient amount of work devoted to it can be explained only by the fact that for the past three decades microbiologists have been occupied almost exclusively in studying the mechanisms of acquired immunity. It is only recently that interest in the humoral factors of specific immunity has increased somewhat, as a result of the discovery of properdine, a protein which apparently plays a considerable role in the mechanisms of natural immunity, by Pillemer and his colleagues.

The stability of certain biologically important substances to temperature is one of their important physicochemical characteristics. In attempting to deal with the problem of the relationships of immune and normal antibodies we thought it wise to investigate the behavior of normal hemolysins on heating.

We may consider it to be established that immune hemolysin is a thermostable substance. According to Yu.A. Finkel'shteyn, the thermostability of immune hemolysin is so great that it can withstand a 12-hour heating at 60°C. There are similar indications in the works of

I.I. Mechnikov, Borde, Erlikh, Morgenrot, Saks, an many others.

As for normal antibodies, statements about their relationship to temperature are rather contradictory. Erlikh and Morgenrot, proceeding from the "side chain" theory, expressed the view that there is no basic difference between normal and immune antibodies. Acknowledging only a quantitative difference between them, these authors categorically denied that there are any qualitative differences. A similar opinion is expressed by Borde in his book "Immunity, Antigens and Antibodies," although his own data contradict this to some extent, as we shall see later. In their handbook "Experimental Bacteriology and Infectious Diseases" Kolle and Getch dismiss Erlikh's view, stating bluntly that "specific hemolysins are the specifically-reproducing receptors of normal serum, this reproduction occurring under treatment with a certain type of blood."

On the other hand, early in the study of normal antibodies data were amassed which clearly contradicted this view. Thus, Borde observed that normal hen serum, which dissolves rabbit erythrocytes, loses this ability after inactivation in the neighborhood of 55-56°C; its hemolytic capacity cannot be restored by adding normal serum. I.I. Mechnikov cites similar data on normal opsonins and Levaditi, Kessler, Rayt, Duglas, Bekher, and certain others report observations of the same type. Apparently attempting to reconcile these observations with the theory that normal and immune antibodies are completely similar qualitatively, Borde advanced the hypothesis that the bactericidal and hemolytic action of normal sera may be caused by alexin alone. In similar fashion Neyfel'd, Rishpau, Bekher, and others identify alexin with normal opsonin.

However, there were apparently no serious arguments available to the supporters of this theory and S.I. Zhitogorov was consequently able

to give a direct proof of the relative thermolability of normal amboceptors in his book "A Study of Microorganisms." This problem was analyzed in special det Saks, who, despite his manifest adherence to Erlikh's theory, concluded that any attempt to apply Erlikh's concepts to an analysis of hemolysis in normal sera would be difficult because of the great thermolability of normal hemolysins. I.I. Mechnikov's statement that "analysis of the hemolytic phenomena which occur in normal sera meets with great difficulties" was obviously not fortuitous.

Summing up all the recent literature on hemolysins available to us we must regretfully note that this interesting problem has not as yet been conclusively solved. Velikanov has superficially indicated the comparatively high thermolability of normal antibodies; the later, more detailed handbooks on immunity (L.A. Zil'ber and Boyd) give no information on this score.

Our experimental method was as follows. Human blood taken sterily from a vein was placed in a refrigerator and surrounded with a snare or glass rod. After 24 hours in the refrigerator the serum was drawn off with a pipette and divided into two portions; one of these was not heated, but the other was heated in a water bath at 56°C for 30 minutes. Both portions of serum were diluted with physiological solution to make 0.5 ml of 1:10, 1:20, and 1:40 suspension; two series of dilutions were prepared for the unheated serum and one for serum heated at 56°C. After this 1 ml of physiological solution, 0.5 ml of standard complement diluted 10 times (the titre of complement being 0.10-0.15-0.20), and 0.5 ml of a 5% by volume suspension of sheep corpuscles were added to each test tube (with the exception of those containing the first series of dilutions of the unheated serum). The same reagents were added to the test tubes containing the first series of dilutions

of the unheated serum, but the diluted standard complement was replaced by physiological solution. After their contents were carefully mixed the test tubes were placed in a heater for 60 minutes, the results then being determined.

The following results were obtained. Of the 44 donor serum specimens investigated 39 contained normal hemolysins in a titre of 1:10 or more. In 37 of these 39 cases the normal hemolysins were seriously damaged during the half-hour heating at 56°C; they disappeared completely in a number of cases and in others their titre dropped, addition of 0.5 ml of standard complement diluted 10 times failing to restore the initial titre. Addition of 0.5 ml of complement diluted 5 times proved to be equally ineffective. Of the 110 serum specimens from patients at a neuropsychiatric hospital which we examined 95 were found to contain normal hemolysin in a titre of 1:10 or more; in 7 cases inactivation of the serum did not affect the titre of normal hemolysin, while complete or partial antibody destruction was observed in 88 cases. We were unable to restore the hemolytic titre of the serum by adding large doses of standard complement (0.5 ml of complement diluted 5 or 10 times). The mean titre of normal hemolysins for both the donor and patient groups was 1:18 for the unheated sera and 1:7 for the inactivated sera.

Thus, in accordance with the data in the literature, we concluded that normal hemolysin is a substance considerably less stable to elevated temperatures than immune hemolysin. As may be seen from a survey of the literature, Saks, Neysser and Borde concurred in acknowledging this fact. However, they gave different interpretations of it. While Borde saw it to be a proof of the direct action of complement on heterogeneous cellular elements, Saks, Neysser and others believed normal hemolysin to be a two-component substance similar to immune hemolysin.

In analyzing the results of our observations we also concluded that normal hemolysins are a two-component system. This is indicated by the following data: first, the hemolytic activity of heated normal serum usually increases sharply after diluted standard complement, which does not have a hemolytic action, is added to it; secondly, in a number of cases inactivated sera, which are not of themselves active, regain some of their hemolytic activity after standard complement is added to them.

The fact that we were unable to detect a clear parallelism between the titre of complement and that of normal hemolysins in our experiments also speaks against the attribution to complement of a capacity for direct action on erythrocytes; there are frequently cases in which the sera contain a considerable quantity of complement and no normal hemolysin or almost none.

Summing up the material presented above, we may conclude that normal hemolysin is a two-component system, both elements of which are very sensitive to heating in the neighborhood of 56-60°C; one of these components corresponds to complement and the other to the antibody. The thermolability of the normal hemolytic antibody considerably exceeds that of the immune antibodies; hence it must be concluded that normal and immune hemolysins are similar but not physicochemically identical substances. Having confirmed the data available in the literature, we hope to revive the interest of investigators in this neglected but important area of the theory of immunity.

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ABOUT THE TEMPERATURE STABILITY OF NORMAL ANTIBODIES

Vasilyev N.V.

The author has shown that normal hemolysins represent two com-

ponent system one part of which corresponds to the complement and the other, to the antibody. It is impossible to explain hemolytic activity of serums by direct action of complement on erythrocytes without the presence of antibodies. Normal hemolysins differ by higher thermolability than immune; a half an hour heating in the zone of 56°C reduces their activity sharply.

RELATIONSHIPS BETWEEN THE BIOELECTRIC POTENTIALS OF INTERNAL ORGANS AND CERTAIN FACTORS OF NATURAL HUMORAL IMMUNITY IN DOGS

N.V. Vasilyev, L.G. Trofimov

The physiology of immunity is one of the least explored branches of immunology; the difficulty in studying this problem results from the necessity of applying purely physiological research methods to investigating the immunity of an organism to infection. The solution of this problem can make a very great theoretical and practical contribution, since the processes of immunity must be considered within the framework of physiological and pathophysiological laws (A.D. Ado, P.F. Zdrodovskiy, and A.G. Speranskiy).

One precise and very graphic method of studying the functional coordination of internal organs is to investigate their bioelectric activity, a technique which can be applied with success both experimentally and clinically. We are now speaking of more than the study of the biocurrents of the brain and heart; research has recently been begun on the bioelectric potentials of other internal organs (kidneys, spleen and liver). According to data obtained by the Department of Animal Physiology of Tomsk State University (L.G. Trofimov, 1956), the biopotentials of these organs are a very sensitive indicator of their functional state and to some extent represent that which is customarily called the "internal environment of the organism."

In 1956 a report was published to the effect that the development of conditioned defensive reflexes in rabbits causes an increase in the titre of complement (L.T. Pronin).

One of us checked these experiments and found that the variation in the titre of complement observed in Pronin's experiments can hardly be attributed to the development of the temporary pathway as such; it is small in extent and may be explained by the slight periodic changes normally observed in this index in rabbits. However, the question of whether or not the phenomena noted by Pronin can be observed in more highly organized animals such as dogs still remained open.

In the work described herein we set ourselves the following tasks: 1) To study the changes in indices of natural humoral immunity in dogs on development of a conditioned defensive reflex and on systematic induction of a well-reinforced temporary pathway; 2) to compare the

changes in these indices with those in the bioelectric indices of the internal organs (brain, liver, spleen, and kidneys).

TABLE 1

Influence of Development of Conditioned Defensive Reflexes on Indices of Humoral Immunity in Dogs

		До опыта A					После опыта B				
		C Титр комплемента	D Общая гемолит. акт.	E Титр норм. термолаб. гемол.	F Титр норм. термост. гемол.	G Титр норм. гемагглют.	Титр комплемента	Общая гемолит. акт.	Титр норм. термолаб. гемол.	Титр норм. термост. гемол.	Титр норм. гемагглют.
H	Опытные животные	0,025	0,03	1:40	0	1:5	0,025	0,03	1:40	0	0
I	Контрольные животные	0,025	0,03	1:40	0	1:5	0,025	0,03	1:33	0	1:5

A) Before experiment; B) after experiment; C) titre of complement; D) general hemolytic activity; E) titre of normal thermolabile hemolysins; F) titre of normal thermostable hemolysins; G) titre of normal hemagglutinins; H) experimental animals; I) control animals.

The experiments were conducted on three dogs. The experimental method was as follows. The indices of natural humoral immunity (titre of complement and normal thermolabile and thermostable hemolysins, general hemolytic activity, and titre of normal hemagglutinins) were determined in all of the animals. The dogs were then operated on by the method described by L.G. Trofimov in 1956; in essence, this method consists in implanting special electrodes in the internal organs of the animals, their flexible leads being run out of the body through a special fistula. Before the biopotentials are plotted the electrodes are soldered to leads connected to a four-channel DC amplifier. The biopotentials are recorded on photographic film with the aid of a MPO-2 loop oscillograph.

After the animals had recovered from the operation their immunobiological indices were again determined and conditioned defensive re-

xes were then developed in them. This latter work was done in a ditioned-reflex room equipped with the necessary signaling and cording apparatus. The conditioned stimulus was an audible 500 cps ne and the unconditioned stimulus was electrical stimulation from induction coil applied to the surface of the skin on the animal's nd leg.

The state of the factors of natural humoral immunity was repeated-checked during development of the conditioned reflex. After the mporary pathway was found to be sufficiently stable the animals were rmitted to rest for 4 or 5 days; experiments were then conducted to udy the influence of development of a well-reinforced defensive reflex the immunobiological reactivity of the organism and the biopoten-als of its internal organs. For this purpose an electrogram was re-rded and the animals' immunobiological indices were determined; on e following day another reflex-induction session was held (5-6 con-ined stimuli coupled with painful reinforcement). Immediately on com-etion of these procedures all of the indices under investigation were gain determined.

In certain cases the experiment was modified in the following shion. After the immunological background had been established con-tioned-reflex-induction sessions were held daily for 5 or 6 days. e immunological indices were then determined once more.

The usual method of complement determination was employed. We took e titre of complement to be the least quantity of serum which was ill capable of producing traces of hemolysis. The titration of normal ermolabile hemolysins was also carried out by the customary method, e only difference being that the sera under investigation were not eliminarily heated. This was done because, as is well known from the terature (Saks, and Neysser), normal hemolysins are easily destroyed

by heating in the neighborhood of 56-60°C; this has also been confirmed by our experimental data.

Nevertheless, at the same time that we determined the normal hemolysins in the heated serum we investigated the titre of normal hemolysins in inactivated sera with the organism in the same functional state.

The general hemolytic activity of the canine serum was determined by a method analogous to the titration of complement, the only difference being that a suspension of inactivated sheep corpuscles of the same consistency as the hemolytic system was used instead of the latter. The normal hemagglutinins were determined by the Paul-Bunnell method.

A total of 14 experiments comprising 124 individual analyses were performed. We obtained the following data: neither development of a conditioned defensive pathway nor induction of a well-developed conditioned reflex had any substantial effect on the indices of natural humoral immunity. This is illustrated by Table 1.

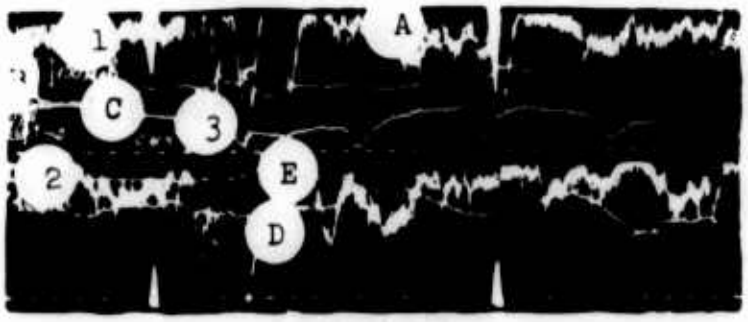

Fig. 1. Bioelectric activity of the brain and internal organs during reinforcement of a conditioned reflex. Electrograms: A) Brain (EEG); B) left kidney ; C) right kidney; D) spleen; E) liver. 1) μv; 2) mv; 3) sec.

This table gives the mean figures obtained for the experimental and control animals in three series of experiments. The control animals were dogs which did not undergo the procedure for inducing conditioned defensive reflexes on the day of the experiment.

We obtained similar data in studying the influence of the deve-

lopment of conditioned defensive pathways on immunobiological factors in dogs subjected to prolonged intensive training in the conditioned-reflex room.

We observed the following changes in the electrographic indices: giving the conditioned stimulus caused changes in the electroencephalogram and the electrograms of the kidneys, liver, and spleen; these took the form of an acceleration of the frequency of the electrical waves and they changed in their amplitude. However, these changes were brief and the biocurrents returned to their initial level 15-20 seconds after the conditioned stimulus was terminated. This is illustrated by the electrograms shown in Fig. 1. We were unable to find any prolonged change in the biopotentials of the brain or other internal organs after the sessions at which the conditioned defensive reflex was induced. In our opinion, this indicates that development of a temporary defensive pathway does not lead to any prolonged marked alteration in the internal environment of the organism, one of the components of which is substances such as complement, normal antibodies, etc. We are inclined to believe that this type of relative constancy in the composition of the internal environment is an important physiological factor which ensures normal functioning of all the systems of the organism.

CONCLUSIONS

1) The development and reinforcement of conditioned defensive pathways in dogs do not lead to any marked change in the factors of natural humoral immunity.

2) Induction of conditioned defensive reflexes in dogs leads to brief (with durations measured in seconds) shifts in the bioelectric potentials of the brain, liver, kidneys, and spleen.

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TO THE QUESTION ABOUT THE RELATION OF BIOELECTRIC POTENTIALS OF INTERNAL ORGANS AND SOME FACTORS OF HUMORAL NATURAL IMMUNITY BY DOGS

Vasilyev N.V., Trofimov L.G.

Attempt of applying biophysical methods of investigation to the study of immunity process is made in this article.

It is shown that producing of conditioned defensive reflex by dogs leads to short-measured in seconds variations in bioelectric activity of brain, liver, kidneys, spleen; such short changes of constancy of internal medium of organism do not lead to the change of its humoral immunobiologic background.

THE SPREADING FACTOR AND HYALURONIC ACID IN INFECTION AND IMMUNITY

Report 1. The Spreading Factor and Hyaluronic Acid in an Experimental Infection

M.I. Alaverdyan

Study of the spreading factor-hyaluronic acid system is now being given a great deal of deserved attention in the works of Soviet and foreign authors. The spreading factor of microbes is actually one of the elements of their aggressiveness, to a large extent determining the characteristics of the course of the infectious process, since it is through the agency of this factor that enzymatic cleavage of hyaluronic acid, the intermediate substance of the connective tissue of the macro-

TABLE 1

Influence of Thermal Factors on Hyaluronic Acid and the Spreading Factor

A Испытуемый препарат		Инактивация наступила через: B 0—6°	17—25°	37°	Препарат C 60°—30′	80°—10′	100°—45′	Автоклав 2 атм.—20 мин. D
E Фактор проницаемости	1G сер.	212 дней H	70 дней	10 дней	активен I	—	—	разрушен J
	II сер.	368 дней	—	—	активен	—	—	—
F Гиалуроновая кислота	I сер.	110 дней	16 дней	10 дней	активен	активен	активен	разрушен
	II сер.	130 дней	—	—	активен	—	—	—

Note: A dash indicates that no test was made.

A) Preparation under investigation; B) Inactivation begun after; C) preparation; D) autoclave, 2 atm., 20 min; E) spreading factor; F) hyaluronic acid; G) series; H) days; I) active; J) destroyed.

organism, occurs. Despite the existence of a large number of works on

the spreading factor and hyaluronic acid, the role of this system in the infectious pathology of man is far from having been exhaustively studied. In the literature available to us we found only a description of the investigations of V.M. Berman etal. (1947) on the influence of the spreading factor on the rate at which an experimental infection developed.

Hyaluronic acid has not been studied at all in this respect.

This article presents the results of a study of the influence of the spreading factor and hyaluronic acid on the development of an experimental staphylococcal infection in rabbits and white mice. These investigations were preceded by a study of certain other problems on which almost no light is shed in the literature. In particular, we were interested in the length of time for which preparations of the spreading factor and hyaluronic acid retain their activity when autoclaved, boiled, or stored at 0-6°, 17-25°, and 37°.

A protein preparation of hyaluronic acid was made from umbilical cords by McLean-Smirnova's method, slightly modified to enable us to obtain a more active preparation. We prepared the spreading factor from bull or ram testicles; these were painted three times with tincture of iodine and washed with alcohol, simultaneously being flamed for several seconds. The tunicae were removed under sterile conditions; the testicles were weighed and cut up in a mortar, an equal quantity of sterile distilled water being added. The mixture was then agitated for 60 minutes in a shaking machine, kept in a refrigerator for 1 day, and centrifuged at 2500 rpm for 45 minutes. The liquid above the precipitate, the spreading factor, was used in the experiment. Both preparations were cold-stored and their absolute sterility was then established by culturing them on sugar broth and sheep sugar agar.

The activity of the two preparations was determined by the well-known McLean-Smirnova reaction. The preparations were then kept in sterile flasks in a refrigerator (at 0-6°), at room temperature (17-25°), and in a heater (at 37°). In addition, both preparations were boiled

in a water bath and autoclaved. The results are shown in Table 1.

It is clear from the data in Table 1 that both the spreading factor and hyaluronic acid are sufficiently stable under cold storage and can retain their activity for several months. This time is considerably shorter (70 and 16 days) at room temperature and is still less (10 days) when the preparations are kept in a heater. The spreading factor decomposes rapidly at 80°, since it is a protein enzyme. Hyaluronic acid, which is a polysaccharide, can withstand boiling for 45 minutes in a water bath, but is decomposed by autoclaving.

TABLE 2

Modified McLean-Smirnova Reaction

A / B — Ингредиенты в мл / № пробирок	C Контроль гиалурон. кис-ты	D Активность фактора проницаемости					E Активность гиалуроновой кислоты		
	1	2	3	4	5	6	7	8	9
F Гиалур. кислота	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
G Дистил. вода	0,8	0,3	0,4	0,5	0,6	0,7	0,8	0,8	0,8
H Фактор проницаемости в смеси с бакпрепаратом (поровну) в мл	—	1,0	0,8	0,6	0,4	0,2	I Только бакпрепарат, без фактора проницаемости, в мл: 0,5	0,3	0,1

A) Ingredients, in ml; B) test tube number; C) hyaluronic acid control; D) activity of spreading factor; E) activity of hyaluronic acid; F) hyaluronic acid; G) distilled water; H) spreading factor mixed with bacterial preparation (in a one-to-one ratio), in ml; I) bacterial preparation alone, without spreading factor, in ml.

It follows from this that, from the practical standpoint, preparations of the spreading factor and hyaluronic acid can be stored and used for a rather long time. Hyaluronic acid can be sterilized by boiling for 15-30 minutes in a water bath, while the production of sterile preparations of the spreading factor is based on sterility in

all operations performed during the extraction of the preparation from testicles. Sterile preparations of the spreading factor and hyaluronic acid must be obtained for experiments on animals, a fact with which we will deal in this and subsequent articles.

The preparations of the spreading factor and hyaluronic acid which we obtained had a very high activity: the working dose of hyaluronic acid was 0.1 ml, 0.05 ml in certain cases, while doses of 0.1 and 0.05 ml of the spreading factor caused destruction of the hyaluronic acid, even when 0.2 rather than 0.1 ml was used as the working dose for the latter.

Having at our disposal sterile high-quality preparations of the spreading factor and hyaluronic acid, we began to study the nature of the influence of these preparations on certain bacterial preparations in test tube experiments and experiments on animals. Not having any information on investigations of a similar nature, we did not limit our selection to any specific bacterial preparation, but attempted to determine the general rules governing the action of various widely used preparations on the spreading factor and hyaluronic acid, regardless of their type and characteristics.

We used 24 preparations in the series of test tube experiments: typhoid, Flexner dysentery, Grigor'yev-Shig dysentery, cutaneous and oral BCG, and rabies vaccines, tetravaccine, typhoid and Flexner dysentery diagnostica, live cultures of typhoid, Flexner and Grigor'yev-Shig dysentery, and paratyphoid A and B bacteria, normal rabbit, horse, and human sera, agglutinative typhoid, antimeasles, antitetanus, precipitated antianthrax, and antidiphtheria sera, and diphtheria and tetanus toxoids.

Each of these preparations was employed in the McLean-Smirnova reaction modified in the manner shown in Table 2.

48 tests were conducted in accordance with the scheme described

in Table 2; 24 of the McLean-Smirnova reactions were set up 30 minutes after the bacterial preparation was mixed with the spreading factor or hyaluronic acid, while 24 were carried out after the mixture had been stored at 0-6° for 5-10 days. The results obtained in both experimental setups were identical, reducing to the following: with the exception of the antimeasles serum, none of the 23 bacterial preparations affected the activity of either the spreading factor or hyaluronic acid. We are inclined to attribute the decomposition of hyaluronic acid by the antimeasles serum to the presence of a preservative, chinosol, in this serum.

Thus, in the test tube experiments none of the aforementioned bacterial preparations reduced the activity of (decomposed) the spreading factor or hyaluronic acid; this indirectly indicates the possibility of using a combination of these elements in an experimental model involving animals.

On the basis of the data obtained we set about the next stage of our work, which was intended to establish the nature of the influence of the spreading factor and hyaluronic acid on the diffusion of certain vaccines and toxoids in experiments on animals. In these experiments we used heat-killed typhoid vaccine (strain 76-75) and dysentery monovaccine and divaccine (from Flexner type X and Grigor'yev-Shig bacteria) which we prepared, diphtheria and tetanus toxoids, cutaneous BCG vaccine, and Fermi antirabies vaccine. We first established that these preparations contained absolutely no hyaluronidase.

Three rabbits were used for the experiment. Each rabbit received 8 intracutaneous injections, 4 in the right side and 4 control injections in the left side, a total of 24 injections. 6 were of the bacterial preparations and the spreading factor, 6 were controls and involved the same preparations and spreading factor inactivated by boiling

TABLE 3

Influence of the Spreading Factor and Hyaluronic Acid on the Rate at Which Certain Vaccines and Toxoids Diffuse in a Rabbit's Skin

A Бактерпрепарат	B Показатель диффузии				G Разница между опытным и контрольным пятном в кв. мм			
	C с фактор. прон.		D с гиалур. кислотой		с фактор. прон.		с гиалур. кислотой	
	E через 8 час.	F через 20 час.	через 8 час.	через 20 час.	через 8 час.	через 20 час.	через 8 час.	через 20 час.
H Брюшнотифозная вакцина	1,1	1,2	0,5	0,5	241	430	408	408
I Дизентерийная вакцина	2,3	2,4	0,5	0,5	942	1030	315	315
J Дифтерийный анатоксин	1,3	1,6	0,9	0,9	242	578	37	37
K Антирабическая вакцина	1,2	1,4	0,5	0,5	121	423	590	650
L Вакцина БЦЖ	1,3	1,2	0,7	0,7	320	288	144	144
M Столбнячный анатоксин	1,3	1,2	0,7	0,6	282	326	258	292
N Среднее из 6 препаратов	1,4	1,5	0,6	0,6	358	512	292	308

A) Bacterial preparation; B) diffusion index; C) with spreading factor; D) with hyaluronic acid; E) after 8 hours; F) after 20 hours; G) difference between experimental and control spots, in mm^2; H) typhoid vaccine; I) dysentery vaccine; J) diphtheria toxoid; K) rabies vaccine; L) BCG vaccine; M) tetanus toxoid; N) average for 6 preparations.

for 15 minutes, six were of bacterial preparations with active hyaluronic acid, and six were controls and involved the same preparations with hyaluronic acid decomposed by autoclaving. A mixture of 0.2 ml of the bacterial preparation, 0.2 ml of a 0.75% solution of trypan blue (an indicator), and 0.2 ml of the spreading factor or hyaluronic acid (in its native or decomposed form) was used for all 24 injections. The area over which the dye had diffused was measured after 8 and 20 hours, using the formula for the area of a circle or ellipse. The area of the experimental spot was compared with that of the control spot and the diffusion index determined. The results are shown in Table 3.

It may be seen from Table 3 that hyaluronic acid retards the diffusion of the bacterial preparations in the rabbit's skin (the diffusion indices are less than one), playing the role of a precipitating agent. Conversely, the spreading factor increases the permeability of the dermal barrier and thus forces the preparations to diffuse in the animal's skin (the diffusion index is greater than one). It must also be noted that the bacterial preparations do not have any inactivating influence on the spreading factor or hyaluronic acid in experiments on animals, so that neither preparation loses its specific action on the tissues of the macroorganism.

These data impelled us to intensify our investigation and pass on to studying the nature of the influence of the spreading factor and hyaluronic acid on the rate at which staphylococcemia develops in rabbits and white mice after subcutaneous injection of a live culture of Staphylococcus albus (Strain F-1).

Twelve animals were used in the first series of experiments on rabbits. A one-million-cell washing from a live day-old hyaluronidase-negative culture of staphylococcus, suspended in physiological solution, served as the test microbe. The injections were given subcutaneously in the left inguinal region: the control animals received 1 ml of the culture mixed with 1 ml of physiological solution, four of the remaining 8 rabbits received the culture mixed with 1 ml of the spreading factor, and four received the culture mixed with 1 ml of hyaluronic acid. Two ml of blood was taken from the heart of each of the first 3 rabbits (Nos. KO, O, and ZhShch) after 10 minutes, 30 minutes, 3 hours, and 24 hours; the samples were cultured in flasks containing 50 ml of meat-extract bouillon. After 48 hours of incubation 0.1 ml of bouillon was used to inoculate sheet agar. The staphylococcus colonies were counted after the flasks were kept in a heater for 48 hours.

It was established that bacteriemia had not yet appeared after 10 minutes in any of the rabbits. Bacteriemia was not noted at any time in the rabbit which received the staphylococcus culture mixed wit hyaluronic acid. Colonies were observed only for the 24-hour sample taken from the control rabbit, 3562 being counted. The 30-minute sample from the rabbit which received staphylococcus mixed with the spreading factor produced 268 colonies, while the 3-hour sample yielde an almost continuous growth and the 24-hour sample a continuous growth.

TABLE 4

Influence of the Spreading Factor and Hyaluronic Acid on the Rate of Development of Staphylococcemia in Rabbits

A Введена культура в смеси с:	B Кролик	C Пробы крови	D Взятие крови из сердца через:				
			30 мин. E	3 часа F	6 часов F	12 часов	24 часа
			G результат посева крови на скошенном агаре				
			H разведения крови				
			ц	1	2	3	ц	1	2	3	ц	1	2	3	ц	1	2	3	ц	1	2	3
J фактором проницаемости	12 M маленький серый N						т	т			+	+			+	+	+	+	+	+	+	+
							-				+	+	+		+	+			+	+	+	+
											+	+			-	+	+	+	+	+	+	+
K физиологическим раствором	14 одноухий O						+				+				+				+	+	+	
															+	+			+			
	10						+	+							+				+			
L гиалуроновой кислотой	коричневый P																					
	кривой Q																					
	большой R																					

Notes: 1) A plus sign indicates staphylococcae were present; 2) the letters ts indicate that whole blood was cultured; 3) the numerals 1, 2, and 3 indicate that blood was cultured in dilutions of 1/100, 1/1000, and 1/10,000, respectively.

A) Culture mixed with; B) rabbit; C) blood tests; D) blood taken from the heart after; E) min; F) hours; G) result of culturing blood on sloped agar; H) blood dilution; I) ts; J) spreading factor; K) physiological solution; L) hyaluronic acid; M) small; N) gray; O) one-eared; P) brown; Q) crookbacked; R) large.

Two ml of blood was taken from the heart of each of the 9 re-
ining rabbits 30 minutes and 3, 6, 12, and 24 hours after inocula-
on; the samples were cultured in a first flask containing 100 ml of
at-extract bouillon. This flask was used for tests involving whole
lood. The blood was then diluted to 1/100, 1/1000, and 1/10,000; the
/100 dilution was prepared by adding 0.1 ml of blood to 10 ml of ster-
le physiological solution, 1 ml of the resultant product was then
ransferred to a second test tube containing 10 ml of physiological
olution (to give a dilution of 1/1000), and 1 ml of this second pro-
uct was added to a third test tube also containing 10 ml of physio-
ogical solution (to yield a dilution of 1/10,000). The contents of
ll three test tubes, i.e., 10 ml, were cultured in 3 flasks each con-
aining 100 ml of bouillon.

Nine animals were thus used in the experiment and 5 blood sam-
les were taken from each of them (a total of 45 samples). Each sample
as cultured in four flasks, i.e., a total of 180 samples were cul-
tured; they were all incubated at 37° for 48 hours. A culture of 0.1 ml
from each flask was made on sloped agar, in order to detect staphylo-
occemia. The presence or absence of bacteriemia in the rabbits was
determined by microscopic examination of smears from the agar. The re-
sults are shown in Table 4.

In analyzing the data given in this table one marked regularity
is striking; none of the blood samples from the rabbits given staphy-
lococcus in conjunction with hyaluronic acid (those designated as brown,
crookbacked, and large) contained staphylococcus. 12 of the 60 flasks
containing blood samples from the control rabbits (14, one eared, and
10) contained staphylococcae, this amounting to 20% positive. The blood
samples from the rabbits which received staphylococcus in conjunction
with the spreading factor yielded 53.3% positive results (32 of 60 cam-

ples).

In summing up the data obtained in this series of experiments, which was performed on 12 rabbits, we may draw the following conclusion. The spreading factor, which increases the aggressiveness of staphylococcae by enzymatic hydrolysis of connective-tissue structures, markedly (by a factor of more than 2.5) intensified the development of staphylococcemia in these animals when the preparation under investigation was injected subcutaneously. Hyaluronic acid had a diametrically opposite effect: it sharply depressed the aggressiveness of the staphylococcae by intensifying the barrier functions of the organism. Hyaluronic acid thus cut short the development of staphylococcemia in the rabbits in our experiments.

In the next series of experiments we studied the influence of the spreading factor and hyaluronic acid on the development of staphylococcemia in an investigation carried out on 110 white mice.

In the first experimental setup we used 63 mice ranging in weight from 14 to 16 g. The animals were divided into 3 groups, with 21 mice in each. Inoculation was performed subcutaneously in the left inguinal region and involved a 500-million-cell culture of hyaluronidase-negative staphylococcus strain K (which we isolated from the puss of an osteomyelitis patient).

The mice in the first group received 0.2 ml of the staphylococcus culture mixed with 0.2 ml of physiological solution (the control), those in the second group received 0.2 ml of the culture mixed with 0.2 ml of the spreading factor, and those in the third group received 0.2 ml of the culture mixed with 0.2 ml of hyaluronic acid. Ten minutes after inoculation the mice were vivisected, 0.1 ml of blood being removed from the heart and cultured on sheet agar. The number of staphylococcus colonies which developed was counted after 48 hours of incubation. The

results are shown in Table 5.

TABLE 5

Influence of the Spreading Factor and Hyaluronic Acid on the Development of Staphylococcemia in Mice. Inoculation was Subcutaneous, in the Inguinal Region. Dissection and Culturing Were Carried Out After 10 Minutes.

Заражение культурой в смеси с гиалур. кислотой A		Заражение культурой в смеси с физиол. раствором. D		Заражение культурой в смеси с фактором проницаемости E	
№ мышей B	колич. колоний C	№ мышей	колич. колоний	№ мышей	колич. колоний
1	180	22	1080	43	1750
2	136	23	73	44	1500
3	100	24	550	45	2350
4	202	25	11	46	3600
5	105	26	∞	47	3800
6	153	27	2900	48	2750
7	23	28	41	49	1980
8	184	29	2800	50	1950
9	112	30	680	51	2100
10	106	31	150	52	∞
11	102	32	∞	53	∞
12	104	33	∞	54	2300
13	роста нет F	34	450	55	
14	118	35	138	56	2500.
15	117	36	1960	57	∞
16	131	37	351	58	1520
17	225	38	70	59	2800
18	109	39	182	60	∞
19	210	40	650	61	2100
20	291	41	120	62	∞
21	111	42	140	63	∞
G Итого	2719	Итого	12346	Итого	33000
H Среднее из 21 опыта	130	Среднее из 18 I	686	Среднее из 14	2357

Notes: 1) ∞ designates a continuous growth of staphylococcus. 2) ~ designates almost continous growth. 3) continuous and almost continous growth are disregarded in determining the mean indices.

A) Inoculation with culture mixed with hyaluronic acid; B) number of mouse; C) number of colonies; D) inoculation with culture mixed with physiological solution; E) inoculation with culture mixed with spreading factor; F) no growth; G) total; H) average of 21 experiments; I) average of.

Analysis of the data in Table 5 clearly shows that hyaluronic acid

TABLE 6

Influence of the Spreading Factor and Hyaluronic Acid on the Development of Staphylococcemia in Mice. Inoculation Given in Palmar Surface of Front Paw. Dissection and Culturing of Blood From Heart After 1 Minute.

Заражение в смеси с фактором проницA		Заражение в смеси с физиол. растворомD		Заражение в смеси с гиалур. кислотойE	
№ чашек (мышей)B	число колоний C	№ чашек (мышей)	число колоний	№ чашек (мышей)	число колоний
64	218	83	4	102	21
65	110	84	2	103	0
66	125	85	4	104	5
67	107	86	0	105	12
68	23	87	128	106	2
69	244	88	5	107	1
70	128	89	117	108	15
71	149	90	185	109	21
72	562	91	136	110	3
73	238	92	8	111	5
74	109	93	11	112	7
75	135	94	9	113	10
76	66	95	25	114	1
77	119	96	8	115	12
78	21	97	1	116	9
79	32	98	11	117	1
80	120	99	16	118	10
81	37	100	7	119	13
82	141	101	131	120	18
F Итого	2681	Итого	808	Итого	166
G Среднее	141	Среднее	42	Среднее	9

A) Inoculation with culture mixed with spreading factor; B) dish (mouse) no.; C) number of colonies; D) inoculation with culture mixed with physiological solution; E) inoculation with culture mixed with hyaluronic acid; F) total; G) average.

reduced the rate at which staphylococcal bacteriemia developed in the white mice by a factor of more than 5 in comparison with the control animals; conversely, the spreading factor intensified the development of staphylococcemia in the mice by a factor of more than 3.

In the second series of experiments we used 57 mice divided into 3 groups (19 animals in each). The only difference in experimental setup was that the inoculation was given in the palmar surface of a front paw rather than subcutaneously. We copied this method from V.M.

Berman (1947), who believes that the microbes enter the blood stream extremely rapidly when this method of inoculation is used.

The first group of mice received 0.025 ml of staphylococcus culture (50 million live cells) mixed with 0.025 ml of the spreading factor, the second group received 0.025 ml of culture mixed with 0.025 ml of physiological solution (the control), and the third group received 0.025 ml of the culture mixed with 0.025 ml of hyaluronic acid. The animals were dissected after 1 minute and 0.1 ml of blood from the heart of each mouse was cultured on sheet agar. The number of staphylococcus colonies was counted after 72 hours of incubation. The data obtained are shown in Table 6.

It is clear from the data given in this table that the most intensive staphylococcemia occurred in the mice injected with staphylococcus mixed with the spreading factor (Nos. 64 to 32); the minimum bacteriemia was noted in the mice which received the culture mixed with hyaluronic acid (Nos. 102 to 120). Just as in the first series of experiments, the control mice (Nos. 83 to 101) yielded intermediate indices.

Summing up the data obtained in these experiments on mice, we may draw the following conclusion: the spreading factor accelerates and hyaluronic acid retards the development of bacteriemia whether the staphylococcus culture is administered subcutaneously or by the intrapalmar method.

CONCLUSIONS

1. It is not especially difficult to obtain sterile preparations of the spreading factor and hyaluronic acid. Cold-storage of the preparations guarantees that they will retain their full biological activity for several months.

2. The vaccines, toxoids, sera, diagnostica, and live microbial cultures listed in this article do not inactivate preparations of the

spreading factor and hyaluronic acid in test tube experiments, even after they have been mixed with the preparations and cold-stored for 5-10 days.

3. The spreading factor facilitates and hyaluronic acid hinders the diffusion of typhoid, dysentery, rabies, and BCG vaccines and diphtheria and tetanus toxoids administered intracutaneously, the two having diametrically opposed effects on the permeability of the dermal barrier in the experimental animals (rabbits).

4. The spreading factor forces and hyaluronic acid inhibits the development of staphylococcemia in rabbits and white mice subcutaneously inoculated with a live staphylococcus culture mixed with them.

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THE FACTOR OF PERMEABILITY AND GYALURONIC ACID IN PHENOMENA OF INFECTION AND IMMUNITY

Information 1. The factor of permeability and gyaluronic acid in experimental infection.

Alaverdyan M.I.

The influence of testicular factor of permeability and umbilical gyalucronic acid on the quickness of spreading some biobacpreparations and living culture of staphylococcus in the rabbit's and white mice's organism was studied. It was established that the factor of permeability quickens but gyaluronic acid slows down the spreading of some vaccines and anatoxins (at intracutaneous injection of them into the rabbit) and the development of staphylococcemia at hypodermic injection of microbe into the rabbits and white mice.

THE SPREADING FACTOR AND HYALURONIC ACID IN INFECTION AND IMMUNITY

Report 2. The Spreading Factor and Hyaluronic Acid in an Experimental Immunological Model

M.I. Alaverdyan

Soviet public health has correctly imposed ever-greater requirements on immunology; these must be satisfied within the shortest possible time and we must work in the direction indicated in the decisions made by the 20th Congress of the Communist Party of the USSR, i.e., toward intensifying the prophylaxis of disease in every way possible. The prime task of immunologists is to devise maximally effective means for the specific prophylaxis of infectious diseases. We now reckon more than 50 vaccines, 15 of them live, in our arsenal of such means (V.M. Zhdanov, 1954).

In addition, not a single reliable method for producing vaccine strains has as yet been developed (V.D. Timakov, 1954). According to the data of S.N. Muromtsev (1953), there is a clear discrepancy between the theory and practice of obtaining live vaccines. It is also well known that many investigators have used filterable forms of microbes, hybrids, a typical strain, etc., in order to develop new types of vaccine for specific prophylaxis. Unfortunately, all this turned out to be unsuccessful, since the attempts ended in the production of negative variants lacking specificity and absolutely useless in immunological practice (L.I. Leshkovich, 1956).

The work of many investigators on the guided mutability of microbes also failed to yield the expected results.

All of the statements made above are completely true of the prospects for developing effective vaccines against enteric infections. It is no exaggeration to include this problem among the weakest links in modern practical immunology. Actually, in recent years the view that dysentery vaccines are of little effectiveness has been advanced more and more often. In prior years vaccination against dysentery led to a sharp reduction in the incidence of this disease, by factors of from 5 to 22, according to the data of different authors. Vaccination against dysentery now decreases its incidence by factors of only 1.5-2, and occasionally to an even lesser extent (A.S. Korshakova, 1948 and others). Certain investigators are inclined to believe that it is generally impossible to set up an immunity to dysentery. Thus, A.B. Aleksanyan (1957) has written "the effectiveness of active immunization against dysentery has been studied over a period of years... The results of this

study have convinced us that vaccination is not justified as a method for the prophylaxis of dysentery."

M.Ye. Lipkin (1957), being dissatisfied with the quality of tetravaccine, believes it necessary to reconsider its composition and, in particular, to improve the quality and increase the quantity of the Sonne antigen. Yu.A.Potapchik (1956) gives the whole antigens of Flexner dysentery bacteria preference over heat-killed vaccines. A.S. Korshakova (1954) believes that ninefold immunization against dysentery is more effective than threefold. A.S. Korshakova and Ye.O. Girshik (1954) note that the NIISI polyvaccine halves the incidence of dysentery. V.N. Kosmodamianskiy (1957) concludes that further improvement in the quality of vaccine antigens is necessary to increase the effectiveness of enteral vaccination against enteric infections.

Even this very brief list of attempts to improve the quality of enteric vaccines shows that we are still far from achieving satisfactory results in the solution of this problem.

With regard to the low immunogenicity of typhoid antigen, the existence of a "polyetiological character" in paratyphoid and dysentery infections, and the doubtful immunogenicity of dysentery microbes, a great many not very successful attempts have naturally been made to develop truly effective enteric vaccines; all this taken together forces us to give serious consideration to the problem of whether we are actually justified in endlessly searching for immunogenic types of enteric microbes among the great number of newly-discovered standard, hybrid, etc., strains of the causitive agents of typhoid, paratyphoid, and dysenteric infections. It seems to us that these attempts will not be successful so long as we are dealing with killed vaccines. At present we have no specific data on live enteric vaccines.

On the basis of the material presented above, we believe it wise to deviate somewhat from this unoriginal way of seeking vaccine strains to be used against enteric infections and to turn to a search for other means of solving this problem; since it has not been possible to find highly effective immunogenic strains among the typhoid, paratyphoid, and dysenteric bacteria, it follows that we should attempt to increase the immunogenicity of existing enteric vaccines by adding substances which have proved themselves in this respect to them.

We selected the spreading factor and hyaluronic acid as substances of this type. We described a modified method for obtaining highly active sterile preparations of these substances in our first report. The selection of the spreading factor as an activator of enteric vaccines was dictated by V.M. Berman's work (1947), the only one of its kind, in

which the author describes his success in experimentally increasing the immunogenic activity of cholera vaccine. He was unable to get uniform results in experiments with typhoid vaccine. The use of hyaluronic acid as a stimulator of the antigenic and immunogenic properties of enteric vaccines had a purely original basis, since we were unable to find any appropriate information in the foreign and Soviet literature available to us.

TABLE 1

Influence of the Spreading Factor and Hyaluronic Acid on the Intensity of the Formation of Specific Agglutinins in Rabbits on Subcutaneous Immunization With Heat-Killed Typhoid Monovaccine

A Пробы крови	B Средние титры агглютининов при иммунизации вакциной в смеси с:		
	C фактором проницаемости	D физиологическим раствором (контроль)	E гиалуроновой кислотой
	1/270	1/130	1/70
II	1/530	1/330	1/230
III	1/670	1/200	1/130
IV	1/1330	1/670	1/530
V	1/930	1/400	1/730
VI	1/670	1/530	1/2400
VII	1/1600	1/2660	1/1200
VIII	1/400	1/930	1/1330
IX	1/670	1/1870	1/3470
X	1/200	1/900	1/400
XI	1/100	1/400	1/500

Note: The mean titres were determined from the sum of the agglutinin titres for 3 rabbits from each of the three groups of subjects.

A) Blood tests; B) mean agglutinin titres following immunization with vaccine mixed with; C) spreading factor; D) physiological solution (control); E) hyaluronic acid.

This report describes the results of an investigation of the antigenic properties of certain enteric vaccines used both in pure form and mixed with preparations of the spreading factor and hyaluronic acid.

On the basis of the data obtained in our prior investigations we

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assumed a priori that, since the spreading factor accelerates and hyaluronic acid inhibits the diffusion of many bacterial preparations in the skin of rabbits and the development of staphylococcemia in rabbits and white mice, it is obvious that both of these preparations will affect the antigenicity of subcutaneously-administered enteric vaccines in the same manner, the spreading factor intensifying and accelerating the formation of specific agglutinins during the early phases of immunogenesis and the hyaluronic acid prolonging the length of time for which they are formed and circulate in the organism because of its retarding influence.

In order to check the validity of this assumption we performed the following chronic experiments on rabbits.

A total of 29 rabbits were used in the experiment, the majority of them being of the chinchilla strain and the remainder of the marters [sic] strain. The animals were divided into three groups. Nine rabbits were used in the first series of experiments, which was intended to determine the comparative effect of subcutaneous immunization with three different types of killed typhoid monovaccine. Three rabbits were immunized with vaccine which we prepared from strain 76-75, a second group of 3 animals was immunized with the same vaccine mixed with the spreading factor, and a third group of 3 rabbits was immunized with the vaccine mixed with hyaluronic acid. The effectiveness of the vaccinations was determined dynamically, with the aid of the agglutination reaction.

All of the animals were vaccinated three times. As their first vaccination the control animals in the first group received 1 ml (100 million units) of typhoid vaccine mixed with 1 ml of physiological solution. The second group was immunized with a mixture of 1 ml of vaccine and 1 ml of the spreading factor. The third group received a mixture of 1 ml of the vaccine and 1 ml of hyaluronic acid. During the second

vaccination (10 days after the first) the rabbits were immunized in the same manner, but were given 500 million rather than 100 million units of vaccine. A third and final vaccination of 1 billion units of vaccine was administered 10 days after the second. Agglutination reactions were set up between blood samples from all the animals and a live culture of the vaccine strain. Eleven blood samples were taken from each rabbit: on the 4th, 7th, and 10th days after the first vaccination, similarly after the second vaccination, and on the 4th, 7th, 10th, 20th, and 25th days after the third vaccination. The results of this series of experiments are shown in Table 1.

In analyzing the data in Table 1 we may see the following basic regularities. From the first blood sample to the fourth the most intensive agglutinin formation was noted in the rabbits immunized with the vaccine mixed with the spreading factor; conversely, antibody formation was greatly retarded in the rabbits which received hyaluronic acid. The control animals occupied an intermediate position. This relationship was reversed in the subsequent blood samples; the hyaluronic acid caused the maximum agglutinin titres, the spreading factor no longer stimulating this process. The control animals again occupied an intermediate position.

The stimulating factor, which promotes extremely rapid resorption of the antigen, thus stimulates the development of typhoid agglutinins soon after vaccination; the hyaluronic acid, which precipitates the antigen, intensifies the formation of immune antibodies at later times.

In the second series of experiments we carried out combined immunization of the same animal with the vaccine and the spreading factor and with the vaccine and hyaluronic acid in order to bring about a summation of these two useful effects.

A total of 10 rabbits (chinchilla) were used in the experiment.

No normal agglutinins (to typhoid antigen) were detected in the serum of these animals in dilutions of 1/100 or more. The animals were divided into two groups, with 5 subjects in each. The control group was immunized subcutaneously with ordinary typhoid monovaccine, 0.5 ml (200 million units) of vaccine being mixed with 1 ml of physiological solution; they were also given similar injections in the opposite side. Each control rabbit consequently received 200 million microbial bodies. The animals in the experimental group received 0.5 ml (200 million units) of vaccine mixed with 1 ml of the spreading factor subcutaneously in the right side and the same quantity of vaccine mixed with 1 ml of hyaluronic acid in the right side. It did not seem possible to give a single injection of the vaccine, the spreading factor, and the hyaluronic acid (in one syringe), since the latter would undoubtedly have been decomposed by the hyaluronidase.

Blood was taken for the agglutination reaction on the 4th, 8th, and 13th days after the first vaccination. All of the animals were again immunized in the same manner on the day when the third sample was taken, but each rabbit received 1 billion rather than 200 million microbial bodies of the same vaccine. Blood was also taken on the 6th, 15th, and 25th days after the second immunization.

The combined method of immunizing the animals produced a considerably greater effect than administration of the vaccine with physiological solution. The antigenicity of the typhoid vaccine always increased when it was administered in conjunction with the spreading factor and hyaluronic acid; the agglutinin titres of the 6 blood samples from the experimental animals were several times higher than those for the control animals. It is sufficient to note that the maximum agglutinin titre for the control animals did not exceed 1/1040, while that for the experimental animals was six times greater (1/6720). The sum

of the agglutinin titres of all six samples from the experimental rabbits (1/16,160) was greater by a factor of 4.1 than that of the agglutinin titres of the six samples taken from the control animals (1/3920).

This series of experiments thus showed even more graphically the agglutinin-formation stimulating role of the spreading factor and hyaluronic acid. When administered simultaneously these two preparations markedly increase the antigenicity of typhoid monovaccine.

The next, third series of experiments was an organic extension of the first two series and was intended to study the influence of the spreading factor and hyaluronic acid on the antigenic properties of typhoid-paratyphoid-dysentery tetravaccine.

Ten rabbits (chinchilla and marters [sic]) were used in the experiment. It was first established that none of the sera from the animals (in dilutions of 1/100 or more) contained agglutinins to the antigens in the tetravaccine. The animals were divided into two groups, 5 in each. Three immunizations were given subcutaneously. In the first immunization the control rabbits received 0.25 ml of vaccine mixed with 0.5 ml of physiological solution in both the left and right sides, a total of 0.5 ml of vaccine or 1.125 billion microbial bodies. The experimental animals were immunized in the same manner, but preparations of the spreading factor and hyaluronic acid were used instead of the physiological solution. The second vaccination was given on the 5th day after the first, in the same manner, the only difference being that each animal received 2.25 billion rather than 1.125 billion bodies of the tetravaccine. The third vaccination, similar to the second, was 5 days after the latter.

Under these chronic experimental conditions 7 blood samples were taken from the rabbits for the agglutination reactions: the first was taken on the 5th day after the first vaccination, the second on the 5th

day after the second vaccination, and the third, fourth, fifth, sixth, and 7th on the 5th, 18th, 30th, 45th, and 60th days after the third vaccination.

The results of this series of experiments are summarized in Table 2.

In analyzing Table 2 we may see the following regularities. Five days after the first immunization and 5 days after the second (first and second blood samples) there was an increase in agglutinin titres in the animals of both groups; the formation of typhoid, Flexner dysentery, and Sonne dysentery agglutinins in the experimental group was markedly more intense than in the animals immunized with the ordinary tetravaccine.

Five days after the third vaccination (III sample) there was a simultaneous decrease in the titre of agglutinins in both groups of animals. On the 18th, 30th, 45th and 60th days after the third vaccination (the IV, V, VI, and VII blood samples respectively) it was found that typhoid, Flexner dysentery, and Sonne dysentery agglutinins developed more intensively in the experimental animals (those which received the vaccine mixed with the spreading factor and hyaluronic acid) than in the control group. The only exception was in the development of paratyphoid B agglutinins, the titre of which differed little from that in the control group.

In determining the mean titre of agglutinins in the seven blood samples we obtained:

	Experimental	Control
For typhoid agglutinins	1/3401	1/1984
For paratyphoid B agglutinins	1/1356	1/1145
For Flexner dysentery agglutinins	1/4347	1/2300
For Sonne dysentery agglutinins	1/2746	1/1553
For the tetravaccine as a whole	1/11850	1/6982

TABLE 2

Intensity of Formation of Typhoid, Paratyphoid B, Flexner dysentery, and Sonne dysentery Agglutinins in Rabbits Immunized With Ordinary Tetravaccine and Tetravaccine Mixed With the Spreading Factor and Hyaluronic Acid

A Пробы крови	B Вид агглютининов	Средние титры агглютининов у кроликов, иммунизированных тетравакциной в смеси с: C	
		фактором проницаемости и гиалуроновой кислотой (опыт) D	физиологическим раствором (контроль) E
I	брюшнотифозные F	1/1040	1/740
	паратифозные G	1/660	1/280
	дизент. Флекснера H	1/1600	1/600
	дизент. Зонне I	1/2160	1/520
II	брюшнотифозные	1/4480	1/1150
	паратифозные	1/1760	1/2100
	дизент. Флекснера	1/7040	1/3600
	дизент. Зонне	1/3680	1/1800
III	брюшнотифозные	1/1360	1/1900
	паратифозные	1/420	1/175
	дизент. Флекснера	1/1920	1/700
	дизент. Зонне	1/1840	1/1100
IV	брюшнотифозные	1/4160	1/2000
	паратифозные	1/1520	1/400
	дизент. Флекснера	1/5440	1/2000
	дизент. Зонне	1/940	1/75
V	брюшнотифозные	1/4500	1/3300
	паратифозные	1/2400	1/2400
	дизент. Флекснера	1/5400	1/3600
	дизент. Зонне	1/4200	1/2850
VI	брюшнотифозные	1/4800	1/2670
	паратифозные	1/2130	1/1730
	дизент. Флекснера	1/5300	1/3200
	дизент. Зонне	1/3730	1/2400
VII	брюшнотифозные	1/3470	1/2130
	паратифозные	1/600	1/930
	дизент. Флекснера	1/3730	1/2400
	дизент. Зонне	1/2670	1/2130

Note: The mean titres were determined from the sum of the titres of the 5 control or the 5 experimental animals.

A) Blood samples; B) type of agglutinins; C) mean agglutinin titres in rabbits immunized with tetravaccine mixed with; D) spreading factor and hyaluronic acid (experimental); E) physiological solution (control); F) typhoid; G) paratyphoid; H) Flexner dysentery; I) Sonne dysentery.

By dividing the titres obtained for the experimental group by the corresponding titres for the control group we obtained the arbitrarily-adopted index of effectiveness (IF): 1.7 for the typhoid agglutinins, 1.2 for the paratyphoid B agglutinins, 1.9 for the Flexner dysentery agglutinins, 1.8 for the Sonne dysentery agglutinins, and 1.7 for the

tetravaccine as a whole.

It may be seen from a comparative analysis of the indices of effectiveness that the combined immunization method which we used markedly increased the antigenicity of the Flexner dysentery, Sonne dysentery, and typhoid components of the tetravaccine. The spreading factor and hyaluronic acid affected the paratyphoid antigen only very slightly in this respect. The antigenicity of the vaccine as a whole increased by a factor of 1.7.

If we consider the problem in its entirety, for the 28 mean (control) titres shown in Table 2, 24 observations (experimental) showed an increase in the titre of agglutinins as a result of addition of the spreading factor and hyaluronic acid to the vaccine. This indicates that when rabbits are immunized with tetravaccine mixed with the spreading factor and hyaluronic acid there is an increase in the antigenicity of the vaccine as a whole (as evaluated from agglutinin formation) in 85.7% of all cases.

CONCLUSIONS

1. When rabbits are subcutaneously vaccinated with typhoid monovaccine mixed with the spreading factor there is a more intensive agglutinin formation (in comparison with the control animals) during the early stages of the immunization process. Conversely, hyaluronic acid delays the development of immune agglutinins (in comparison with the control group), stimulating their formation at a comparatively late time.

2. Subcutaneous immunization of rabbits with typhoid monovaccine by the combined method (the vaccine being mixed with the spreading factor and hyaluronic acid together) causes a marked increase in the antigenicity of the vaccine strain: the index of effectiveness equals 4.1.

3. Subcutaneous immunization of rabbits with typhoid-paratyphoid

dysentery tetravaccine mixed with the spreading factor and hyaluronic acid markedly intensifies the formation of Flexner dysentery, Sonne dysentery, and typhoid agglutinins (the indices of effectiveness equal 1.9, 1.8, and 1.7 respectively). In our opinion, the low antigenic effectiveness of the paratyphoid B antigen of the tetravaccine, both in ordinary vaccination (the mean titre for seven samples being 1/1145) and in the combined method (the mean titre for seven samples being 1/1356), resulted both from an unfortunate choice of vaccine strain and from the low dosage (250 million cells per ml of vaccine). This latter fact necessitates a qualitative and quantitative reexamination of the paratyphoid component of the tetravaccine.

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THE FACTOR OF PERMEABILITY AND HYALURONIC ACID IN PHENOMENA OF INFECTION AND IMMUNITY

Information 2. The factor of permeability and hyaluronic acid in experimental immunologic model

Alaverdyan M.I.

In the experiments with rabbits it was established that at hypodermic immunization of them by typhus mono- and tetravaccines the quickened formation of specific agglutinins takes place at the beginning of immunogenesis at injection of vaccine with the permeability factor and vice versa, slowing down at the beginning and longer producing of agglutinins – in last phases of immunizing process at injection of vaccine with gyabiromc asi. At simultaneous injection of vaccine with both preparations the summation of pointed out useful effects takes place.

THE SPREADING FACTOR AND HYALURONIC ACID IN INFECTION AND IMMUNITY

Report 3. The Spreading Factor and Hyaluronic Acid in an Experimental Immunological Model in Poikilotherms

M.I. Alaverdyan

In our preceding reports (1 and 2), which described experiments on homotherms, we showed that the spreading factor—hyaluronic acid system has a definite effect on the course of an experimental infectious process and on the antigenicity of certain enteric vaccines.

This report presents the results of experiments (on frogs), intended to clarify the nature of the influence of the spreading factor and hyaluronic acid on the immunization process in poikilotherms.

I.I. Mechnikov was the first to establish that, while phagocytosis is present in living organisms in all stages of evolutionary development, the ability to form antibodies appears only in the vertebrates; when kept under normal conditions, the cold-blooded vertebrates develop almost no antibodies. The ability of these animals to form antibodies increases when they are kept at temperatures of approximately 37°C. L.A. Zil'ber (1952) points out that Mechnikov's observations have been confirmed in all subsequent investigations and have entered the theory of immunity as solidly established facts.

The virtually complete absence of an antibody-forming function in frogs kept under natural conditions is thus indisputable. Considering

the fact that the literature sheds almost no light on the role of the spreading factor - hyaluronic acid system in infection and immunity in poikilotherms, we decided to attempt to activate the humoral component of immunity in frogs by immunizing them with a vaccine enriched with preparations of the spreading factor and hyaluronic acid.

The Department of Microbiology of the Yerevan Medical Institute isolated from the cloaca of a frog a pure culture of a microbe which, when administered parenterally (intramuscularly) to frogs, led in the overwhelming majority of cases to the development of a diffuse inflammatory process (erubescence and edema of the thigh) which usually terminated in the development of septicemia and death. Cultures of these bacilli, which the authors (B.G. Avetikyan and E.G. Shekoyan) arbitrarily called bacteria P_2, were regularly cultured from the hearts of the frogs which died.

Having at hand the means of constructing a convenient experimental model for reproducing a lethal infection in frogs with the aid of bacillus P_2, we decided to produce a vaccine from this microbe and use it in conjunction with the spreading factor and hyaluronic acid for the active parenteral immunization of frogs, later testing the effect of vaccination by inoculating the animals with a live culture of the same strain.

In essence, our experimental model was as follows. As the killed vaccine we used a heated physiological-solution washing of 4 billion cells from a 24-hour agar culture of bacterium P_2 grown at 28-30°C. Three different variants of the P_2 vaccine were employed in the experiments: 1) ordinary P_2 vaccine, 0.5 ml of the 4-billion-cell vaccine mixed with 0.5 ml of physiological solution; 2) the spreading factor and P_2 vaccine, 0.5 ml of the 4-billion-cell vaccine mixed with 0.5 ml of the spreading factor; 3) hyaluronic acid and P_2 vaccine, 0.5 ml of

the 4-billion-cell vaccine mixed with 0.5 ml of hyaluronic acid.*

We used 66 frogs, divided into four groups, in the experiment. The first, control group consisted of 30 frogs, which were not immunized. The second, third, and fourth groups were immunized: the second group (12 frogs) with ordinary P_2 vaccine, the third (12 frogs) with P_2 vaccine and the spreading factor, and the fourth (12 frogs) with P_2 vaccine and hyaluronic acid. Three immunizations were given, with intervals of five days between injections. The animals received 1 ml of vaccine intramuscularly, in the left thigh, in each immunization. Only one frog, which had been vaccinated with P_2 vaccine and hyaluronic acid died during the immunization period. No microbes were found in its bloo the cause of death was not determined. No pathological symptoms whatsoever were noted in the other 65 frogs during immunization.

Five days after the third injection all 35 vaccinated frogs were given a lethal dose (1 ml of a 4-billion-cell culture) of P_2 bacillus in the right thigh.

The intact frogs of the first (control) group were divided into 3 groups, 10 frogs in each. These 30 frogs were also inoculated with the culture of P_2 bacillus, the first group with 1 ml of the 4-billion-cell culture mixed with 0.5 ml of physiological solution, the second with 1 ml of the 4-billion-cell culture mixed with 0.5 ml of hyaluronic acid, and the third group with 1 ml of the 4-billion-cell culture mixed with 0.5 ml of the spreading factor.

Inoculation of intact (unvaccinated) frogs with a virulaent culture of P_2 bacillus mixed with the spreading factor and hyaluronic acid thus served our purpose, to study the nature of the influence of these preparations on an infectious process in frogs. There was another reason behind the immunization of the experimental frogs with P_2 vaccine mixed with the spreading factor and hyaluronic acid; we wished to

etermine the characteristics of the effect of these preparations on he development of artificial active immunity in experimental animals.

After inoculation of all the animals, which were kept under natural onditions, we conducted prolonged observations (for 1 month). All of he frogs which died were dissected and the cause of death (P_2 bacillus) as determined by culturing blood from their hearts on simple nutri-ive media.

The results of the investigations made on the immunized frogs are given in Table 1.

In analyzing Table 1 we may see the following. Of the 35 immunized animals 22 died and 13 survived, regardless of the type of vaccination (with physiological solution, with the spreading factor, or with hyaluronic acid). In the animals immunized with P_2 vaccine alone death occurred, on the average, on the 19th day; in the frogs which received the vaccine mixed with the spreading factor or with hyaluronic acid death occurred, on the average, on the 25th day. This slight difference may be neglected for all practical purposes.

It is clear from what has been said that addition of the spreading factor and hyaluronic acid to P_2 vaccine yields absolutely no results.

Table 2 shows the results of the experiments on the unimmunized control animals.

It may be seen from Table 2 that of the 30 intact frogs inoculated with a culture of P_2 bacteria 24 died and 6 survived. All of the frogs inoculated with P_2 vaccine alone died; 2 frogs survived in the group inoculated with the microbial culture mixed with the spreading factor and 4 frogs in the group inoculated with the culture mixed with hyaluronic acid. It may be seen from this that there is no marked regularity in this case either; it would seem that inoculation of frogs with the culture of P_2 bacillus mixed with the spreading factor caused

a higher mortality, while the frogs which received the microbial culture alone exhibited the greatest mortality. On the average, death occurred among the animals inoculated with the culture of bacillus P_2 alone on the 21st day; among the frogs which received the culture mixed with the spreading factor death occurred, on the average, on the 28th day and among the frogs injected with the culture mixed with hyaluronic acid death occurred, on the average, on the 16th day.

TABLE 1

Outcome of an Infectious Process Induced by Intramuscular Injection of a Culture of P_2 Bacteria in Frogs Immunized With Various Types of Homologous Vaccine

Вакцина P_2 с физиологическим раствором A		Вакцина P_2 с фэктором проницаемости D		Вакцина P_2 с гиалуроновой кислотой E	
B № лягушек	C день наступления смерти после заражения	№ лягушек	день наступления смерти после заражения	№ лягушек	день наступления смерти после заражения
1	12	13	12	25	*)
2	12	14	15	26	14
3	12	15	21	27	19
4	15	16	30	28	19
5	19	17	32	29	20
6	23	18	32	30	25
7	28	19	33	31	26
8	31	20	выжила	32	29
9	выжила F	21	"	33	выжила
10	"	22	"	34	"
11	"	23	"	35	"
12	"	24	"	36	"

*This frog died during immuniation, from an indeterminate cause.

A) P_2 vaccine with physiological solution; B) frog number; C) day after inoculation on which death occurred; D) P_2 vaccine with the spreading factor; E) P_2 vaccine with hyaluronic acid; F) survived.

In other words, the animals which received the microbial culture mixed with hyaluronic acid died sooner. The situation should have been precisely the opposite, since hyaluronic acid precipitates

TABLE 2

Outcome of an Infectious Process Induced by Intramuscular Injection of a Culture of P_2 Bacteria in Unvaccinated Frogs

№ лягушек (A)	День наступления смерти после заражения (B)	№ лягушек	День наступления смерти после заражения	№ лягушек	День наступления смерти после заражения
37	5	47	19	57	8
38	10	48	20	58	12
39	14	49	22	59	14
40	22	50	32	60	19
41	24	51	32	61	22
42	25	52	32	62	24
43	27	53	32	63	выжила
44	27	54	32	64	"
45	27	55	выжила (C)	65	"
46	29	56	"	66	"

A) Frog number; B) day after inoculation on which death occurred; C) survived.

microbes and should have inhibited the development of septicemia in the frogs. In similar fashion the death on the 28th day of the frogs inoculated with the culture mixed with the spreading factor is contrary to our generally-accepted concepts of the nature of this substance; instead of accelerating the development of the infectious process it prolonged it.

The data obtained in these experiments thus make it clear that it is impossible to detect in an experimental infection in poikilotherms the characteristic biological effect which we customarily attribute to the spreading factor and hyaluronic acid; both of these preparations behave atypically in an infected frog and lose their widely known importance in infection and immunity.

On the basis of the above we believe it logical to assume that the permeability factor – hyaluronic acid system does not play the important and widely known role in infection and immunity in poikilotherms which it undoubtedly fulfills in homotherms. It seems to us that this results from three facts: first of all, we have established that it is

impossible to detect any traces of hyaluronic acid in the body (muscles and skin) of a frog and this naturally greatly limits the possibility of enzymatic action on the part of the corresponding enzyme (hyaluronidase). Secondly, we have not found any indications in the literature accessible to us of the presence of substances similar to the spreading factor in the bodies of frogs. Thirdly, the biological environment which surrounds the bodies of poikilotherms prevents any manifestation of the enzymatic function of the spreading factor.

All of the material which has been presented gives us reason to evaluate the spreading factor - hyaluronic acid system as an obligatory attribute of homotherms and calls into doubt its importance (if not its very existence) for poikilotherms.

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THE FACTOR OF PERMEABILITY AND HYALURONIC ACID IN PHENOMENA OF INFECTION AND IMMUNIZATION

Information 3. The factor of permeability and hyaluronic acid in experimental immunologic model on cold-blooded animals

Alaverdyan M.I.

In result of studying the character of influence of the permeability factor and hyaluronic acid on the course of infection process and efficiency of active immunization by frogs an assumption is made that by cold-blooded system permeability factor-hyaluronic acid does not play that important part that it plays by warm-blooded. In particular, this system proves to be indifferent at growth, in experiments on frogs, infection and immunizing processes.

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[Footnote]

263 The method for obtaining preparations of the spreading factor and the hyaluronic acid is described in Report I.

SENSITIZING ACTION OF EXTRACTS OF LISTERELLA CULTURES ON ERYTHROCYTES*

A.A. Tripolitova

The Soviet and foreign literature now contains a great many works in which it is shown that extracts of cultures of various bacteria and viruses sensitize human and animal erythrocytes, causing them to be agglutinated by specific sera. Since agglutination occurs as a result of interaction between the antigens adsorbed on the erythrocytes and the antibodies to these antigens, it has come to be called indirect, passive, or conventional hemagglutination.

Either normal erythrocytes or erythrocytes preliminarily treated with tannin are used for treatment with antigens. Normal erythrocytes adsorb primarily the polysaccharide and lipopolysaccharide components of microbial antigens, while the protein components are adsorbed best by "tanned" erythrocytes.

This report presents data obtained in studying the sensitizing properties of extracts of various strains of listerella with respect to sheep corpuscles. This problem is of some interest, since when specific positive data are obtained sensitized erythrocytes may be used as antigens for detecting antibodies to listerella in sera and, when the reaction has acquired sufficient sensitivity, for detecting antigens in various materials to be analyzed.

After preliminary experiments we used the following method for solving the problem we had set outselves.

TABLE 1

Agglutination of Sensitized Erythrocytes With Antilisterellosis Sera

Количество испытанных антигенов A	№№ сывороток B	Титр сывороток C	Количество антигенов агглютинирующихся D			Количество неагглютинирующихся антигенов H
			выше титра E	до титра F	ниже титра G	
12	2741	1:640	10	2	—	—
37	14,57	1:320	—	15	22	—
37	15,57	1:320	—	4	33	—
37	16,57	1:160	11	21	5	—
37	17,57	1:80	—	3	34	—

A) Number of antigens tested; B) serum number; C) titre of serum; D) number of antigens agglutinated; E) higher titre; F) same titre; G) lower titre; H) number of nonagglutinated antigens.

1. Preparation of antigen for erythrocyte sensitization. Listerella were cultured on dishes containing glucose agar (0.1% glucose); after being permitted to grow for 18-24 hours at 37° the culture was washed with physiological solution (4-5 ml per dish) and heated in a water bath containing boiling water for one hour. This procedure caused the microbial bodies to be precipitated on the bottom of the vessel, while an almost clear yellowish liquid remained above the precipitate; this liquid was used for sensitizing the erythrocytes. When the liquid above the precipitate was not completely clear it was centrifuged. Antigens were prepared in this manner from standard and freshly-isolated strains of listerella.

2. Preparation of sensitized erythrocytes. Two parts of a 2.5% suspension of washed sheep corpuscles in physiological solution were added to one part of the clear extract; the mixture was kept at 37° for one hour and the erythrocytes were then removed by centrifuging. After being washed twice with physiological solution to eliminate any excess of antigen the residue of sensitized erythrocytes was resuspended in physiological solution in such fashion that a 2.5% suspension was produced.

3. Preparation of specific sera. Rabbits were immunized either with a suspension of the killed listerella or with extracts from the boiled cultures. At the beginning of the work we used strain 2741, which G.F. Pogonyaylo isolated from the corpse of an immature sow,* for preparing the antigen; we later used standard strains of listerella obtained from Czechoslovakia. The titres of the sera were determined by indirect hemagglutination reactions with erythrocytes sensitized with antigens of the appropriate strains.

4. Setting up the reactions. We prepared a series of dilutions, in multiples of two, of inactivated antilisterelosis serum and added 0.1 ml of a 2.5% suspension of sensitized erythrocytes to 0.5 ml of each dilution. Normal erythrocytes were added to the same serum dilutions as a control. The test tubes were agitated vigorously and kept at room temperature. The reaction was noted every two hours, but clearer results were obtained on the following day. The intensity of the reactions was indicated by crosses. The reaction was easily read with the aid of a concave mirror.

The purpose of the first series of experiments was to determine whether erythrocytes were sensitized by antigens from different strains of listerella, whether it was possible to detect changes produced in the erythrocytes by the sera used in the experiments, and at what serum dilutions agglutination occurred. In these experiments, in addition to the strains used for immunizing the rabbits, we tested strains of different origins which we had available, including those isolated from various animals (sheep, young pigs, sable, guinea pigs, etc.) in Tomskaya Oblast. For graphicness, in Table 2 the antigens (sensitized erythrocytes) tested are arranged in accordance with the degree of serum dilution at which marked hemagglutination was observed. The dilutions of serum 2741 ranged from 1:40 to 1:10,260, while those of the

other four ranged from 1:20 to 1:320.

As the data cited show, there was not a single one of the antigens which we tested which failed to enter into reaction with the specific antilisterellosis sera. The only difference noted was in their distribution with respect to the serum dilutions at which marked agglutination began. Sera 2741 and 16/57 proved to be the most active. The former agglutinated all of the antigens and the latter the majority (32 of 37) of the antigens in the same titre or higher. Serum 16/57 agglutinated the remaining 5 antigens in a dilution of 1:80; sera 14/57 and 17/57 agglutinated the majority of antigens in dilutions amounting to one-half or one-fourth of their titres. Serum 15/57 agglutinated only four of the 37 strains when diluted to 1/8 of its titre, agglutinating the others in greater dilutions. There was not a single case in which the normal erythrocytes and the erythrocytes sensitized with antigen from Erysipelothrix rhusiopathiae, which were used as the control, were not agglutinated at the lowest serum dilution.

TABLE 2

Attenuation of Indirect Hemagglutination Reaction by Extracts of Listerella Cultures

Количество испытанных антигенов A	Реакция с сывороткой 14/57 B				Реакция с сывороткой 14/57, обработанной экстрактом культуры 14/57 C			
	1:40	1:80	1:160	1:320	1:40	1:80	1:160	1:320
14	—	2	6	6	2	2	—	—

A) Number of antigens tested; B) reaction with serum 14/57; C) reaction with serum 14/57, treated with extract of culture 14/57.

The next series of experiments was carried out in order to establish the specificity of the hemagglutination reaction from its attenuation by the appropriate antigens.

This reaction was set up in the following fashion. The antilisterellosis sera were diluted with physiological solution to produce

dilutions of 1:20 to titre. To 0.25 ml of each dilution we added 0.25 ml of extract from the corresponding culture diluted to 1:10. Physiological solution was added to the control test tubes in place of the culture extracts. The test tubes were kept in a heater for 30 min., and sensitized erythrocytes were then added to them. The procedure thenceforth was the same as in the first series of experiments.

Table 2 gives the composite results for one of these investigations.

With this experimental setup we observed either a complete lack of hemagglutination or the formation of hemagglutinate at low serum dilutions. Similar results were obtained in experiments with other antigens.

Our data thus indicated that it is possible to sensitize erythrocytes with soluble listerella antigens and confirmed the specificity of this reaction.

The ease of preparing the antigen and the convenience of observing the reaction gives us a basis for studying it further in order to find out whether it is possible to use it to determine antibodies to listerella and listerellosis antigen.

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Tomsk Scientific Research Institute for Vaccines and Sera

ABOUT SENSIBILIZED ACTION ON ERYTHROCYTES OF EXTRACTS FROM LISTERIA CULTURES

Tripolitova A.A.

The possibility of sensibilization of ram's erythrocytes by extracts of boiled cultures of listeria with the help of reaction of indirect hemagglutination and specific of this reaction by the method of extinguishing it by extracts of corresponding cultures were studied. It is shown that extracts of boiled cultures of listeria sensibilize

erythrocytes, in consequence they are agglutinated by specific antiserums. Preliminary adding to serums the extracts of corresponding cultures extinguishes the reaction of hemagglutination that is a witness of its specific.

Obtained data give the base for further study of reaction of indirect hemagglutination with the aim of discovering antibodies against listeria in serums and listeria antigens in different materials.

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[Footnotes]

268 Presented at a combined conference of the TomNIIVS [Tomsk Scientific Research Institute of Vaccines and Sera] and the N.F. Gamaley Institute of the Academy of Medical Sciences USSR, 29 May 1958.

270 We are obliged to express our gratitude to Doctor A.P. Tarasova (LenNIVI [Leningrad Scientific Research Veterinary Institute]) for the strain that he contributed.

THE INDIRECT HEMAGGLUTINATION REACTION IN THE IMMUNOLOGY OF LISTERELLOSIS*

A.A. Tripolitova

The in vivo bacteriological diagnosis of listerellosis is associated with certain difficulties. These result, on the one hand, from the fact that when physicians are not sufficiently well acquainted with the symptomatology of this disease the material for bacteriological examination is taken too late, frequently after various antibacterial preparations have been administered, and, on the other hand, from the fact that insufficient research has apparently been done on the fate of listerella in the human body, there being no exhaustive data on the phases of the disease during which listerella are present in the peripheral blood where they are most frequently sought, and on how long they persist in the spinal fluid in the meningoencephalitic forms of the disease.

Study and evaluation of various methods for the serological diagnosis of listerellosis are extremely important in this connection. The works of Soviet (P.P. Sakharov and Ye.I. Gudkova, A.A. Smorodintsev and V.I. Il'yenko, S.P. Karpov, A.P. Tarasova, O.G. Shpinbakh, G.I. Khomenko, V.A. Matsiyevskiy, and O.P. Lebedeva) and foreign (Seeliger, Potel, Drew, Seeliger, and Linzenmeier) investigators describe comparative studies of the agglutination, precipitation, and complement-fixation reactions.

Various authors have obtained data which force us to approach agglutination-reaction indices very carefully in diagnosing listerellosis. The precipitation reaction has not been widely used. The complement-fixation reaction has given encouraging results and is being subjected to ever wider study.

Our attention was struck by the indirect, or passive, hemagglutination reaction. According to the data of many investigators, this reaction is a convenient, precise, and sensitive diagnostic method. There is very little information on its importance with respect to listerellosis. In the literature accessible to us we found a report that Potel and Griziben verified the suitability of this reaction for the ordinary diagnosis of listerellosis, while Seeliger says that it may possibly be used to improve the diagnosis of this disease.

This report presents the results of a study of sera from humans and various animals with the aid of the indirect hemagglutination reaction (RNGA). The method of preparing the sensitized erythrocytes and

TABLE 1

Titres of Antibodies in Guinea Pig Sera

№ A серий	Вид сыворотки B	Количество свинок C	D Титр сыворотки	
			E в РНГА	F в РА*)
I	Нормальная G	10	1:4	1:10
II	Иммунная H	19	1:64	1:40
III	"	10	1:512	1:80
IV	"	20	1:512	1:40

*RA designates agglutination reaction.

A) Series number; B) type of serum; C) number of guinea pigs; D) titre of serum; E) in RNGA; F) in RA.*

of setting up the reaction are described in the article "Sensitizing Action of Extracts of Listerella Cultures on Erythrocytes" (see p. 268, this book). These sera were simultaneously tested with a corpuscular antigen (a formalized listerella culture) in the ordinary agglutination reaction.

We were interested in the relationship of the titres of antibodies to soluble and corpuscular listerella antigens in normal and immune sera. We had at our disposal sera from normal and immunized guinea pigs, rabbits, horses, and cows. In addition, serum obtained from donors at the oblast blood-transfusion station were tested to detect natural antibodies to soluble listerella antigens. The data obtained are given below.

Investigation of Guinea Pig Sera

Four series of sera were studied: one was normal, having been taken before immunization, while three were from animals immunized with various preparations containing corpuscular listerella antigen in addition to simple antigens. The sera were tested in dilutions ranging from 1:2 to 1:512. The serum titres are shown in Table 1.

It may be seen from the table that the serum from the normal guinea pigs contained only a small quantity of natural antibodies to

soluble listerella antigens. Immunization of the animals led to an increase in the titre of antibodies, which was occasionally very substantial (by a factor of 128 in series III and IV). Normal hemagglutinins were detected only in the serum of series III diluted to 1:2.

It is interesting to note that agglutinins were detected in the sera of all series except the normal series in lower titres than in the RNGA. This was found only in investigating the guinea pig sera. In all other cases the titres of agglutinins exceeded the titres of antibodies to the soluble listerella antigen.

Investigation of Rabbit Sera

A study of the accumulation of antibodies during immunization and of natural antibodies in rabbit sera was made by R.S. Karpov, a fourth-year student and member of the Science Students' Association in the Department of Microbiology of the Tomsk Medical Institute. The data which he obtained are reported in the article "The Indirect Hemagglutination Reaction as a Method of Determining Increases in Antibodies to Listerella"(in this book).

We must call attention to the fact that the titres of agglutinins in the rabbits usually exceeded the titres of antibodies to soluble listerella antigens. This apparently resulted from the fact that the rabbits were immunized with a corpuscular rather than a soluble antigen. The ratio of the RA indices to the RNGA indices varied considerably for the different sera, indicating almost complete correspondence at one extreme and a difference amounting to a factor of 10 at the other.

We obtained similar data in studying sera which we prepared from rabbits immunized with extracts of enteric cultures; these confirmed that there are no normal hemagglutinins to sheep corpuscles in rabbit sera diluted to 1:10 or more, that there is only a small quantity of

TABLE 2

Antibody Titres in Horse Sera

№ лошадей B / Этапы иммунизации A	148	147	149
После I цикла C	1:50	.	—
Перед началом II цикла D	1:50	.	—
Проба после II цикла E	1:800	1:100	—
I кровопускание после II цикла F	> 1:800 — < 1:1600	1:100	—
II кровопускание после II цикла G	> 1:800 — < 1:1600	1:100	—
После III цикла H	> 1:800 — < 1:1600	.	—

A dot indicates that no investigation was made; a dash indicates that there were no antibodies.

A) Stage of immunization; B) horse number; C) after cycle I; D) before cycle II was begun; E) sample taken after cycle II; F) phlebotomy I after cycle II; G) phlebotomy II after cycle II; H) after cycle III.

natural antibodies to soluble listerella antigen (the RNGA was positive for dilutions of 1:10-1:20), and that there is a sharp rise in the titre of these antibodies during immunization (to 1:1280 or more). The titre of agglutinins in the sera of the rabbits immunized with soluble listerella antigen was the same as or somewhat lower than the titre of antibodies to soluble antigens.

Investigation of Horse Sera

Sera from three horses (Nos. 148, 147, and 149) were studied. The first horse was immunized with a complex corpuscular antigen which included killed listerella, the second with a still more complex preparation consisting of various corpuscular antigens and a mixture of toxoids, and the third with a mixture of toxoids. Serum from this last horse was used as the control. The results of the investigation are shown in Table 2.

The data given indicate that antibodies accumulated in the sera of the immunized horses. This was especially striking in horse No. 148. Antibodies were detected in lower titres in horse No. 147, possibly as

a result of the complexity of the antigen but perhaps because of individual peculiarities; however, they were clearly observed in serum diluted to 1:100, while they were never found in the serum of horse No. 149.

Further investigations were carried out to detect natural antibodies in the sera of horses and cows. Ye. Nam, a third-year student, and Yu. Odintsov, a second-year student, both members of the Science Students' Association of the Department of Microbiology of the Tomsk Medical Institute, participated in this portion of the work. The data obtained are shown in Table 3.

The data given in the table show that the majority (227 of 237) of the horse sera agglutinated the erythrocytes in dilutions up to 1:80. Only isolated specimens markedly agglutinated the sensitized erythrocytes in higher dilutions (10 sera in dilutions of 1:160-1:320). The titres of the cow sera were even lower. 14 of the 15 samples agglutinated the erythrocytes in dilutions of up to 1:20. The small number of cow serum samples investigated makes it impossible to draw definite conclusions about the titres of antibodies to soluble listerella antigens.

The ratio between the titres of antibodies to soluble and corpuscular listerella antigens may be illustrated by the results of titration of sera from two groups of horses, one comprising animals in quarantine and the other producers of antitetanus serum (see Table 4).

It may be seen from the table that the titres of agglutinins in the majority of the sera were higher than the titres of antibodies to soluble listerella antigens, as determined by the RNGA. The ratio of these titres varied in different horses, indicating complete correspondence at one extreme and a difference amounting to a factor of eight or sixteen at the other. These data on the high titres of agglutinins

in horse sera agree with those in the literature. It is interesting to note that when the sera were purified and concentrated by the "Diaferm 3" method developed by the Academy of Medical Sciences they were almost entirely freed of listerellosis agglutinins. Before purification the 7 series of sera which we tested agglutinated listerella in dilutions of 1:160-1:320; after purification the agglutinins either disappeared entirely or appeared in dilutions of 1:10-1:20.

TABLE 3

Natural Antibodies to Listerella in Sera of Various Origins

A Количество исследованных сывороток	B Вид сыворотки	C Происхождение сыворотки	D Положительных в разведении 1:10	1:20	1:40	1:80	1:160	1:320
82	E Лошадиная	от карантинных лошадей ТомНИИВС G	3	7	38	25	7	—
34	•	от колхозных лошадей H	•	6	2	—	1	—
28	•	• I	•	15	3	6	—	1
36	•	от продуцентов п/столбнячной сыворотки	•	19	3	4	—	—
43	•	от продуцентов п/дифтерийн. сыворотки J	•	12	2	—	—	—
14	•	от продуцентов п/энцефал. сыворотки K	—	6	5	2	1	—
15	F коровья	от колхозных коров L	3	3	1	—	—	—

A) Number of sera investigated; B) type of serum; C) origin of serum; D) positive in dilutions of; E) horse; F) cow; G) from quarantined horses, Tomsk Scientific Research Institute for Vaccines and Sera; H) from farm horses; I) from producers of antitetanus serum; J) from producers of antidiphtheria serum; K) from producers of antiencephalitis serum; L) from farm cows.

TABLE 4

Results of Comparative Titration of Sera

A Количество образцов	B Положительных в РНГА 1:20	1:40	1:80	1:160	C Положительных в РА 1:40	1:80	1:160	1:320	1:640	1
29	12	3	4	—	1	1	10	10	5	1
77	7	35	23	7	4	5	19	35	12	—

A) Number of specimens; B) positive in RNGA; C) positive in RA.

TABLE 5

Serum Titres in Hemagglutination Reactions With Normal and Sensitized Erythrocytes

Количество сывороток (A)	Титры в реакции гемагглютинации (B)											
	с сенсибилизированными эритроцитами (C)						с нормальными эритроцитами (D)					
	1:10	1:20	1:40	1:80	1:160	1:320	1:10	1:20	1:40	1:80	1:160	1:320
91	4	10	26	30	12	7	5	20	34	16	7	2

A) Number of sera; B) titres in hemagglutination reaction; C) with sensitized erythrocytes ; D) with normal erythrocytes.

We wish to dwell separately on the results which we obtained in investigating human sera. Donor sera were used for this study. It must be noted that normal agglutinins to sheep corpuscles are detected in human sera in rather high dilutions. It was stated above that this phenomenon was no hindrance to us in animal sera, since at the dilutions at which the sera were tested they did not agglutinate normal erythrocytes.

In order to avoid this in the human sera it was necessary to study them after first depleting these agglutinins with normal sheep corpuscles or human erythrocytes (group O) used for preparing sensitized erythrocytes. However, we deliberately worked with sheep corpuscles for the following reasons. Certain investigators have noted changes in the indices of the Paul-Bunnell reaction during listerellosis. These changes reduce to an increase in the titre of antibodies to sheep corpuscles in the patient's serum. In order to have some basis for evaluating the indices of the Paul-Bunnell reaction in various patients, as antigens in investigating the donor sera we used sensitized (in the RNGA) and normal (in the RGA, or hemagglutination reaction) erythrocytes. We were interested in establishing what causes hemagglutination to occur in the sera of healthy persons, the hemagglutinins to the sheep corpuscles or the antibodies to the soluble listerella antigens.

Table 5 shows the results of this investigation of donor sera with normal and sensitized erythrocytes.

It may be seen from the table that the serum titres established by the normal and sensitized erythrocytes are very close in value, the discrepancy between them being slight. Thus, in the 61 cases in which the titres did not correspond a difference of one dilution occurred 45 times, a difference of two dilutions 10 times, and a difference of more than two dilutions 2 times. The high RNGA indices in the donor sera thus resulted from the presence of hemagglutinins to the sheep corpuscles rather than from antibodies to the soluble listerella antigens.

The titres of agglutinins to corpuscular listerella antigens in the donor sera varied from 1:40 to 1:320. The majority of the sera agglutinated the listerella culture in a dilution of 1:80.

Our data are still far from sufficient to enable us to draw definitive conclusions; more material must be amassed for this. However, we may state with certainty that the indirect hemagglutination reaction can be used for detecting antibodies to listerella in human and animal sera.

Our next task is to study sera from patients with various diseases, using antigens from standard listerella strains.

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THE REACTION OF INDIRECT HEMAGGLUTINATION IN IMMUNOLOGY OF LISTERIOSIS

Tr olitova A.A.

Men's and animals' serum were studied with the helf of reaction of indirect hemagglutination and agglutination with corpuscular antigen. It is established that in serums of normal guinea-pigs, rabbits

and healthy people antibodies to soluble antigens of listeria are found out in very small amounts. Some higher titres are determined in serums of horses. The immunization by listeria antigen leads to considerable growth of the titre of antibodies defined by RIHA. The titres of antibodies to corpuscular antigens of listeria defined by the reaction of agglutination considerably exceed titres of antibodies to soluble antigens. The reaction of indirect hemagglutination may be used for defining natural and artificial antibodies to listeria in men's and animals' serums.

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274 Presented at a combined conference of the TomNIIVS [Tomsk Scientific Research Institute of Vaccines and Sera] and the N.F. Gamaley Institute of the Academy of Medical Sciences USSR, 29 May 1958.

PREPARATION OF LISTERELLA ANTIGEN FOR THE INDIRECT HEMAGGLUTINATION REACTION

L.A. Burenkova

A great many works have recently appeared in the literature on the use of the indirect hemagglutination reaction for the serological diagnosis of disease. Bacterial hemagglutination of this type was first described in 1947 by Keogh, North, and Warburton. These authors showed that antigens obtained from Hemophilus influenzae are adsorbed well on human and animal erythrocytes; erythrocytes sensitized in this manner acquire the ability to agglutinate in the presence of a homologous antibacterial serum. This indirect, or passive, hemagglutination was then employed by a number of other investigators (Middlebrook and Dubo, Hayes, Neter, Rytsay, Shwartzman and Perova, et al.). It was demonstrated that this reaction is very sensitive in determining bacterial antibodies.

Since A.A. Tripolitova's investigations (1957) of the possibility of using the indirect hemagglutination reaction (RNGA) for listerellosis yielded positive results we decided to devise a method of preparing the antigen for this reaction and to determine how long it retains its activity.

The bacterial antigens which can be adsorbed by erythrocytes are the soluble components liberated by bacteria treated in various ways. We used the following method of preparing the antigen for the RNGA: a day-old agar culture of listerella was heated in a bath containing

boiling water for one hour, the heated suspension was centrifuged, and the liquid above the precipitate was used as the antigen, 0.5% carbolic acid or merthiolate in a dilution of 1:10,000 being added as a preservative.

We first had to decide what density to use for the microbial suspension employed in preparing the antigen. In our experiments we used microbial suspensions containing from 500 million to 40 billion microbial bodies.

Our observations showed that an antigen prepared from a microbial suspension containing from 500 million to 10 billion microbial bodies causes hemagglutination in a titre of 1:640. Denser microbial suspensions (in our investigation, those containing from 11 billion to 40 billion microbial bodies) reduce the hemagglutination titre to 1:320.

In subsequent experiments erythrocytes were sensitized with reduced concentrations of soluble antigen, the latter being diluted with physiological solution to from 1:5 to 1:320 for this purpose.

The data obtained in these investigations showed that the hemagglutination titre of the serum was the same when the erythrocytes were sensitized with the undiluted antigen and the antigen diluted to 1:5. When the concentration of the antigen was further reduced, beginning with a dilution of 1:10, the hemagglutination titre dropped in parallel with the increase in dilution and disappeared completely at an antigen dilution of 1:320.

The next series of experiments was carried out to study the time necessary for sensitization of erythrocytes with the soluble antigen. Erythrocytes were treated for different periods of time (15, 30, 60, 90, and 120 min.). Our observations showed that the erythrocytes treated with antigen for these different periods of time (15, 30, 60, 90, and 120 min.) were agglutinated in the same dilution (1:640) by

antilisterellosis serum.

We also studied the importance of the concentration of the erythrocytes used to set up the RNGA. The erythrocytes were added to the centrifugate of the boiled listerella culture so as to obtain erythrocyte concentrations of 5, 2.5, 1.25, and 0.625%. The best results were obtained with an erythrocyte concentration of 2.5%.

After the observations described above had been made we prepared 7 series of listerella antigen for the RNGA. Carbolic acid (0.5%) and merthiolate diluted to 1:10,000 were tested as preservatives, being added to the antigen on the day of its preparation. After one week we checked the sterility of the antigen and tested it in the RNGA (antigen without preservative was used as the control). The activity of the antigen was checked monthly.

Our observations showed that the activity of RNGA listerella antigen preserved with 0.5% carbolic acid or merthiolate diluted to 1:10,000 is maintained for no less than 12 months (the observation period).

The data obtained enable us to draw the following conclusions:

1. A microbial suspension containing from 500 million to 10 billion microbial bodies may be used for preparing listerella antigen for the RNGA.

2. Soluble listerella antigen diluted to 1:5 with physiological solution is just as effective as undiluted antigen.

3. Prolonged sensitization of the erythrocytes does not affect the increase in the titre of specific antiserum. It is quite sufficient to treat the erythrocytes with the antigen for 15 minutes.

4. RNGA listerella antigen preserved with merthiolate diluted to 1:10,000 or 0.5% carbolic acid retains its original properties for no less than 12 months (our observation period).

Tomsk Scientific Research Institute for Vaccines and Sera

TO THE QUESTION OF PREPARING LISTERIAL ANTIGEN FOR REACTION OF INDIRECT HEMAGGLUTINATION

Burenkova L.A.

The precipitation got by centrifugation of boiled culture of listeria the activity of which was preserved no less than five months was used as antigen.

THE INDIRECT HEMAGGLUTINATION REACTION AS A METHOD OF DETERMINING INCREASES IN ANTIBODIES TO LISTERELLA*

R. Karpov

It is well known that human and animal erythrocytes may be successfully used for diagnosing infectious diseases.

In 1941 Hirst and McClelland and Hare independently described viral hemagglutination. They observed an agglutination of erythrocytes in the presence of influenza virus. This hemagglutination is specifically suppressed by homologous antiviral serum. A large number of other viruses having hemagglutinative properties were later discovered. Viral hemagglutination has come into wide use for the identification and titration of hemagglutinative viruses and for the determination and titration of the corresponding antibodies.

A whole series of bacteria which cause agglutination of erythrocytes have been described. This direct hemagglutination does not require the presence of antibodies. It has not found any use in bacteriological diagnosis.

A considerable number of works describing studies of so-called indirect (passive or conditional) hemagglutination have recently appeared in the bacteriological literature. Various authors used this reaction for different diseases (tuberculosis and diseases caused by enteric bacteria, staphylococci, streptococci, etc.). Investigators have noted its high specificity and sensitivity in a number of cases.

Rytsay's data are extremely interesting; using the indirect hemagglutination reaction this author was able to determine botulin toxin in canned foods in quantities too small to be detected by the biological methods ordinarily employed. Because of its sensitivity the indirect hemagglutination reaction can be used to settle immunological problems which cannot be solved by ordinary methods. Thus, Stavisskiy employed it to determine antitoxin formation in tissues outside the organism (tissue cultures). In this connection it is of both theoretical and practical interest to test this method of serological analysis with respect to listerellosis.

Sera from rabbits immunized with various listerella antigens* were studied. A total of 207 serum samples were investigated with the aid of the RNGA. At the beginning of the work we obtained sera from different groups of animals. The rabbits in the first two groups passed through a cycle of immunization with corpuscular vaccines. One group received a suspension of listerella killed by heating (at 65°C for one hour), while the other received microbes killed by exposure to chloroform vapor. We were able to follow the titres of antibodies to soluble listerella antigens in the sera of these two groups only after immunization ended. Immunization of the rabbits in the third and fourth groups had just begun and we were able to follow the changes in the titre of antibodies to the soluble listerella antigen during immunization.

In this work we used the method described in A.A. Tripolitova's article "Sensitizing Action of Extracts of Listerella Cultures on Erythrocytes" (p. 268, this book).

Results obtained. Using the RNGA we detected antibodies to soluble listerella antigens in the sera of the first and second groups of rabbits,which were immunized with the heat- and chloroform-killed

vaccines. The titre of these antibodies varied from 1:80 to 1:2560. It did not always depend directly on the titre of agglutinins determined by the ordinary agglutination reaction. Thus, a titre of antibodies to the soluble antigen of 1:1280 was noted in rabbits with agglutinin titres of 1:1600 and 1:12,800. From our first investigations we noted a gradual decrease in the titres of antibodies to soluble antigens in the first two groups. The titres of agglutinins continued to increase and began to decrease somewhat later. In these experiments our attention was struck by the fact that, when the titres of agglutinins were considerably lower than those in the first group (see the second group), the titres of antibodies determined by the indirect hemagglutination reaction were higher.

TABLE 1

Titres of Agglutinins and Antibodies to Soluble Listerella Antigens

№№ групп A	Количество кроликов B	Антиген, послуживший для иммунизации C	Период иммунизации D	E Средние титры сывороток при кровопускании I	II	III	IV	V	VI	VII	VIII
I	5	Гретый F	По окончании цикла иммунизации K	1200/6000	688/1024	650/11570	488/3850	272/3520			
II	4	Хлороформированный G		1600/1150	1120/4000	1120/3730	1120/11750	1200/1300			
III	5	Мертиолятовый H	В процессе иммунизации L	14/24	6/60	240/17920	704/25600	768/35850	1920/15360	704/10240	320/5760
IV	5	Карболизованный I	.	—	4/90	128/2240	1152/2560	1600/5760	3072/5120	1472/3280	736/3520
V	4	Формализированный J	.	0/100	10/175	185/325	1280/4600	2880/28800	2080/22400		
VI	4	.		20/120	128/175	70/175	1280/32555	1120/38400	587/17070		

* The numerator shows the titres determined from the RNGA and the denominator those determined from the RA.

A) Group number; B) number of rabbits; C) antigen used for immunization; D) immunization period; E) mean serum titres on pnlebotomy; F) heat-killed; G) chloroform-killed; H) merthiolate-killed; I) carbolic-acid-killed; J) formalin-killed; K) at end of cycle; L) during immunization.

The rabbits in the third group were immunized with vaccine killed with merthiolate; vaccine killed with carbolic acid was used as the antigen for the fourth group. There was a gradual increase in anti-

bodies to the soluble listerella antigen in the sera of these groups. The highest antibody titres were noted after the sixth phlebotomy; in this experiment the ordinary agglutinins reached their highest level after the fifth phlebotomy and, consequently, began to decrease somewhat earlier. In this group of sera we noted higher antibody titres, as determined from the RNGA, in those series in which the agglutinin titres were somewhat lower.

We obtained similar data in the fifth and sixth groups of sera, where the rabbits were immunized with the same antigen, the only difference being in the immunization scheme.

Table 1 shows a comparison of the mean antibody titres which we determined with the aid of the indirect hemagglutination reaction and the data obtained from the agglutination reaction.

Conclusion. The investigations which we conducted showed that when rabbits are immunized with corpuscular listerella vaccines an increase in antibodies to soluble listerella antigens occurs simultaneously with agglutinin formation. These antibodies are very clearly determined by the indirect hemagglutination reaction. It is a matter of some interest to study other variants of this reaction, particularly that involving tannin-treated erythrocytes, for evaluating immune antilisterellosis sera.

Science Students' Association, Department of Microbiology, Tomsk Medical Institute

REACTION OF INDIRECT HEMAGGLUTINATION AS THE METHOD OF DEFINITION OF ANTIBODY'S GROWING ON LISTERIA

Karpov R.

The pcssibility of using the reaction of indirect hemagglutination for the definition of dynamics of antibodies at immunization of rabbits by corpuscular antigens of listeria was studied. It was shown

that at the same time with the growing of titre of agglutinins the increase of the quantity of antibodies to soluble antigens of listeria, which are clearly defined by the reaction of indirect agglutination, takes place.

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[Footnotes]

287 Presented at student scientific conferences of the Tomsk and Novosibirsk Medical Institutes (April 1958).

288 We take this opportunity to express our gratitude to TomNIIVS [Tomsk Scientific Research Institute for Vaccines and Sera] Graduate Fellow L.A. Burenkova, who provided us with the rabbit sera and data on their titres, which were established by the conventional agglutination method.

DATA ON THE PRODUCTION OF ANTIENCEPHALITIS SERUM

Ye.N. Rodyukova

Production of antiencephalitis serum at the Tomsk Institute for Vaccines and Sera began in 1954. Horses from 4 to 11 years old were selected for immunization. M.K. Tyushnyakova and M.G. Baybarodova have shown that it is necessary to use horses from natural nidi of tick-borne encephalitis for the production of antiencephalitis serum, since they yield high serum titres from the first cycle of hyperimmunization, the neutralization indices reaching five figures. Horses were consequently obtained over a three-year period from Chainskiy or Tomskiy Rayons, where tick-borne encephalitis is encountered, for serum production.

In 1957 we selected 5 horses obtained from Stavropol'skiy Kray for our experiment. They were all of the same strain, ranging in age from 4 to 6 years and in weight from 410 to 460 kg. Appropriate investigations carried out before the beginning of the first immunization cycle showed that the sera of these horses did not contain any natural antibodies. After the first cycle serum samples from the producers in this group neutralized tick-borne encephalitis virus with a neutralization index of 10,000-436,500. It is obvious that horses not from nidi of tick-borne encephalitis whose sera do not contain natural antibodies to spring-summer tick-borne encephalitis may be used for producing antiencephalitis serum.

It is well known that it is possible in serum production prac-

TABLE 1

Results of Immunization of Horses Put Into Service In 1956

№ A лошади	B I цикл		E II цикл		F С какого цикла продуцент
	к-во антигена за цикл C	индекс нейтрализации D	к-во антигена за цикл	индекс нейтрализации	
635	310	5150	160	100000	1
647	310	5152	160	100000	1
652	310	5000	160	594400	1
658	310	5152	160	100000	1
659	310	5000	160	100000	1
662	310	5000	160	653200	1
648	310	30	160	100000	2
650	310	81	160	56230	2
657	310	162	160	594400	2
665	310	51	160	болъна G	—

A) Horse number; B) cycle I; C) number of antigens in cycle; D) neutralization index; E) cycle II; F) producer from which cycle; G) became ill.

tice to switch producers which do not yield the requisite titres from one type of immunization to another. We obtained 12 horses for antiencephalitis serum production which had undergone 4-7 cycles of diphtheria immunization and had not yielded the necessary titres of antidiphtheria serum. When immunized with live tick-borne encephalitis virus all 12 horses became good producers of antiencephalitis serum. The neutralization indices reached 5 and 6 figures. Prior immunization with diphtheria toxoid consequently had no negative effect on the development of antibodies to tick-borne encephalitis virus.

We were interested in finding out whether refractory horses could be found on immunization with tick-borne encephalitis virus. Our observations were made on a group of 10 horses put into service in 1956. During the first cycle all 10 horses received 310 ml of antigen each. After the first cycle 6 of the horses had neutralization indices of 5000 (Nos. 652, 659, and 662) to 5122 (Nos. 647, 658, and 638). Four of the ten horses had neutralization indices of from 30 to 162 after

the first cycle. During the second cycle the horses received 160 ml of antigen and all had high neutralization indices. The data obtained are given in Table 1.

It may be seen from the data in Table 1 that 6 of the horses became good producers of antiencephalitis serum after the first cycle. Nine horses became producers of antiencephalitis serum after the second cycle. The exception was No. 665, which developed clinical symptoms of tick-borne encephalitis after the second immunization cycle. This horse was killed. Beginning with the fourth cycle, the remaining 9 horses received from 20 to 60 ml of antigen per cycle. The neutralization indices of their sera were 2754-676,100.

TABLE 2

Results of Immunization with Small Antigen Doses

Группа (A)	После какого цикла определялись показатели (B)	1957 г. к-во лошадей (C)	1957 г. дозы антигена (D)	1957 г. индекс нейтрализации (E)	1958 г. к-во лошадей	1958 г. дозы антигена	1958 г. индекс нейтрализации	Примечание (F)
I	2—18	20	20—90	1000—812800	20	20-60	1000—416600	G
II	11—17	8	100-140	1000—707900	4	20-60	1820—645700	4 лошади в 1958 г. пропускались без иммунизации

A) Group number; B) cycle after which observations were made; C) number of horses; D) antigen doses; E) neutralization index; F) note; G) phlebotomies were performed on 4 unimmunized horses in 1958.

In an analysis (carried out during a period beginning in 1955) of immunization of horses with a 10% suspension of brain cells from white mice inoculated with strains of tick-borne encephalitis virus we noted that a number of the horses developed symptoms of a neural disorder involving pareses and paralyses of the front and hind legs at different times. Thus, there were two such horses in 1955, 10 in 1956, and 4 in 1957. In 1957-1958 two strains of virus were isolated

from the brains of horses Nos. 531 and 539, which were exsanguinated at the height of the illness; these strains did not differ in biological properties from standard tick-borne encephalitis strains. Horse No. 511 died on the third day after the onset of the disease, exhibiting acute clinical symptoms of tick-borne encephalitis (complete paralysis of the hind legs, loss of sensitivity in the lumbar region of the spinal cord, severe retraction of the head, and pareses of the lips). Virus was isolated from the brain, lumbar section of the spinal cord, urine, and kidneys of this horse. All this gives us reason to assume that it is possible to induce tick-borne encephalitis in horses by intensive immunization with the appropriate virus and intensive phlebotomy.

O.G. Andzhaparidze, Z.F. Zubova, N.V. Moskovicheva, and V.D. Nikitin (1954) investigated 21 urine samples from horses producing antiencephalitis serum and concluded that horses immunized with live tick-borne encephalitis virus do not excrete it with their urine. The virus obviously appears in the urine of an immunized horse during the illness.

In order to prevent encephalitis in horses producing antiencephalitis serum in 1957-1958 we altered the exploitation scheme, using the data obtained by O.G. Andzhaparidze, Z.F. Zubova, N.M. Gordiyenko, and R.M. Milyutina as our basis; these authors showed that the total antigen dose used to obtain antiencephalitis serum should not exceed 120-150 ml in one immunization cycle.

In the production scheme which we employed in 1954-1955 the total antigen dose in the first cycle amounted to 200-495 ml, while in subsequent cycles the horses received up to 200 ml of antigen per cycle for 10 cycles. In 1956 the antigen dose was reduced to 140-160 ml. The duration of this immunization cycle was 30-45 days. The authors men-

tioned above recommend additional phlebotomies with repeated immunization being avoided if the blood taken in the main phlebotomy contains a high titre of antibodies.

In the work described herein we set ourselves the task of, firstly, using the method developed by these authors under the conditions which obtained in our production scheme for antiencephalitis serum and, secondly, determining how long antibodies can develop in the blood of producers if they are not immunized for an extended period.

In order to solve the first problem we made observations on 28 horses divided into 2 groups. The first group, containing 20 horses, were placed under observation when the individual producers had undergone 2-18 cycles and received no less than 140 ml of antigen in each cycle. The doses given this group of horses were reduced in 1957; thus, they received 20-90 ml of antigen per cycle. The neutralization indices for their sera varied from 100 to 812,800 in different cycles.

The second group, comprising 8 horses, was placed under observation after 11-17 immunization cycles, having received antigen doses similar to those for the first group. The antigen dose was reduced considerably in 1957, to 100-140 ml. The neutralization indices of their sera varied from 1000 to 707,900. The data obtained are given in Table 2.

It may be seen from Table 2 that there is no difference in the serum titres for the two groups. Our observations showed that use of antigen doses of from 20 to 60 ml per cycle is wisest; if a decrease in titre is noted it is possible to increase the dose to 140 ml for 1-2 immunization cycles.

These observations made it possible for us to switch the horses to immunization in doses of 20-60 ml of antigen per cycle in 1958. The immunization cycles were reduced to 15-25 days in this scheme. The

sera obtained neutralized various strains of tick-borne encephalitis virus with neutralization indices of 1000-645,700. Using smaller doses for immunization, we simultaneously reduced the rest between cycles to 10-15 days. The horses' general condition remained good and there was no temperature reaction. No cases of tick-borne encephalitis were observed among the animals once administration of small antigen doses was started.

TABLE 3

Results of Phlebotomies Performed on Horses Given Antigen

A № лошади	B С какого времени эксплуатируется	C Сколько прошла циклов	D Период времени неполучения антигена	E К-во кровопусканий	F К-во крови за 1957 г.	G К-во крови за 1958 г.—1-IV	H Индекс нейтрализации	I Примечание
422	17/VIII-54 г.	16	Май 1957 г. — Март 1958 г. (J, K)	48	263	113	1995—309000	22/III-58 г. пала, сердечн. недостаточн. (N)
423	17/VIII-54 г.	16	Май 1957 г. — Апрель 1958 г.	46	280	124	1000—389000	IV-58 г. получила 60 мл. антигена (O)
657	3/X-56 г.	6	Октябрь 1957 г. — Май 1958 г.	32	192	149	2570—100000	
658	3/X-56 г.	7	Октябрь 1957 г. — Май 1958 г.	32	210	148	2754—100000	

A) Horse number; B) date put into service; C) number of cycles undergone; D) period of time for which animal did not receive antigen; E) number of phlebotomies; F) quantity of blood taken during 1957; G) quantity of blood taken during January-April 1958; H) neutralization index; I) note; J) May; K) March; L) April; M) October; N) died of cardiac insufficiency; O) received 60 ml of antigen.

As usual, each immunization cycle terminated in a triple or, less frequently, double phlebotomy with a one-day interval, in which 7-8 liters of blood were taken. During 1957-1958 all of the producers then in service were subjected to 5-6 phlebotomies at intervals of 1-12 days after the immunization cycle, 6-8 liters of blood being taken at each phlebotomy; as a result, 31-58 liters of blood could be obtained from each horse after the immunization cycle, the neutralization indices of the sera reaching 5 and 6 figures. In order to clarify the feasibility

of subjecting producers to multiple phlebotomies with no additional administration of antigen we made observations on four producers. The results are given in Table 3.

It may be seen from the material in Table 3 that producers Nos. 422 and 423 were put into service in August 1954. After they had undergone 16 cycles of immunization with live virus they were subjected to 48 phlebotomies at intervals of 1-7-14 days. As a result of this production scheme during 1957 we obtained 283 liters of blood from horse No. 422 and 280 liters from horse No. 423. The other two producers (Nos. 657 and 658), which were put into service in October 1956, underwent 6 and 7 immunization cycles respectively, then being given no antigen for 6 months. During 1957, 192 liters of blood were obtained from horse No. 657 and 210 liters from horse No. 658. During the first 4 months of 1958, 534 liters of blood were obtained from these four producers without administration of antigen. The neutralization indices of their sera varied from 389,000 to 1000 and remained high for 6-10 months.

After the serum titre of producer No. 423 dropped to 1000 in April 1957, this animal was immunized in doses of 20 and 40 ml. A neutralization index of its serum increased to 4266. Producer No. 422, which underwent 16 immunization cycles and 48 additional phlebotomies, died after a regular phlebotomy, exhibiting symptoms of cardiac insufficiency.

The remaining horses felt well during this entire exploitation period, exhibiting no temperature reaction; their hemoglobin contents did not drop below 56%.

The observations which we made enable us to draw the following conclusions.

1. Horses which have no natural antibodies in their blood and

horses rejected for another type of immunization can be used for producing antiencephalitis serum.

2. When horses are immunized with live tick-borne encephalitis virus all of them become producers of antiencephalitis serum.

3. When horses are immunized with antigen doses of 20-60 ml per cycle during exploitation, none of them contract tick-borne encephalitis.

4. It is wise to carry out phlebotomies for 6-10 months (so long as the serum titre remains stable) without administering antigen; this makes it possible to obtain a large quantity of serum.

Tomsk Scientific Research Institute for Vaccines and Sera

TO THE QUESTION OF PRODUCTION ANTIENCEPHALITIS SERUM

Rodyukova H.N.

Making the immunization of horses by 10 percent suspension of cerebrum of white mice, infected by the strain of virus of tick encephalitis it was noted that symptoms of nervous disorder with paresis, paralysis of forelegs and hind legs appear by the number of horses.

By the sick horses virus is isolated from cerebrun and spinal cord, state and kidney. With the aim of preventing described phenomena the changes in scheme of hyperimmunization of horses were proposed.

USE OF ENCEPHALITIS VACCINE AS THE ANTIGEN IN THE PRODUCTION OF ANTIENCEPHALITIS SERUM

B.G. Trukhmanov and Ye.N. Rodyukova

Live tick-borne encephalitis virus in the form of a 10% suspen-ion of mouse brain cells is used as the antigen for immunizing pro-ucer horses in the production of antiencephalitis serum. The bri:f seful life of this antigen (2 hours) makes it inconvenient to use it n production. In addition, its use makes it necessary to maintain a igid producer-care regime during the entire time for which the horses re in service.

In the work described herein we set ourselves the task of elu-idating the possibility of obtaining antiencephalitis serum from orses by immunizing them with inactivated virus in the form of en-ephalitis vaccine. For immunizing the horses we used spring-summer ick-borne encephalitis vaccine prepared from the brains of white mice nd from chick embryos. Both preparations were manufactured at the omsk Institute for Vaccines and Sera.

The vaccines used for immunizing the horses had a high index of mmunogenicity, were harmless when tested on mice, and satisfied the equirements imposed on them by the instructions now in force. Thus, he cerebral vaccine, which was prepared in 1956, consisted of the wo strains "Sof'in" and "Obor" and had an index of immunogenicity of rom 2138 to 8,511,000 for different series; the vaccine prepared in 957 (series Nos. 2, 9, 11, 14, and 17) consisted of the strains

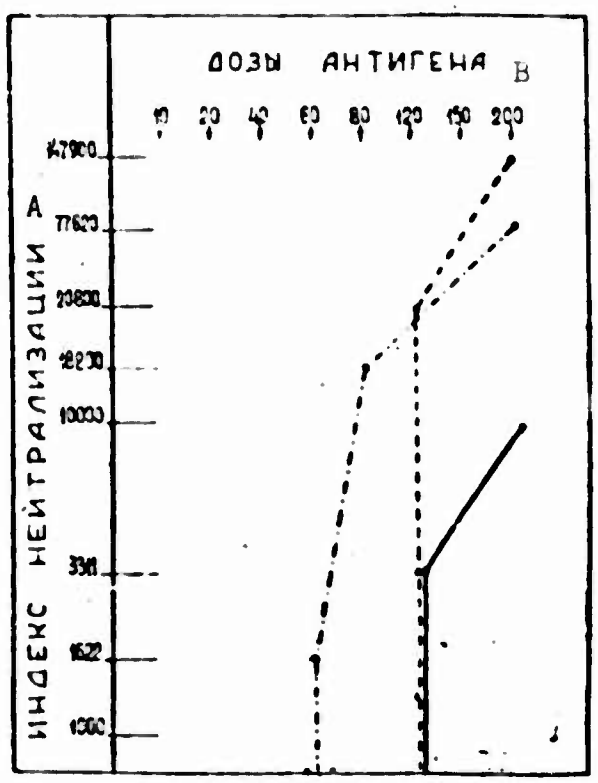

Fig. 1. Arbitrary symbols: -·-·- horse No. 111; ---- horse No. 112; ——— horse No. 114. A) Neutralization index; B) Antigen doses.

"Sof'in,""Absettarov," and "L-3" and had an index of immunogenicity of from 6026 to 144,500. The activity of the strains composing the vaccine varied from $10^{-7.0}$–$10^{-8.75}$.

The embryonic vaccine (series Nos. 1, 5, 7, and 8) consisted of 43 "Ikh-10" "Yas-8," and "L-3" strains and had an index of immunogenicity of from 1585 to 251,200; the activity of the strains was $10^{-4.0}$–$10^{-7.5}$.

In this experiment we initially used three horses (Nos. 111, 112, and 114) which were obtained from Stavropol'skiy Kray in 1956 and had not previously been subjected to any other type of immunization.

No natural antibodies to tick-borne encephalitis virus were detected in the blood serum of these horses before immunization was begun. The horses underwent 3 cycles of immunization with cerebral encephalitis vaccine. During the first cycle they received 680 ml of vaccine in doses of 10, 20, 40, 60, 80, 120, 150, and 200 ml, in the second they received 600 ml in doses of 100, 200, and 300 ml, and in the third they received 500 ml in doses of 200 and 300 ml. The intervals between injections were 4-5 days. Phlebotomies were performed on the 7-9th day after the last injection of antigen. The virus-neutralizing antibodies in the horses' sera were determined by the neutralization reaction after every other vaccination during the first cycle. The results of the titration of the sera from this group of horses are given in Fig. 1.

It may be seen from the data cited that the content of virus-neutralizing antibodies in the blood serum of horse No. 111 increased after the 60-ml dose was administered, the neutralization index being 1622. After this horse received the 80-ml dose the neutralization index of its sera was 18,200; the index then increased markedly after each subsequent injection and amounted to 77,620 at the end of the first immunization cycle. Antibodies were detected after the sixth vaccination in two other horses (Nos. 112 and 114, which exhibited neutralization indices of 20,890 and 3311, respectively). After these horses received the 200-ml dose their neutralization indices were 147,900 and 10,000, respectively. The concentration of virus-neutralizing antibodies in the sera thus rose with the increasing vaccine dosage in successive injections.

TABLE 1

Neutralization of Tick-Borne Encephalitis Virus By Serum From Horses Immunized With Vaccine

A	B	C	D		E
№ лошади	Цикл иммунизации	Доза вакцины	Дата кровопускания	LD –50	Показатель нейтрализации
111	2	600	19/II–57 г.	3,41	1995
	3	500	7/V–57 г.	4,67	46770
112	2	600	19/II–57 г.	5,0	100000
	3	500	7/V–57 г.	3,56	4571
114	2	600	19/II–57 г.	3,25	1778
	3	500	7/V–57 г.	3,57	3715
F	Смесь сывороток от лошадей № 111, 112 и 114			4,55	26300
G	Контроль – нормальная лошадиная сыворотка			9,0	–

A) Horse number; B) immunization cycle; C) vaccine dosage; D) date of phlebotomy; E) neutralization index; F) mixture of sera from horses Nos. 111, 112, and 114; G) control, normal horse serum.

After the second and third vaccine-immunization cycles the neutralization index rose to 1995-100,000, as is shown in Table 1.

After three cycles of immunization with the vaccine the horses

were shifted to immunization with live tick-borne encephalitis virus, receiving 60 ml of antigen per cycle. The neutralization indices of the sera reached four to six figures (1000-229,100).

We then immunized a new batch of horses, which had been shifted from diphtheria immunization in which they had not yielded the necessary titres, with both the cerebral and embryonic vaccines.

TABLE 2

Serum Titres of Producer Horses Immunized With Cerebral and Embryonic Vaccines

Мозговая вакцина A						Эмбриональная вакцина I					
№ лошади B	Цикл C	Дата кровопускания D	Нейтрализация вируса клещевого энцефалита E			№ лошади	Цикл	Дата кровопускания	Нейтрализация вируса клещевого энцефалита		
			„Софьин" F	„Л-3" G	„Абсеттаров" H				„Софьин"	„Л-3"	„Абсеттаров"
60	0	17/X—57 г.	281	—	—	13	0	17/X—57 г.	100		—
	1	2 XI—57 г.	1000	—	—		1	2 XI—57 г.	Отриц. J	1479	—
	2	17/XII—57 г.	—	—	—		2	12/XII—57 г.	2692	537	575
	3	13 I—58 г.	28180	166000	—		3	7/II—58 г.	741	114800	2630
	4	8 II—58 г.	141300	112200	23990		4	8 III—58 г.	3715	2344	3162
	5	27/II—58 г.	100000	281800	31620		5	29 III—58 г.	—	—	4677
	6	19/III—58 г.	100000	—	416200						
87	0	17/X—57 г.	100	—	—	14	0	17/X—57 г.	Отриц.	147	—
	1	2 XI—57 г.	21880	3715	—		1	2 XI—57 г.	1000	7244	—
	2	17/XII—57 г.	44670	61660	6166		2	12/XII—57 г.	575	—	363
	3	13 I—58 г.	—	—	—		3	7 II—58 г.	2291	—	30900
	4	8 II—58 г.	100	173300	3802		4	8 III—58 г.	2250	—	—
	5	27/II—58 г.	100000	28180	10000		5	29 III—58 г.	—	—	46770
	6	19/III—58 г.	2570	—	—						
121	0	17/X—57 г.	234	61	—	46	0	17 X—57 г.	23	125	—
	1	2 XI—57 г.	436	1175	—		1	2 XI—57 г.	23	3981	—
	2	17/XII—57 г.	1000	39810	1660		2	12 XII—57 г.	100	83	363
	3	13 I—58 г.	588	33880	—		3	7/II—58 г.	316	66070	—
	4	8 II—58 г.	14790	1862	—		4	8/III—58 г.	100	13490	—
	5	27/II—58 г.	1995	5129	5495		5	29/III—58 г.	—	—	3715
	6	19/III—58 г.	—	—	1698						

Note: A zero indicates the titres after the preparatory injections.

A) Cerebral vaccine; B) horse number; C) cycle; D) date of phlebotomy; E) neutralization of tick-borne encephalitis virus; F) Sof'in; G) L-3; H) Absettarov; I) embryonic vaccine; J) negative.

For this experiment we used 6 horses divided into two groups. The

first group was immunized with cerebral encephalitis vaccine prepared in 1957. The second group was immunized with embryonic encephalitis vaccine. Both groups of horses were given two preparatory injections of 20 ml of 1 vaccine or the other at an interval of 2 weeks during the month before the first immunization cycle was begun. One month after these preparatory injections the sera of the horses in the first group had neutralization indices of from 100 to 281, while those of the horses in the second group had indices of 23-147. The horses in the first group underwent 6 cycles and those in the second group 5 cycles. The first cycle consisted of injections of 20,40, and 80 ml of antigen at intervals of 4-5 days; in the subsequent cycles the horses were given 300-800 ml of vaccine per cycle, in two or three injections. The virus-neutralizing activity of the sera was determined with three strains, "Sof'in,""L-3," and "Absettarov." Table 2 shows the results of titration of the sera obtained in the phlebotomies at the end of each cycle.

In comparing the data obtained it may be seen that the serum titres for the horses immunized with the embryonic vaccine (100 to 114,800) were somewhat lower than those for the horses immunized with the cerebral vaccine (100 to 166,000).

The results of the titration of the sera obtained from the horses immunized with the encephalitis vaccine enable us to conclude that this vaccine has a sufficient immunogenic activity to be used as an antigen for producer horses. It must also be noted that the horses withstood immunization with the vaccine very well, the only symptom being a slight rise in the temperature of certain of the producers during the first cycle.

Two series (Nos. 194 and 200) were prepared from the sera produced with the aid of the vaccine ; these series were purified and concentrated by the "Diaferm 3" method. Thus, series No. 194 consisted of

sera from the horses immunized with the cerebral vaccine, while series No. 200 consisted of sera from the horses immunized with the embryonic vaccine. The virus-neutralizing properties of these sera are shown in Table 3.

Before concentration the sera of series No. 194 neutralized strains "L-3" and "Absettarov" with indices of 54,950 and 10,000; after concentration they neutralized strains "Sof'in" (with an index of 28,840), "L-3" (107,200), and "Absettarov" (61,660). Before concentration the sera of series No. 200 neutralized strain "Absettarov" with an index of 8128; the neutralization index after concentration was 100,000-17380.

The data cited indicate that sera from horses immunized with this vaccine have a sufficiently high virus-neutralizing activity and can also be purified and concentrated by the "Diaferm 3" method.

TABLE 3

Characteristics of Series of Sera Obtained From Vaccine-Immunized Horses

A № серии	B От каких лошадей	C Вид сыворотки	D Индекс нейтрализации со штаммом		
			E „Софьин"	F „Л-3"	„Абсетта-G ров"
194	№ 111, 112, 114, 60, 87, 121	до концентрации H	не титров.	54950	10000
		после концентрации	28840	107200	61660
200	№ 13 и 14	до концентрации I	не титров.	не титров.	8128
		после концентрации	100000	-	17380

A) Series number; B) horses from which obtained; C) serum form; D) neutralization index with strain; E) Sof'in; F) L-3; G) Absettarov; H) before concentration; I) after concentration; J) no titres.

This experiment thus demonstrated that it is possible to use cerebral and embryonic encephalitis vaccine under production conditions as the antigen for immunizing producer horses to obtain antiencephalitis

serum. There are always waste products in the manufacture of encephalitis vaccine and these can be employed as the antigen.

Use of the vaccine makes it possible to begin immunization of horses even before they are moved to a special-purpose immunology clinic.

Tomsk Scientific Research Institute for Vaccines and Sera

THE USAGE OF ENCEPHALITIC VACCINE AS AN ANTIGEN IN PRODUCTION OF ANTIENCEPHALITIC SERUM

Trukhmanov B.G. and Rodyukova H.N.

Encephalitic cerebral and embryonic vaccines were used as antigens for immunization of horses-producers.

Serums received from these horses possessed enough virus neutralized activity and were concentrated by the method of "Diaferm 3."

CERTAIN CAUSES OF THE PYROGENICITY OF SERA CONCENTRATED BY THE "DIAFERM 3" METHOD AND POSSIBLE WAYS OF ELIMINATING IT

S.M. Preger, A.P. Dutova, N.G. Turlyantseva

Antitoxic sera purified and concentrated by the "Diaferm 3" method developed by the Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR frequently have a pyrogenic effect. The reasons why the sera become pyrogenic have not as yet been sufficiently well explained

Certain authors (Chertkova, Shapovalova, Raykher, Panin, Sirbiladze, Ramishvili, Drozdova, Myl'nikova, and Karkhadze) believe that the pyrogenic action of the sera results from bacterial contamination during purification and concentration. In this connection it is recommended that the extent to which the sera are contaminated during the various stages of the technological process be studied and the conditions under which they are prepared be made as sterile as possible.

Other authors (Mirskova, Gitterman, Khrustaleva, and Kalugina) were unable to establish any relationship between the pyrogenicity of the sera and the extent of bacterial contamination.

A number of authors (Budylina, Torban, Anisimova, Sosina, Izumrudova, and Kondrashova) believe that the pyrogenicity of the sera results not only from microbial contamination, but also from the process of peptic digestion. The problem of the pyrogenicity of these sera thus has not as yet been the subject of a conclusive study.

The investigation described herein was a study of the causes of the pyrogenicity of such sera and of possible ways of eliminating it.

In order to study this problem we analyzed data obtained in determining the degree of contamination which occurs during concentration. Of 104 batches of sera concentrated between October 1957 and May 1958 which we examined 4 batches of diphtheria serum bacame pyrogenic. Only one or two test tubes were contaminated during concentration. At the same time, 3 batches concentrated after preliminary removal of all

globulins and subsequent contamination in a five-test tube culture during concentration proved to be apyrogenic.

TABLE 1

Purification of Sera From Pyrogens With Ammonium Sulfate

A Название сыворотки	B № серии	C Пирогенность: D до переосаждения	E после переосаждения	% выхода F
Противодифтерийная	315	пироген. H	пироген.	40,1
• G	121	пироген.	апироген. I	38,6
•	149	пироген.	пироген.	40,3

A) Serum type; B) series number; C) pyrogenicity; D) before reprecipitation; E) after reprecipitation; F) % yield; G) antidiphtheria; H) pyrogenic; I) apyrogenic.

We employed the resources of our epidemiology department to check the microflora with which the sera became contaminated during concentration. Thirteen strains of various microbes were isolated from the 10 batches studied. Staphylococcus albus, Sarcina, gram-negative Shigella alkalescens, and Bacterium coli were found in one case. All 10 batches were found to be apyrogenic and were released.

Since it was possible for microbes to enter the sera from the air during purification and concentration, we checked the importance of the atmospheric microflora in the development of pyrogenicity in the sera. Normal serum was inoculated with various quantities of the microbes most frequently encountered in indoor air.

We isolated 2 strains of Staphylococcus albus and 4 strains of Sarcina. The former were nonpathogenic (did not produce zones of hemolysis on blood agar within 24-48 hours and did not cause plasma coagulation or necrosis when administered intracutaneously to rabbits).

The microbial suspension was added to the serum in quantities of 1,5, 30, and 100 billion microbial bodies per liter. The serum con-

taminated in this fashion was kept in a heater for 2-7-14 days, then being filtered and tested for pyrogenicity.

We were unable to obtain a pyrogenic serum on contamination with this flora, even when the inoculation consisted of 100 billion cells per liter of serum and when the serum and suspension remained in contact for 14 days.

We were thus unable to establish any relationship between the pyrogenicity of the sera and the degree of bacterial contamination and type of microbe from this comparatively small amount of material.

We also checked the pyrogenicity of 26 batches of native serum before concentration. All of the sera proved apyrogenic before treatment and all but one were released after purification and concentration. This one serum (antidiphtheria serum No. 125) was apyrogenic before concentration and pyrogenic afterward.

The development of pyrogenicity in a concentrated serum is thus independent of the apyrogenicity of the native sera.

We know from the literature (Kofanov and Revenko) that pyrogenicity may result from the use of poor-quality distilled water in purification and concentration. We prepared physiological solution, sterilized it, and administered it to rabbits in a dose of 0.1 ml per kg of body weight. Nine water samples were checked at various times of year (summer, winter, and spring). All of them proved to be apyrogenic.

The next stage in the work was a study of possible ways of purifying the sera of pyrogens. For this purpose we tested various methods of acting physically and chemically on the pyrogenic serum. Assuming that the pyrogens might possibly be associated with the products of microbial vital activity, we heated the serum at 60° for 2 hours. Three antidiphtheria serum samples heated in this manner did not lose their pyrogenicity.

We tried to purify the serum of pyrogens by reprecipitating it with ammonium sulfate. The serum sample was diluted with twice the amount of distilled water and 140 g of dry ammonium sulfate was added for each liter of the mixture. To the filtered liquid which was obtained we added 200 g of ammonium sulfate per liter. After being filtered through a cotton filter the treated protein was pressed, dialyzed, standardized, and subjected to a sterilizing filtration; it was then checked for pyrogenicity. Of the three series of antidiphtheria serum reprecipitated in this manner two remained pyrogenic and one became apyrogenic (see Table 1). These data indicate that the method is not uniform. The serum loss was slight.

We made an attempt to purify the serum of pyrogens with various adsorbants (starch, carbolene, calcium carbonate, magnesium sulfate, and talc). From 50 to 300 g of adsorbant was added per liter of pyrogenic serum. The sera were agitated for 30 minutes. They were centrifuged and filtered on the following day. The protein content, titre, and pyrogenicity of the sera were determined before and after treatment (see Table 2).

With the exception of the magnesium, none of the adsorbants used freed the sera of pyrogens. The magnesium adsorbed the pyrogens completely, but also reduced the protein content from 12.5 to 2.75% and caused a fivefold decrease in serum titre.

It is well known that aluminum hydroxide is used for purifying and concentrating toxins, adsorbing antigens used in immunization, and adsorbing peptidases. We attempted to use aluminum hydroxide for adsorbing the pyrogens. However, we used the aluminum hydroxide produced by the Stalinsk Pharmaceutical Plant rather than that manufactured by the Kazanskiy Pharmaceutical Plant, as the Stavropol' Institute for Vaccines and Sera recommends. Using their method, we treated anti-

TABLE 2

Purification of Serum From Pyrogens With Adsorbants

A Название сыворотки	B Адсорбент	C Содержание белка, в %		F Титр в АЕ		G Пирогенность	
		D до обработки	E после обработки	до обработки	после обработки	до обработки	после обработки
H Противодифтерийная	I Крахмал	12,5	11,96	1700	1700	N пир.	пир.
•	J Карболен	12,5	10,87	1700	1700	•	•
•	K Углекислый кальций	12,5	12,5	1700	1700	•	-
•	L Тальк	12,5	12,5	1700	1700	•	•
•	M Магнезия	12,5	2,75	1700	400	•	апир. O

A) Serum type; B) adsorbant; C) protein content, %; D) before treatment; E) after treatment; F) titre, units; G) pyrogenicity; H) antidiphtheria; I) starch; J) carbolene; K) calcium carbonate; L) talc; M) magnesium; N) pyrogenic; O) apyrogenic.

TABLE 3

Results of Addition of Sugars to Pyrogenic Serum

A Материал для исследования	B % прибавления сахаров	C Пирогенность сыворотки	
		Ср. повышение температуры через 1 м-ц D	Ср. повышение температуры через 1 год E
Пирогенная противодифтерийная сыворотка, серия 315 F	—	—	I кролик 1,9 II кролик 2,5 III кролик 3,0 I
Пирогенная сыворотка с глюкозой G	1,0	I кролик 1,8 II кролик 1,3 III кролик 2,1	—
Пирогенная сыворотка с глюкозой	1,5	I кролик 0,2 II кролик 1,9 III кролик 2,3	I кролик 1,8 II кролик 1,6 III кролик 1,8
Пирогенная сыворотка с сахарозой H	1,0	I кролик 1,6 II кролик 1,2 III кролик 0,6	—
Пирогенная сыворотка с сахарозой	1,5	I кролик 1,6 II кролик 1,3 III кролик 2,3	I кролик 0,9 II кролик 1,3 III кролик 2,3

A) Material under investigation; B) percentage of sugars added; C) pyrogenicity of serum; D) mean increase in temperature after 1 month; E) mean increase in temperature after 1 year; F) pyrogenic diphtheria serum series 315; G) pyrogenic serum with glucose; H) pyrogenic serum with sucrose; I) rabbit.

iphtheria serum twice with the aluminum hydroxide. However, we were nable to free the serum of pyrogens.

We know from the literature (Chertkova, Shapovalova, and Rayk) hat this method does not free all sera of pyrogens. Additional ob- ervations must be made on the purification of serum from pyrogens with luminum hydroxide prepared by different methods (particularly from luminum sulfate and sheet aluminum).

We conducted a series of experiments on the possibility of re- lucing the pyrogenicity of the sera by adding glucose and sucrose in arious percentages. The sera remained pyrogenic over the one-year ob- servation period (see Table 3).

In order to purify the sera of pyrogens we reconcentrated them by the "Diaferm 3" method developed by the Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR (see Table 4).

Of the 18 series of reconcentrated pyrogenic sera (antidipytheria, antitetanus, antiperfringens, antiedimatiens, and polyvalent antigangrene) all except antiperfringens serum No. 14 were released as apyrogenic. Series No. 14 remained pyrogenic after reconcentration.

The reconcentration caused the serum yield to decrease to an average 30.6%, varying from 25.1 to 44.4%.

The following conclusions may be drawn from our work.

1. We were unable to establish any relationship between the pyrogenicity of the sera and the extent of bacterial contamination and type of microbe.

2. Native serum sterily prepared with distilled water having an oxidizing capacity within normal limits is apyrogenic.

3. Heating, reprecipitation with ammonium sulfate, and treatment with adsorbants and sugars did not eliminate the pyrogenicity of the

serum. Magnesium sulfate accomplished this, but sharply reduced the serum titre.

TABLE 4

Reconcentration of Pyrogenic Sera

Название сывороток A	B № серии	Титр до переконцентр. C	Титр после переконцентр. D	выхода E	Пирогенность F
Противодифтерийная G	158	1800	2400	30.0	апирогенна L
Противодифтерийная	3	2200	3200	28.8	апирогенна
Противодифтерийная	136.124 127.133 134	1800	2200	25.1	апирогенна
Противодифтерийная	138.140 141	1800	2300	26.3	апирогенна
Противостолбнячная H	76.78	2000	2000	30.0	апирогенна
Противоперфрингенс I	10.11 14	800	1300	28.6	апирогенна
Противоэдематиенс J	9	4000	4500	34.8	апирогенна
Противоэдематиенс	7	5000	5000	30.2	апирогенна
Поливалентная противогангренозная K	3	1300	1500	44.8	апирогенна

A) Serum type; B) series number; C) titre before reconcentration; D) titre after reconcentration; E) % yield; F) pyrogenicity; G)antidiphtheria; H) antitetanus; I) antiperfringens; J) antiedimatiens; K) polyvalent antigangrene; L) apyrogenic.

4. In the majority of our experiments reconcentration of the pyrogenic sera by the "Diaferm 3" method reduced their pyrogenicity, but also decreased their yield.

Tomsk Scientific Research Institute for Vaccines and Sera

THE STUDY OF SOME REASONS OF PYROGEN OF CONCENTRATED SERUMS AND THE POSSIBILITIES OF ITS REMOVAL

Preger S.M., Dutova A.P., Turlyantseva N.G.

In this work the authors did not succeed in find the connection between the degree of sowing the serums on the stages of concentration,

microflora, with pyrogenic properties of serums.

Sterile prepared native serums, distilled water having oxidation in normal limits, do not possess pyrogenic properties. The influence upon pyrogenic serum by physical and chemical factors such as: warming thoroughly, overprecipitation by ammonium sulfate, adsorbants (starch, carbolen, carbonic calcium, talc), glucose and saccharose do not free it from pyrogen. The authors succeeded in purifying from 18 pyrogenic series - 17 by overconcentration by the method of diaferm 3 but in this case the outlet decreases in average to 30 percent.

EXPERIENCE IN THE PRODUCTION OF EMBRYONIC SPRING-SUMMER TICK-BORNE ENCEPHALITIS VACCINE

L.D. Ovchinnikova, N.I. Ponomarenko, N.A. Sonchik

Since 1940 vaccination of the populace against spring-summer tick-borne encephalitis has been carried out with vaccine prepared from the brains of mice inoculated with encephalitis virus. The production of this vaccine necessitates special breeding of a large quantity of white

TABLE 1

Comparative Data on the Activity of Vaccines Prepared by Different Methods

Эмбриональная вакцина A			Мозговая вакцина E		
Название штамма B	Число серий C	Средний показатель активности D	Название штамма	Число серий	Средний показатель активности
F Ix_{10}	11	$10^{-6,0}$	I «Софьин»	27	$10^{-8,2}$
G $Яс_{8}$	14	$10^{-6,3}$	J «Абс»	52	$10^{-7,7}$
H Лз	1	$10^{-6,0}$	K «Лз»	65	$10^{-7,6}$

A) Embryonic vaccine; B) strain designation; C) series number; D) mean activity index; E) cerebral vaccine; F) Ikh_{10}; G) Yas_{8}; H) Lz; I) Sof'in; J) abs; K) Lz.

mice.

Mice are subject to various diseases (ectromelia, spontaneous mouse encephalitis, paratyphoid, etc.). This makes vaccine production extremely difficult, complicating it and increasing its cost. It has become necessary to develop new methods of preparing vaccine for the prophylaxis of spring-summer tick-borne encephalitis.

In 1949 A.K. Shubladze and O.G. Andzhaparidze made use of the

ability of tick-borne encephalitis virus to multiply in chick embryos to develop a new method of vaccine preparation; it has come to be called the embryonic method.

In 1949-1950 the authors used this vaccine to vaccinate the inhabitants of a number of areas of the Soviet Union. The results obtained showed that this vaccine is not inferior to the cerebral vaccine in immunogenic quality and is completely areactive. The vaccine was prepared from the virus strains "Sof'in," "YaMz," and "90-E."

In 1954 the instructions for the preparation of the embryonic vaccine were approved by the Commission on Sera and Vaccines, but it has not been manufactured or studied since that time.

The encephalitis department of the Tomsk Scientific Research Institute for Vaccines and Sera began to use this method of preparing embryonic vaccine in June 1957, under the direct supervision of Prof. A.K. Shubladze. The vaccine was produced from virus strains "Ikh_{10}" and "Yas_{8}" in accordance with the instructions approved by the Ministry of Health USSR on 25 June 1957.

During 1957 the department treated 25,858 embryos and produced 340 l of vaccine from them. The rejection rate for the embryos was 21.2% (5495) before inoculation and 34.3% (6984) when the eggs were opened; 39 embryos were required to produce one l of vaccine.

Hens' eggs from poultry farms near Moscow were used at first. A total of 104 l of vaccine was prepared from 3868 such embryos, the average rejection rate for the group being 16.2%. Production of one l of vaccine from the Moscow group required 31 embryos.

We later employed eggs from suburban farms near Tomsk, with no preliminary sorting.

During the autumn months, while the hens were molting, the eggs received were small and of low quality, having thin, poorly formed shells

and poorly developed embryos. When screened before inoculation and on opening these eggs usually yielded a high rejection rate (36.7-66%). At the same time that it was producing the embryonic vaccine the department prepared a vaccine from the brains of white mice. During the period in question 524,000 white mice were treated and 3127 l of vaccine were prepared. The cerebral vaccine was produced from the virus strains "Sof'in," "Lz," and "Absettarov."

Table 1 shows comparative data on the activity indices of the cerebral and embryonic vaccines.

It may be seen from Table 1 that the mean activity of the embryonic suspensions was somewhat lower than that of the cerebral suspensions.

A test of the immunogenic properties of the embryonic vaccine showed that its mean resistance indices were the same as those for the cerebral vaccine. The results obtained are given in Table 2.

In analyzing the material available we were unable to find any direct relationship between the resistance indices of the vaccine and the activity indices of the embryonic suspensions.

TABLE 2

Comparative Data on the Immunogenicity of Vaccines Prepared in Different Ways

Название штамма A	Название вакцины B	Число серий C	Средний индекс иммунизации D
E. Софьин"	Эмбриональная H Мозговая I	2 3	13.875 8.514
F. Lz"	Эмбриональная Мозговая	6 10	50.646 1448.369
G „Абсеттаров"	Эмбриональная Мозговая	14 8	105.011 30.000

A) Strain designation; B) vaccine designation; C) number of series; D) mean immunization index; E) S[illegible]'in; F) Lz; G) Absettarov; H) embryonic; I) cerebral.

Table 3 gives comparative data on the activity and immunogenicity indices of the embryonic vaccine.

TABLE 3

Comparative Data on the Activities and Immunogenicity Indices of the Embryonic Vaccine

№ серий [A]	Средн. показатель активности [B]	Индекс иммуноген. [C]	С каким штаммом [D]
1	$10^{-6.7}$	10.463	„Софьин" [E]
2	$10^{-6.4}$	1.995	„Лз" [F]
4	$10^{-5.8}$	1.950	„Абсеттаров" [G]
5	$10^{-6.4}$	2.752	"
6	$10^{-6.3}$	7.079	
7	$10^{-6.4}$	263.853	"
8	$10^{-6.3}$	178.043	"

A) Series number; B) mean activity index; C) immunogenicity index; D) with which strain; E) Sof'in; F) Lz; G) Absettarov.

TABLE 4

Comparative Data Obtained in Checking the Immunogenicity of the Embryonic Vaccine With Various Strains

№ серий моновакцин [A]	С каким штаммом проверено [B]	Индекс резистентности [C]
1	[D] „Софьин" „Лз" [E]	6.310 254.200
2	„Софьин" „Лз"	1.585 125
6	„Софьин" „Лз"	10.000 15
18	[F] „Абсеттаров" „Софьин"	7.943 10.000
21	„Абсеттаров" „Софьин"	1000.000 31.620
22	„Абсеттаров" „Софьин"	31.620 7.943

A) Number of monovaccine series; B) with which strain checked; C) resistance index; D) Sof'in; E) Lz; F) Absettarov.

Testing immunogenicity with various strains frequently yields different results; it is consequently necessary to conduct a parallel control test with several (a minimum of two) strains in order to obtain greater reliability. The data available on this problem are given

in Table 4.

The master suspensions of the embryonic vaccine were decontaminated in accordance with the instructions, i.e., held for 6 days at a temperature of +10-13°C, and then for 15 days at 4°C. Tests showed the preparation to be satisfactorily uncontaminated.

None of the 26 mice on which the safety of the suspension was tested were discarded for encephalitis.

In conclusion it must be pointed out that the production of embryonic vaccine is more profitable than the production of cerebral vaccine. Thus, for example, the cost of the cerebral vaccine is almost three times as high as that of the embryonic vaccine. In addition, it is more hygienic to produce vaccine in embryos than in mice.

CONCLUSIONS

1. It is necessary to use high-quality eggs for producing embryonic vaccine.

2. In working with strains "Ikh_{10}" and "Yas_3" the activity of the master suspensions of the embryonic vaccines varied from 10^{-6} to 10^{-67}.

3. The immunogenicity indices of the embryonic vaccine satisfied the requirements set by the instructions and were precisely the same as those of the cerebral vaccines.

4. We were unable to establish any direct relationship between the immunogenicity and activity indices.

5. In order to obtain more reliable results in immunogenicity testing it is necessary to set up a parallel control with several strains.

6. The method suggested by the instructions for checking the safety of the vaccine ensures its inactivation.

7. Production of the embryonic vaccine is more profitable than

production of the cerebral vaccine.

Tomsk Scientific Research Institute for Vaccines and Sera

THE EXPERIENCE OF PRODUCTION OF EMBRYONIC VACCINE AGAINST SPRING-SUMMER (TICK) ENCEPHALITIS

Ovchinnikova L.D., Ponomarenko N.I., Sonchik N.A.

The material of production experience of preparing vaccines against spring summer tick encephalitis from virus received on hen embryos was given. The production of it is more profitable than cerebral vaccine.

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REACTOGENICITY OF PREPARATIONS FOR THE PREVENTION OF SPRING-SUMMER TICK-BORNE ENCEPHALITIS

B.G. Trukhmanov, L.A. Yegorshina and N.F. Zel'tina

Various preparations have come into wide use for the prophylaxis of spring-summer tick-borne encephalitis. The severity of the clinical course of this disease and the subsequent severe disablement of persons who contracted it required highly effective drugs. All of this led to a sharp increase in the production of known preparations and an acceleration of the development of new, more highly perfected ones. However, the reactogenicity of these new drugs has still not been studied very thoroughly and their allergenic properties have not been investigated at all.

Considering this and the fact that cases of intensified reactions to administration of antiencephalitis vaccine have recently occurred, we decided to check the reactogenicity of the preparations recommended for the prophylaxis and treatment of encephalitis.

In our institute, which is now producing all types of antiencephalitis preparations (for diagnosis, treatment, and prophylaxis), the safety of all series of preparations manufactured is carefully checked both during production and by a State quality-control unit. According to the data of the MGKL [Moscow State Control Laboratory], the results of the tests on the antiencephalitis preparations are as follows (see Table 1).

In our experiments we decided to somewhat broaden the tests on the safety of the preparations and, specifically, to vary the method by which they were administered to the experimental animals.

In the first series of observations, which were made on 90 white mice, 168 guinea pigs, and 29 rabbits, we checked the primary reactogenicity of ordinary antiencephalitis vaccine, a 5% suspension of brain

cells from white mice inoculated with encephalitis virus, and a new antiencephalitis vaccine prepared in chick embryos (a 10% suspension of chick-embryo tissue in physiological solution).

We are using the term primary reactogenicity to refer to the reaction which develops immediately after administration of the preparation.

TABLE 1

A Препарат	B Год освоения	Выпущено C		F Не разрешены к выпуску
		D серий	E литров	
G Сыворотка (нативная)	1951	384	L св. 7000	1 серия D
H Сыворотка „Диаферм 3"	1957	7	50	—
I Гамма-глобулин	1957	39	св. 200	2 серии D
J Вакцина мозговая	1955	76	св. 5000	—
K Вакцина эмбриональная	1957	7	св. 200	—

A) Preparation; B) year put into production; C) output; D) series; E) liters; F) not released; G) serum (native); H) "Diaferm 3" serum; I) gamma-globulin ; J) cerebral vaccine; K) embryonic vaccine; L) more than.

Table 2 shows the test methods, test doses, modes of administration, and results obtained. The only phenomena noted were slight infiltrations caused by intracutaneous injection of 0.2 ml of either vaccine and, in one experiment, by subcutaneous injection of the embryonic vaccine. All of the other tests yielded very good results, demonstrating the extremely slight reactogenicity of both variants of the encephalitis vaccine.

In the second series of observations we decided to check the effect of repeated administration of the antiencephalitis preparations to an experimental animal, selecting the Arthus phenomenon, a model of a local anaphylactic reaction, for this purpose.

The experiment was performed on 15 rabbits. Both vaccines were employed for the investigation and native antiencephalitis serum was

TABLE 2

Results of Tests on the Immunogenicity of Encephalitis Vaccine Administered by Different Methods

A Животные		D Вакцина		G Способ введения препарата	H Результат испытания			
B Вид	C Кол-во	E Тип	F Доза (мл)		I гладко	J инфильтрат	K уплотнение	L погибли
M Белые мыши	30	мозговая P	0,5—2,0	подкожно S	30	—	—	—
—.—	30	—.—	0,5—1,0	внутрибрюшинно T	27	—	—	3
—.—	30	—.—	0,2—0,4	внутривенно U	30	—	—	—
—.—	35	—.—	0,25—1,0	подкожно	35	—	—	—
N Свинки	6	эмбриональная Q	1,0	подкожно	1	3	2	—
—.—	6	—.—	5,0	—.—	0	4	2	—
—.—	15	мозговая	9,0*)	внутримышечно V	15	—	—	—
—.—	14	—.—	9,0*)	подкожно	14	—	—	—
—.—	21	мозговая	0,2	внутрикожно W	3	6	12	—
—.—	8	эмбриональная	0,2	—.—	—	1	7	—
—.—	7	очищенная**) R	0,2	—.—	5	2	—	—
—.—	48	мозговая	2,0	внутрибрюшинно	20	—	—	—
—.—	15	—.—	4,0	—.—	15	—	—	—
O Кролики	5	мозговая	3,0	подкожно	5	—	—	—
—.—	5	эмбриональная	3,0	—.—	5	—	—	—
—.—	13	мозговая	5,0	внутривенно	13	—	—	—
—.—	6	эмбриональная	5,0	—.—	6	—	—	—

* This quantity was administered in three doses of 13 and 5 ml.
** Vaccine purified with methyl alcohol by the Tomsk Scientific Research Institute for Vaccines and Sera method (see p. 86).

A) Animals; B) species; C) number; D) vaccine; E) type; F) dose (ml); G) method of administration; H) results of test; I) smooth; J) infiltration; K) thickening; L) death; M) white mice; N) guinea pigs; O) rabbits; P) cerebral; Q) embryonic; R) purified; S) subcutaneous; T) intraperitoneal; U) intravenous; V) intramuscular; W) intracutaneous.

used as an independent control. The preparations were administered subcutaneously in the same body area.

The experimental method and the results obtained are shown in Table 3.

As may be seen from the table, the Arthus phenomenon developed on administration of the serum and the cerebral vaccine. The reaction to the serum began somewhat earlier; thus, after 3 April the Arthus phenomenon developed in four of the rabbits which received injections of the serum and in only one of those which received the cerebral vaccine. In addition, one rabbit exhibited a thickening and one a slight thickening at the injection site.

The complete absence of the Arthus phenomenon in the animals which received the embryonic vaccine under precisely the same conditions is striking. In a subsequent similar test purified vaccine did not cause the Arthus phenomenon.

We wish to call attention to the fact that administration of encephalitis vaccine for the treatment of progressive forms of tick-borne encephalitis may occasionally cause an undesirable complication of the Arthus-phenomenon type. It may be necessary to produce a special embryonic vaccine no more anaphylactogenic than the purified preparation for specific therapy.

In the third series of observations the antiencephalitis preparations (the two vaccines and gamma-globulin) were tested in a model of an active anaphylactic reaction. The literature contains no reports on this method of testing virus preparations, so we used the method which we had employed in corresponding investigations of complex associated vaccines.

We used 117 guinea pigs in the experiment. Different doses of the preparations were used for both the sensitizing and shock-induction (reacting) injections and were tested in different groups.

In connection with the fact that the antiepidemic service has been using the cerebral vaccine for less than a year and is just beginning to employ the embryonic vaccine, we sensitized the guinea pigs princi-

TABLE 3

Antiencephalitis Preparations in the Arthus Phenomenon in Rabbits

A Испытуемый препарат	B Разовая доза (мл)	C Реакция на введение препарата							
		4/III	10/III	16/III	22/III	28/III	3 IV	9 IV	15 IV
D Сыворотка	3	гл G	упл. H	упл.	некроз I	некроз	некроз	некроз	некроз
—.—	3	гл	гл	гл	гл	[illegible]	некроз	некроз	некроз
—.—	3	гл	гл	гл	гл	гл	[illegible]	упл.	упл.
—.—	3	гл	гл	гл	гл	гл	некроз	некроз	некроз
—.—	3	гл	гл	упл.	упл.	некроз	некроз	некроз	некроз
E Вакцина мозговая	3	гл	гл	гл	гл	гл	гл	гл	гл
—.—	3	гл	упл.	упл.	некроз	некроз	некроз	некроз	некроз
—.—	3	гл	гл	гл	гл	гл	гл	упл.	некроз
—.—	3	гл	гл	гл	гл	гл	упл. J	некроз	некроз
—.—	3	гл	гл	гл	гл	гл	н/упл.	упл.	некроз
F Вакцина эмбриональная	3	гл	гл	гл	гл	гл	гл	гл	гл
—.—	3	гл	гл	гл	гл	гл	гл	гл	гл
—.—	3	гл	гл	гл	гл	гл	гл	гл	гл
—.—	3	гл	гл	гл	гл	гл	гл	гл	гл
—.—	3	гл	гл	гл	гл	гл	гл	гл	гл

A) Preparation being tested; B) single dose (ml); C) reaction to administration of preparation; D) serum; E) cerebral vaccine; F) embryonic vaccine; G) smooth; H) thickening; I) necrosis; J) necrosis and thickening.

pally with the cerebral vaccine and used both types of vaccine for shock induction.

Since it is primarily native antiencephalitis serum which is now employed and it is extremely probable that gamma-globulin may be given on top of a previously administered serum, we also used the ordinary serum for sensitizing the guinea pigs in this portion of the experiments and employed encephalitic gamma-globulin for shock induction. Table 4 shows rather completely the experimental scheme and the data obtained.

TABLE 4

Antiencephalitis Preparations in Experiments on Active Anaphylaxis in Guinea Pigs

	Сенсибилизация A				Шокизация F			Явления анафилаксии H			
	Препарат B	Способ введения C	Доза (мл) D	Интервал (в днях) E	Препарат	Доза (мл)	Кол. животных G	I отсутствуют	J слабые	K тяжелые	L смертельный шок
M	Мозговая вакцина	Подкожно P	9	55	Мозговая вакцина M	0,5—1,0	7	7	—	—	—
	"	Внутримышеч. Q	9	55	"	1,0	6	6	—	—	—
	"	"	9	55	Эмбрион. вакцина N	0,5—1,0	10	10	—	—	—
	"	Подкожно	9	55	"	1,0	5	5	—	—	—
	"	Подкожно	0,25	14	Мозговая вакцина	1,0	8	8	—	—	—
	"	"	0,5	14	"	1,0	6	6	—	—	—
	"	"	1,0	14	"	1,0	8	8	—	—	—
	"	"	0,25	28	"	1,0	4	4	—	—	—
	"	"	0,5	28	"	1,0	4	4	—	—	—
	"	"	1,0	28	"	1,0	4	4	—	—	—
	"	Внутри брюшинно	2,0	14	Мозговая вакцина	0,05	6	6	—	—	—
	"	" R	2,0	14	"	0,1	7	7	—	—	—
	"	"	2,0	14	"	0,2	7	7	—	—	—
	"	"	2,0	23	"	0,2	4	4	—	—	—
	"	"	2,0	23	"	0,1	4	4	—	—	—
N	Эмбрион. вакцина	Подкожно	1,0	32	Эмбрион. вакцина	1,0	4	2	2	—	—
	Мозговая вакцина	"	1,0	32	Мозговая	1,0	2	2	—	—	—
	"	"	5	32	"	1,0	3	1	2	—	—
O	Сыворотка	"	5	30	Гамма-глобулин S	0,5—1,0	18	5	2	11	5
	"	"	5	30	Сыворотка O	0,5	1	—	—	1	1

A) Sensitization; B) preparation; C) mode of administration; D) dose (ml); E) interval (in days); F) shock induction; G) number of animals; H) anaphylactic symptoms; I) none; J) mild; K) severe; L) lethal shock; M) cerebral vaccine; N) embryonic vaccine; O) serum; P) subcutaneous; Q) intramuscular; R) intraperitoneal; S) gamma-globulin.

In the experiments with gamma-globulin we noted differences in the actual anaphylactic reactions. Of the 18 guinea pigs 11 exhibited severe symptoms of anaphylaxis, ranging up to convulsions, and five died displaying the characteristic symptoms of acute shock.

Dissection of the animals also revealed specific signs indicating death from anaphylaxis: distended, decolorized, "floppy" lungs, severe hyperemia of the large vessels of the internal organs and the liver, etc. We also noted a phenomenon which we had previously described, severe engorgement of the cervical portion of the spinal column with

venous blood (see p. 175).

All of the remaining guinea pigs soon returned to normal and exhibited no delayed reactions.

The data obtained in this experiment indicate the necessity of taking native antiencephalitis serum out of production.

At the same time, contrary to our assumptions, anaphylactic reactions were completely lacking in almost all of the experiments with vaccines and mild symptoms of anaphylaxis were recorded in only one experiment involving 4 guinea pigs; however, there was not a single case of severe symptoms, much less anaphylactic shock.

The remaining 94 guinea pigs exhibited virtually no reaction at all to the shock-inducing injection of the preparation; this indicates the weak allergenic properties of the antiencephalitis vaccines and confirms our impression of their low specific reactogenicity.

We have set ourselves the task of continuing our investigations and, in particular, believe it especially necessary to check the reactogenicity of antiencephalitis vaccines against a background of administration of other bacterial preparations, as well as substances of the sulfanilamide and antibiotic types, which have come into wide use in everyday practice.

CONCLUSIONS

1. When tested under various experimental conditions both the cerebral and embryonic antiencephalitis vaccines proved to be of low reactogenicity.

2. Antiencephalitis serum and then the cerebral vaccine proved to be most reactogenic in the Arthus phenomenon. The embryonic vaccine proved to be an absolutely areactogenic preparation.

3. In the experiments on active anaphylaxis neither type of vaccine caused an active anaphylactic reaction. Against a background of sensi-

tization with native encephalitis serum gamma-globulin caused a clear anaphylactic reaction.

4. It is necessary to use a purified preparation for intracutaneous immunization with encephalitis vaccine.

5. The observations which we made confirmed our prior data on the low reactogenicity of both types of antiencephalitis vaccine.

ABOUT THE REACTOGENITY OF PREPARATIONS AGAINST SPRING-SUMMER TICK ENCEPHALITIS

Trukhmanov B.G., Egorshina L.A., Zeltina N.F.

On experiments on guinea-pigs, white mice and rabbits an initial reactogenity and allergenic properties of antiencephalitis preparations were examined that proved to be insignificant.

Arthus's phenomenon was developed after introducing antiencephalitis serum and cerebrum encephalitis vaccine (embrionic vaccine proved to be inactive). On experiments of active anaphylaxis shock was obtained at laying on the hemoglobulin on serum, both vaccines being inactive.

A PAPER ELECTROPHORETIC STUDY OF THE PROTEIN COMPOSITION OF RABBIT BLOOD SERA ON IMMUNIZATION WITH DYSENTERY ANTIGEN VACCINES

O.A. Vasil'yeva

Study of the protein fractions of animal blood serum is of considerable importance in immunology. This method can be used to determine the ratios in the fractional composition of serum during immunization and, by relating them to the increase in antibody titre, to show in which protein fractions the antibodies to a given causitive agent accumulate. Knowing this, we can successfully purify and concentrate immune sera without risking loss of the necessary fraction during processing.

Electrophoretic fractionation is especially valuable in this respect; this method is based on the different rates at which protein particles move in an electric field. However, because of its complexity, the classical method of separating proteins in an analytic vessel by Tiselius' method can be used in only a small number of laboratories.

Electrophoretic fractionation on filter paper, a method proposed several yea s ago (in 1948-1952) by a number of investigators (Wieland, Durrum, Kra er and Tiselius, and Cunnci and Tiselius) working independently, is far more widely used. Proteins, lipoids, polysaccharides, bacterial toxins, etc., have been studied by this method.

Fractionation on filter paper makes it possible to use a small quantity of material without any preliminary treatment and to investigate simultaneously several specimens under absolutely identical conditions. This method is not inferior in precision to the classical method (V.I. Oyvin, V.P. Smolichev, et al.), while the low cost of the apparatus, its small size, and its lack of complexity facilitate its wide use in laboratories.

The literature contains articles by both domestic (I.A. Oyvin, Yu.V. Sergeyev, N.A. Ponomareva, M.P. Durasova, S.N. Babayeva, L.Ye. Khundayev, Yu.Z. Gendon, G.A. Annenkov, Shpolyanskaya, et al.) and foreign (Rutkvist, Tal', Oberg, Yamane, Shrared-Beyel'shteyn, Zeyelinger, Patnod, et al.) authors on the use of electrophoresis in immunology.

In working on the preparation of diagnostic agglutinative sera we were naturally interested in their fractional composition. We found no information on this problem in the literature accessible to us.

We consequently set ourselves the task of determining the extent of the changes which occur in the fractional composition of the blood serum of rabbits when they are immunized with dysentery antigens. In addition, we deemed it necessary to clarify the protein spectrum of the blood of normal unimmunized animals.

Experimental Methodology

We used 62 immunized and 60 unimmunized rabbits in the experiment. The animals weighed 3-3.5 kg, were of both sexes, and belonged to the Vienna blue and chinchilla strains. The experimental group was immunized with antigen vaccines prepared from dysentery microbes by the ordinary method. The times for which the animals served as subjects

TABLE 1

Electrophoresis of Serum From Rabbits Immunized with Dysentery Vaccines

	A Вид иммунизации	B Количество животных	C Титр антител, по +++	D Вес в кг	E Общий белок, г %	F Фракционный состав сыворотки относит. %			
						G альбумины	H глобулины α	β	γ
I	Нормальные кролики	60	—	3—3,5	6,7	53,1	13,4	15,2	15,5
J	Ньюкестль	12	1:5000	.	6,79	46,5	13,5	13,5	26,0
K	Бойд-Новгородской	16	1:3200	.	7,1	48,1	12,0	12,5	23,9
L	Флекснер	8	1:20000	.	7,4	51,8	12,1	13,4	23,1
M	Зонне	18	1:12800	.	6,88	47,7	14,2	14,5	23,4
N	Шмитц-Штуцер	8	1:6400	.	7,52	48,4	14,7	13,9	23,3

A) Type of immunization; B) number of animals; C) titre of antibodies, from + + +; D) weight in kg; E) total protein, g-%; F) fractional composition of blood, relative %; G) albumin; H) globulins; I) normal rabbits; J) Newcastle; K) Boyd-Novgorodskoy; L) Flexner; M) Sonne; N) Schmitz-Shtutser.

differed. When the titre of antibodies was at its highest the serum of each animal was investigated in the following manner: the antibody titre and total protein were determined, electrophoretic fractionation, photometry , and gravimetry were carried out, and the relative percentages of the protein fractions were calculated.

Blood was taken from the marginal vein of the ear, left to coagulate, and then centrifuged and examined. The total protein content was determined with a type RLU refractometer. The titre of antibodies was determined from the agglutination reaction.

The electrophoresis was performed in a paraffin chamber, as described in Prof. L.D. Kashevnika's manual of biochemistry (1957). We made the following technical changes: 1) the paraffin chamber was enclosed in a wooden case (after I.I. Balasheva) with a removable lid and dismountable electrodes, the latter being platinum rather than carbon, 16-20cm long, and 1 mm in diameter; the platinum electrodes made it possible to use the same uncontaminated buffer four or five times; 2) the stained strips were washed with a 0.5% rather than a 2% solution of acetic acid.

The quantitative determination of the fractions was made with the densitometer developed by V.Z. Gorkin and Levitin (1955), and the strips were then cut and weighed on torsion balances (the gravimetric method). The quantitative protein-fraction composition was calculated in relative percentages.

The rabbits in the experimental group were immunized with antigens prepared from Flexner, Schmitz-Shtutser, Sonne, Boyd-Novgorodskoy, and Newcastle dysentery bacteria by the ordinary production method. The results of these investigations are shown in Table 1, as averages.

It may be seen from the data in Table 1 that the following differences from normal rabbits were observed in the rabbits immunized with the vaccines prepared from dysentery microbes:

1) An increase of 12% in total protein content (except in the animals vaccinated with the Sonne and Newcastle antigens, in which the total protein content remained unchanged);

2) A marked general decrease in the albumin fraction, most marked

on immunization with the Newcastle antigen (46.5%) and least marked with the Flexner antigen (51.8%), normal being 53.1%. According to current notions, albumins are synthesized in the liver and the slight decrease in albumin content noted without exception in the animals consequently indicates a general reaction on the part of the liver;

3) the globulin fractions yielded the following picture:

a) a marked increase in the gamma-globulin fraction in all series of experiments (by 50-60% in comparison with the normal rabbits);

b) a decrease in the quantity of beta-globulins in all cases (by 4-12%);

c) an increase in the alpha-globulin fraction on immunization with the antigens prepared from Sonne and Schmitz-Shtutser bacteria, a decrease on immunization with the Flexner and Boyd-Novgorodskoy antigens, and no change on immunization with the Newcastle antigen.

The literature contains a number of works on the protein-fraction content of normal rabbit sera. The data in the literature and the mean values which we calculated are shown in Table 2.

On comparison of our data with the mean figures obtained by various authors using different electrophoresis methods we should note a slight decrease in the quantity of albumins and a proportional increase in the alpha-, beta-, and gamma-globulin fractions.

Comparing the results which we obtained in our experiment (shown in Table 1) with the data in the literature on normal rabbit sera (shown in Table 2), we may note that the changes in the albumin and gamma-globulin fractions run in the same direction (the albumins decrease by 15-24% and the gamma-globulins increase by 75-97%); the same is true of the remaining fractions, the quantity of beta-globulins remaining unchanged and the alpha-globulins increasing slightly.

In conclusion we might point out that when animals are given cor-

TABLE 2

Protein Fractions of Normal Rabbit Sera From Data in the Literature

Авторы [A]	Метод [B]	Альбумины [C]	Глобулины [D]		
			α	β	γ
[E] И. А. Ойвин, В. И. Ойвин, В. И. Сомин (1951)	Классический по Тизелиусу [O]	64,3—60,3 (62,3)*	7,8—10,5 (9,15)*	12,3—16,4 (14,35)*	15,3—12,9 (14,1)*
[F] Н. А. Троицкая (1953)	"	52,5—66 (59,25)*	7—10,7 (8,85)*	14,5—20,1 (17,3)*	9,8—22,8 (18,4)*
[G] Г. В. Троицкий, Л. С. Тарасова (1955)	"	64,8	10,7	11,1	13,2
[H] Г. В. Троицкий, Л. А. Соркина (1951)	"	70,2—50,0 (64)*	6,9—21,2 (12,5)*	12,5—18,5 (12)*	10,3—17,7 (12)*
[I] Мор (1945)	"	63—65 (64)*	10—15 (12,5)*	11—13 (12)*	10—14 (12)*
[J] Паттнод, Каммингс, Хаджинс (1956)	"	57,2	14,9	14,5	13,2
[K] М. С. Суровкина (1956)	"	60,5	11,5	16,7	11,3
[L] М. В. Бавина, М. Г. Крицман (1953)	на фильтровальной бумаге [P]	55,5—63,0 (62,25)*	10—16 (13)*	6—15,6 (10,8)*	8—14,0 (11)*
[M] Ю. З. Гендон (1956)	"	67,3	12,1	9,7	11,9
[N] О. А. Васильева (1957)	"	61,8*	11,6*	13,5*	18,2*

* Calculated by us

A) Authors; B) method; C) albumins; D) globulins; E) I.A. Oyvin, V.I. Oyvin, V.I. Somin; F) N.A. Troitskaya; G) G.V. Troitskiy, L.S. Tarasova; H) G.V. Troitskiy, D.A. Sorkina; I) Mor; J) Patnod, Kammings, Khadzhins; K) M.S. Surovkina; L) M.V. Bavina, M.G. Kritsman; M) Yu.Z. Gendon; N) O.A. Vasil'yeva; O) clasical Tiselins; P) filter paper.

puscular dysentery vaccines, which do not have exotoxins, there is a marked increase in the gamma-globulin fraction. This agrees with the data in the literature on other corpuscular vaccines (Khadzhins, Kammings, Patnod, Pesheti, and Orlandi for immunization with BCG; Rutkvist, Tal', and Overg for immunization with Pasteurella pseudotuberculosis; and Yamane for immunization with sheep corpuscles).

We may thus assume that antibodies to these vaccines are concentrated in the gamma-globulin fraction of rabbit serum. However, the question of whether the increased gamma-globulin fraction is strictly specific for the given agent or whether the antibodies are only included in it is a matter for further investigation.

CONCLUSIONS

1. The total quantity of protein in intact nonexperimental rabbits averages 6.7 g-%.

2. The protein-fraction composition of the blood serum of intact rabbits has been shown to be: albumins - 53.1%, alpha-globulins - 13.4%, beta-globulins - 15.2%, and gamma-globulins - 15.5%.

3. When the antibody titres were at their heights in the rabbits immunized with Flexner, Sonne, Schmitz-Shtutser, Boyd-Novgorodskoy, and Newcastle dysentery antigen vaccines there was in all cases an increase of 50-65% in the quantity of gamma-globulins and a decrease in the quantities of beta-globulins (of 4-12%) and albumins (of 2-10%). In some cases (Flexner and Boyd-Novgorodskoy vaccines) the alpha-globulins increased, while on immunization with the antigens prepared from Sonne and Schmitz-Shtutser dysentery bacteria the content of this fraction increased somewhat.

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THE STUDY OF ALBUMIN COMPOSITION OF SERUM OF RABBITS BLOOD BY THE METHOD OF ELECTROPHORESIS ON THE PAPER AT IMMUNIZATION OF THEM BY DYSENTERIC VACCINES ANTIGENS

Vasilyeva O.A.

Fractional composition of serum of the blood of normal rabbits and immunized by dysenteric antigens was studied.

The increase of gamma-globulin fraction on the height of standing of antibodies was shown.

IMMUNOBIOCHEMICAL PARALLELS IN THE PRODUCTION OF SONNE AGGLUTINATIVE SERUM

O.A. Vasil'yeva

In our preceding report (see p. 329, this book) we showed that the blood sera of rabbits serving as donors of agglutinative dysentery sera, being kept under this type of immunization for several months, exhibit an increase in serum gamma-globulins and occasionally in alpha-globulins, accompanied by a decrease in albumins and beta-globulins.

Simultaneous determination during immunization of the changes in certain immunological indices (antibody titres) and of total protein, the albumin-globulin coefficient, and the fractional serum composition is of undoubted interest.

The literature accessible to us contains almost no reports on this problem, the exception being M.I. Shpolyanskaya's article on diphtheria antitoxin, which was published very recently. This author used Roemer's method to investigate the background and total contents of diphtheria antitoxin and attempted to determine its relationships to the serum protein factors. She found that there are definite correlations between these indices.

This work presents data on the immunobiological parallels in the production of diagnostic agglutinative Sonne dysentery serum using various types of antigen vaccines.

Experimental Method

18 rabbits selected by weight, breed, and sex were divided into three identical groups. The first group was immunized with antigen prepared from Sonne dysentery bacteria Nos. 714 and 1714 killed with formalin vapor. The second group was immunized with a live culture (a physiological-solution washing of one-day cultures of the same strains). The last group was immunized with merthiolated antigen, i.e., merthiolate in a final dilution of 1:10,000 was used as a preservative in this case. Three intravenous injections of antigen were given at 4 or 5 day intervals. Blood samples were taken from each rabbit before each injection of antigen and at the end of the immunization cycle for determination of the total protein content and antibody titre and electrophoretic investigation. The method used for these determinations was described in the aforementioned article (see p. 329).

The results of the experiments, expressed as averages for each group, are given in Table 1.

As may be seen from the table, the greatest antibody titre during immunization was obtained when the merthiolated antigen was used and amounted to 1:36,100; the live culture yielded the second highest titre, 1:25,600, and the formalin-killed culture the third highest. As usual, the total protein content varied slightly during immunization. The tendency of the albumin-globulin coefficient to decrease may be seen quite clearly. Our attention was struck by the fact that there was a considerable increase in all serum globulins in each group of rabbits after the first injection of antigen. This protein reaction became less intense after the second antigen injection and more selective (by groups) as a result of immunization.

In reviewing the results of our experiments we should first note that the intact rabbits exhibited an increase in alpha- and beta-

globulins as well as gamma-globulins during immunization. As a result, we were led to hypothesize the specificity of these changes (this will be the subject of further investigations). In addition, we should note that there was no direct proportionality between the high antibody titre and the gamma-globulin content. In a whole series of cases the titres of the agglutinative sera differed by 300-400%, while their gamma-globulin contents were virtually identical.

In our opinion, this fact can be explained in the following manner. It is difficult to imagine that the process of antibody formation could involve only the quantitative aspect of gamma-globulin synthesis, leaving its qualitative side intact. As may be seen from the work of Pauling's school, there are severe shifts in the spatial configuration of protein molecules during antibody synthesis. It may be supposed that in those cases in which we obtain high titres of antibodies with a comparatively low gamma-globulin content we are dealing not so much with a process of quantitative accumulation as with a change in their stereochemical configuration.

CONCLUSIONS

1. Immunization of the rabbits with the merthiolated antigen yielded the highest antibody titres, the greatest increase in the quantities of total serum protein and gamma-, beta-, and alpha-globulins and a considerable decrease in the quantity of albumins and the albumin-globulin coefficient.

2. Immunization with the live culture yielded a marked increase in serum gamma- and beta-globulins, the total protein and alpha-globulin levels remaining virtually unchanged in this case; the albumin content and the albumin-globulin coefficient decreased sharply.

3. Vaccination with the "formalin-vapor" antigen yielded a less marked increase in all of the globulin fractions and a decrease in the

TABLE 1

Immunobiochemical Indices of Immunization

A Показатели	B Определения *)	Вид иммунизации C		
		парофомалиновый антиген D	живая культура E	мертиолятный антиген F
G Титры	1	1:50	1:100	1:50
	2	1:100	1:3500	1:700
	3	1:6400	1:12800	1:14400
	4	1:8000	1:25600	1:36100
H Общий белок, в г. %	1	6,37	6,83	6,58
	2	6,50	6,55	6,64
	3	6,59	6,83	7,66
	4	6,52	6,65	7,22
I Альбумино-глобулиновый коэффициент	1	1,1	1,1	0,8
	2	0,9	0,7	0,6
	3	1,0	1,0	0,9
	4	0,8	0,7	0,6
J Альбумины, в %	1	52,3	53,2	47,2
	2	49,0	41,1	39,6
	3	50,1	51,4	48,0
	4	46,9	42,6	37,6
K Альфа-глобулины, в %	1	14,3	15,8	16,0
	2	18,7	18,7	20,2
	3	15,7	16,0	19,8
	4	15,4	19,6	19,8
L Бета-глобулины, в %	1	16,7	15,4	17,9
	2	16,0	17,6	21,1
	3	17,4	15,0	17,8
	4	17,8	15,9	20,2
M Гамма-глобулины, в %	1	16,2	16,2	17,1
	2	18,4	18,1	26,0
	3	16,7	17,4	18,0
	4	19,9	21,9	22,2

*The numerals in the "determinations" column indicate: 1) Background investigation; 2) investigation before second antigen injection; 3) investigation before third antigen injection; 4) investigation of results of immunization.

A) Indices; B) determinations; C) type of immunization; D) antigen killed with formalin vapor; E) live culture; F) merthiolated antigen; G) titres; H) total protein, in g-%; I) albumin-globulin coefficient; J) albumins, in %; K) alpha-globulins, in %; L) beta-globulins, in %; M) gamma-globulins, in %.

albumin content and the albumin-globulin coefficient.

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IMMUNO-BIOCHEMICAL PARALLELS IN THE PROCESS OF GETTING AGGLUTINATING SONNE SERUM

Vasilyeva O.A.

In the process of immunization the quantity of gamma-globulins in the serum of rabbits gradually increases and so do (but in less degree) alpha-globulins and beta-globulins albumen is gradually and steadily decreasing. The common serum albumen almost does not change.

A METHOD OF PREPARING LISTERELLOSIS AGGLUTINATIVE SERUM

L.A. Burenkova

Bacteriological, biological, and serological research methods are used for the diagnosis of listerellosis. The most convenient and precise diagnostic method for this disease is isolation and identification of a culture of the causitive agent. A high-quality specific agglutinative serum is necessary for the serological identification of a listerella culture; development of a method for preparing such a serum was the object of the work described herein.

TABLE 1

Importance of Immunization Schemes in the Production of Listerellosis Agglutinative Serum

A № схем	B Вид антигена	C Количество взятых в опыт кроликов	Доза антигена за один цикл иммунизации в млрд. микробных тел D	Средний титр сыворотки E
1	Пароформалиновый, штамм 2741 F	7	10	1:13600
2	" "	9	5	1:6500
3	"	7	7	1:5750
4	" "	8	6	1:5200
5	"	4	2,6	1:3200
6	"	4	10	1:57600

A) Scheme number; B) type of antigen; C) number of rabbits used in experiment; D) antigen dose over one immunization cycle, in billions of microbial bodies; E) mean serum titre; F) formalin-vapor-killed, strain 2741.

Listerellosis agglutinative serum has been prepared by several

investigators (P.P. Sakharov and Ye.I. Gudkova, A.P. Tarasova and G.F. Pogonyaylo, Zeyeliger, Potel, et al.).

We made a detailed study of various antigens and immunization schemes for the production of listerellosis agglutinative serum. The experiments were conducted on rabbits weighing from 1.7 to 4.2 kg. Strain 2741, isolated in 1951 from the corpse of a young pig by G.F. Pogonyaylo, was used as the basic strain. After a preliminary determination of normal serum agglutinins the animals were immunized intravenously with a microbial suspension prepared by a given method. It was first necessary to select the most suitable doses and intervals for the antigen injections. For this purpose the rabbits were immunized with an antigen consisting of a suspension of microbes (1 billion cells per ml) killed with formalin vapor, in accordance with the following schemes.

Scheme 1. The antigen was administered four times, the intervals between the first three injections being two days and that between the third and fourth injections 7 days. The doses administered were 1, 2, 3, and 4 billion microbes. The animals consequently received 10 billion microbial bodies during the immunization cycle. P.P. Sakharov and Ye.I. Gudkova obtained listerellosis agglutinative serum by this method.

Scheme 2. The immunization doses were halved, 5 billion microbial bodies per cycle being administered. The intervals between injections were the same as in the first scheme.

Scheme 3. The antigen was administered at 3 or 4 day intervals. The doses given were 1, 1.5, 2, and 2.5 billion microbes. The total dose was 7 billion microbes.

Scheme 4. The rabbits were immunized four times, at seven-day intervals, in doses of 0.5, 1, 2, and 2.5 billion microbes. A total

of 6 billion microbes were given during the immunization cycle.

Scheme 5. This scheme was based on administration of small antigen doses: 0.2, 0.4, 0.8, and 1.2 billion microbial bodies. The antigen was given at the same intervals as in scheme 4. The rabbits received 2.6 billion microbes during the immunization cycle.

Scheme 6. The rabbits were immunized 6 times, in doses of 1, 1, 2, 2, 2, and 2 billion microbes. The interval between the first two injections was two days, the antigen subsequently being given daily. The animals received 10 billion microbial bodies during the immunization cycle.

In order to study the various immunization schemes the rabbits were divided into groups having the same average weight. The data obtained in studying different doses and different intervals between individual injections are given in Table 1.

It may be seen from the data in Table 1 that Scheme 6 (which yielded a mean serum titre of 1:57,600) is the most rational, Scheme 1 (a mean titre of 1:13,600) occupying second place, Scheme 2 (mean titre of 1:6500) third place, Schemes 3 and 4 (mean titres of 1:5750 and 1:5200) fourth place, and Scheme 5 (mean titre of 1:3200) last place.

After determining the best scheme for immunizing the rabbits we turned to a study of various antigens in order to find the most effective method of treating the microbial suspension. For this purpose physiological solution was added to a washing from an agar listerella culture to make a one-billion-cell suspension and the microbes were killed by various methods.

We prepared the following antigens for the immunization:

1) a microbial suspension killed with formalin vapor;

2) a heat-killed antigen, a microbial suspension heated at 65° for one hour;

TABLE 2

Importance of Antigen Processing in the Production of Listerellosis Agglutinative Serum

A Вид антигена	Количество взятых в опыт B кроликов	Средний титр сыворотки C
D Бактерии, убитые парами формалина	7	1:13600
E Бактерии, убитые температурой 65°	5	1:13000
F Бактерии, убитые парами хлороформа	5	1:3200
G Бактерии, убитые формалином	6	1:8500
H Бактерии, убитые 0,5% карб. кислоты	5	1:5100
I Полный антиген из листерий	4	—
J Бактерии, обработанные 1/4 N раствором трихлоруксусной кислоты	5	1:10200
K Солянокислая вакцина	5	1:2200

A) Type of antigen; B) number of rabbits used in experiment; C) mean serum titre; D) bacteria killed with formalin vapor; E) bacteria killed by heating at 65°; F) bacteria killed with chloroform vapor; G) bacteria killed with formalin; H) bacteria killed with 0.5% carbolic acid; I) whole listerella antigen; J) bacteria treated with 1/4 N trichloracetic acid; K) vaccine treated with hydrochloric acid.

3) an antigen treated with chloroform vapor;

4) a formalized antigen, a one-billion-cell listerella suspension to which 0.25% formalin was added;

5) a microbial suspension killed by addition of 0.5% carbolic acid;

6) a whole antigen prepared by the method devised by Buaven and Mesrobeanu;

7) a microbial suspension treated with 1/4 N trichloracetic acid (after extraction of the whole antigen);

8) a vaccine extracted with 10 times its volume of 0.2 N hydrochloric acid.

The results obtained on immunization of the rabbits with the various listerellosis antigens are shown in Table 2.

It may be seen from this table that the formalin-vapor-killed antigen has the best antigenic properties (a mean serum titre of 1:13,600), the heat-killed vaccine occupying second place (mean titre of 1:13,000) the antigen treated with 1/4 N trichloracetic acid third place (mean

itre of 1:10,200), the formalized vaccine fourth place (mean titre of 1:8500), the bacteria killed with carbolic acid fifth place (mean titre of 1:5100), the bacteria killed with chloroform vapor sixth place (mean titre of 1:3200), and the vaccine killed with hydrochloric acid last place (mean titre of 1:2200). The whole listerella antigen, which we obtained by the method devised by Buaven and Mesrobeanu, proved to be ineffective in immunizing rabbits; it did not cause the formation of agglutinins in their sera.

During immunization we made systematic observations of the condition of the vaccinated rabbits; their temperatures and weights were measured and their appetites noted. It might be pointed out that the rabbits withstood immunization with the listerellosis antigen well, no epizootic occurring. Blood samples were taken before each immunization for determination of the increase in serum agglutinins.

A study of the dynamics of the increase in antibody titre during hyperimmunization showed that the first antigen injection does not cause any marked rise in serum antibodies; the titre of agglutinins increases sharply after the 2nd, 3rd, and 4th injections, reaches its maximum on the 16th day after the beginning of immunization, and remains at this level until the 18th day, then dropping sharply.

On the basis of the observations which we made we may draw the following conclusions.

1. In preparing listerellosis agglutinative serum it is wisest to use an immunization scheme consisting of six antigen injections in doses of 1, 1, 2, 2, 2, and 2 billion microbes; the interval between the first two injections should be two days and the antigen should then be given daily. Of the other immunization schemes the best is that consisting of four antigen injections in doses of 1, 2, 3, and 4 billion microbes with intervals of two days between the first three

injections and an interval of seven days between the third and fourth injections.

2. Listerella killed with formalin vapor had more marked antigenic properties than the microbes treated by other methods.

3. Phlebotomies to obtain listerellosis agglutinative serum must be carried out on the 6th-8th day after the end of immunization.

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TO THE METHOD OF PREPARING LISTERIAL AGGLUTINATING SERUM

Burenkova L.A.

The scheme of immunization and the influence of the way of cultivating microbe suspension were studied. The best results were received at using the scheme based on summation of antigen irritation and bacteria killed by the vapors of formalin.

INFLUENCE OF VARIOUS PRESERVATIVES AND STORAGE TIME AND CONDITIONS ON THE QUALITY OF LISTERELLOSIS AGGLUTINATIVE SERUM

L.A. Burenkova

There are many data in the literature on the preservation of immune sera. Thus, I.S. Rubinshteyn and D.Kh. Zibitsker (1938) studied the preservative properties of chloroform in a concentration of 0.5%, phenol in a concentration of 0.5%, and chinisol in concentrations of 0.1, 0.05, and 0.02%. They concluded that chinisol is a better preservative than chloroform or phenol, since it alters the physicochemical properties of the serum considerably less, causes a smaller drop in antibody titre, and has good bactericidal properties. Of the 3 chinisol concentrations tested the authors believe that 0.05% is sufficient for the preservation of serum.

Z.A. Yakubovich (1931) investigated the influence of chinisol on thermolabile and thermostable agglutinins. From his experiments the author found that this preservative does not affect the H- and O-agglutinins of paratyphoid B serum, which contains all group agglutinins.

R.P. Lopatitskaya and A.M. Gluzman (1941) studied the Zbarskiy bactericide, streptocide, and chinisol; they believe that these substances are quite suitable for the preservation of immune sera.

R.I. Vayndrakh (1943) suggests that rabbit serum be preserved by adding recrystallized boric acid to make 1-2% of the total volume, carboglycerine, or chinisol.

In preparing listerellosis agglutinative serum Zeyeliger (1955) used phenol in a concentration of 0.5% for preserving the O-serum and glycerine in a concentration of 50% and merthiolate in a dilution of 1:10,000 for preserving the H-serum.

In our work we used 1% recrystallized boric acid, 0.05% chinisol, 0.5% chloroform, 40% glycerine, and merthiolate diluted to 1:10,000 for preserving listerellosis agglutinative serum.

As is well known a preservative must first of all protect the serum against microbial growth and should not cause any decrease in its titres, regardless of the storage time, or alter its physicochemical

properties. Each of the preservatives which we used was consequently tested for: 1) bactericidal properties; 2) its influence on the serum titre; 3) its influence on the physical properties of the serum.

TABLE 1

Change in the Titre of Listerellosis Agglutinative Serum on Storage in a Refrigerator

Срок наблюдения в месяцах (A)	Консервант (B)	Количество серий (C)	Сохранился прежний титр (D)	Снизился титр в 2 раза (E)
3—4	Борная кислота (F)	19	19	—
	Хинозол (G)	10	10	—
	Хлороформ (H)	9	9	—
	Мертиолят (I)	11	11	—
	Глицерин (J)	7	7	—
5—7	Борная кислота	19	19	—
	Хинозол	10	10	—
	Хлороформ	9	9	—
	Мертиолят	11	11	—
	Глицерин	7	7	—
8—10	Борная кислота	17	17	—
	Хинозол	9	9	—
	Хлороформ	8	8	—
	Мертиолят	10	8	2
	Глицерин	7	7	—
12	Борная кислота	16	16	—
	Хинозол	9	8	1
	Хлороформ	8	7	1
	Мертиолят	9	6	3
	Глицерин	7	6	1
15	Борная кислота	10	8	2
	Хинозол	4	1	3
	Хлороформ	2	—	2
	Мертиолят	3	1	2
	Глицерин	3	2	1
18	Борная кислота (F)	6	5	1
	Хинозол (G)	1	1	—
	Мертиолят (I)	1	1	
21	Борная кислота (F)	3	2	1

A) Observation time, in months; B) preservative; C) number of series; D) retained prior titre; E) titre halved; F) boric acid; G) chinisol; H) chloroform; I) merthiolate; J) glycerine.

We prepared a total of 22 series of listerellosis agglutinative serum. After the serum had been processed it was poured into several flasks for addition of the different preservatives and kept in a refrigerator for seven days; the serum titre was then determined, a culture was made under sterile conditions, and a portion of the serum in

each flask was exposed to room conditions.

Boric acid was tested as a preservative in 19 series of sera, chinisol in 10 series, chloroform in 9 series, glycerine in 7 series, and merthiolate in 11 series.

As has already been stated, in order to study the bactericidal properties of the preservatives the sera were cultured on sterile media after being kept in contact with the preservatives for one week. Microbial growth did not occur in a single one of the 22 series.

Tables 1 and 2 show data on the influence of the different preservatives on the serum titre during storage in a refrigerator and at room temperature.

It may be seen from the material in Tables 1 and 2 that the titre of the listerellosis agglutinative serum did not decrease in a single case on storage for up to 7 months, regardless of which of the five preservatives was used and under what conditions the serum was kept.

On further storage the titre of the serum preserved with merthiolate decreased earliest (after 8-10 months). The titres of the sera preserved with boric acid and chloroform also decreased at this time, but only when they were kept under room conditions.

The sera which were preserved with boric acid and refrigerated completely retained their prior titre over a 12-month storage period; we must consequently note that they are preferable to sera with other preservatives.

A study of the influence of the various preservatives on the physical properties of the sera showed that the latter usually became cloudy after the preservatives were added, as a result of protein denaturation. The degree of cloudiness varied in accordance with the preservative. In addition, when the sera were stored residues formed as a result of a change in the serum colloids. The quality of the glass used

in the ampule is of great importance in this process. The extent of the cloudiness and the quantity of residue were independent of the character of the preservative. The residue was greater when the serum

TABLE 2

Change in Titre of Listerellosis Agglutinative Serum as a Result of Storage at Room Temperature

A Срок набл-дения	B Консервант	C Количество серий	D Сохранили прежний титр	E Снизили титр в 2 раза
3 – 4	Борная кислота F	15	15	—
	Хинозол G	9	9	—
	Хлороформ H	8	8	—
	Мертиолят I	9	9	—
	Глицерин J	6	6	—
5 – 7	Борная кислота	15	15	—
	Хинозол	9	9	—
	Хлороформ	8	8	—
	Мертиолят	9	9	—
	Глицерин	6	6	—
8 – 10	Борная кислота	15	14	1
	Хинозол	9	9	—
	Хлороформ	8	7	1
	Мертиолят	9	7	2
	Глицерин	6	6	—
12	Борная кислота	14	13	1
	Хинозол	9	7	2
	Хлороформ	8	5	3
	Мертиолят	8	3	5
	Глицерин	6	5	1
15	Борная кислота	7	3	4
	Хинозол	4	1	3
	Хлороформ	2	1	1
	Мертиолят	3	1	2
	Глицерин	3	1	2
18	Борная кислота F	3	2	1
	Хинозол G	1	1	—
	Мертиолят I	1	1	—
21	Борная кислота F	1	—	1

A) Observation time; B) preservative; C) number of series; D) retained prior titre; E) titre halved; F) boric acid; G) chinisol; H) chloroform; I) merthiolate; J) glycerine.

was stored at room temperature than when it was refrigerated.

On the basis of the observations which we made we may draw the

following conclusions.

1. Recrystallized 1% boric acid, 0.05% chinisol, 0.5% chloroform, 40% glycerine, and merthiolate diluted to 1:10,000 may be used as preservatives for listerellosis agglutinative serum. These preservatives have good bactericidal properties, do not cause any sharp decrease in agglutinin titre, and do not materially affect the physical properties of the serum. Boric acid had the best properties of all the preservatives studied.

2. Listerellosis agglutinative serum must be stored in a refrigerator at a constant temperature of 4-5°C. Under these conditions it usually retains its titre for no less than 10-12 months.

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THE STUDY OF INFLUENCE OF CONSERVANTS, TIME AND CONDITIONS OF KEEPING ON THE QUALITY OF LISTERIAL AGGLUTINATING SERUM

Burenkova L.A.

To preserve agglutinating serum it is possible to use boric acid, chinisol, chloroform, merthiolate and glycerine.

The best conservant is boric acid.

DATA ON THE PREPARATION OF TYPE-SPECIFIC ADSORBED LISTERELLOSIS SERA

L.A. Burenkova

We know from the data in the literature that listerella have a complex antigenic structure and that they may be classified into several serological types in accordance with their content of O- and H-antigens.

Dzhulianelle and Pons (1939) established that listerella comprised two serotypes, a "rodent" type common to cultures from rabbits and man and a "ruminant" type common to cattle, sheep, and other animals. On the basis of a study of the antigen structure of 54 strains of listerella Peterson (1939, 1940) concluded that listerella may be classified into four types in accordance with their content of flagellate and nonmotile antigens: the first has the flagellate antigens AB and the nonmotile antigens I and II (III), the second has the flagellate antigens BD and the nonmotile antigens I and II (III), the third has the antigens AB and II, and IV (III), and the fourth has the antigens ABC and V (III). Griffin and Robbins (1944), who also studied the formation of antibodies to individual components of the antigenic mosaic, arrived at similar results.

P.P. Sakharov and Ye.I. Gudkova (1948) distinguished three serotypes of listerella, the principal and most common type in the USSR being the "rodent" type, while the "ruminant" and "equine" types develop as a result of adaptation of listerella to ruminants and horses.

Zeyeliger's investigations (1953) confirmed Peterson's data. The former believes that it is sufficient to use two types of listerella (Peterson's 1st and 4th types) to make a serological diagnosis, these having very similar flagellate antigens but different O-antigens. Types 2 and 3 differ only slightly from type 1, so that it is not obligatory to use them in serological diagnosis.

Our institute obtained the 4 standard serotypes of listerella: strain 14/57 (type 1), strain 15/57 (type 2), strain 16/57 (type 3), and strain 17/57 (type 4). We set ourselves the task of preparing type-specific adsorbed listerellosis sera. For this purpose we produced OH- and O-sera against these four strains by immunizing rabbits with formalized and boiled antigen.

In order to ensure saturation of the sera we prepared OH- and O-adsorbants from these same strains. The OH-adsorbants were produced by culturing listerella on meat-extract agar containing 0.1% glucose for two days at room temperature. The cultures were washed with physiological solution and killed with 0.5% formalin.

The O-adsorbants were prepared by culturing listerella on the medium at heater temperature (37°) for one day. The cultures were washed with physiological solution and heated in a bath containing boiling water for one hour.

After addition of the adsorbants the sera were placed in a heater at 37° and left there until the following day. In order to determine the extent of adsorption the serum samples were centrifuged and checked in the agglutination reaction on glass.

For serotyping of the listerella we had to obtain the so-called nonmotile serum factors I-II, I, IV, and V and the flagellate factors A and D against the corresponding antigens. Adsorption was carried out in the following manner.

Factors I-II: O-serum against type 1 was saturated with OH-adsorbant type 4.

Factor I: O-serum type 1 was saturated with OH-adsorbant type 3.

Factor IV: O-serum type 3 was adsorbed with OH-adsorbant type 1.

Factor V: O-serum type 4 was adsorbed with OH-adsorbant type 2.

Factors AB: OH-serum type 1 was saturated with O-antigen type 1.

Factor A: OH-serum type 1 was adsorbed with OH-antigen type 2.

Factor D: OH-serum type 2 was saturated with OH-adsorbant type 1.

As a result of adsorption of the native listerellosis agglutinative sera we obtained 7 type-specific sera containing the aforementioned factors.

The criterion for the finished serum was a clear positive agglutination reaction with a homologous culture within 1/2-1 minute at room temperature and no agglutination reaction with a heterologous culture within 20 minutes (in a humid room).

TABLE 1

Agglutination of Listerella Strains With the Adsorbed Sera Prepared

A № штаммов	Факторы сыворотки B						
	I, II	I	IV	V	AB	A	Д
4930	++++	++++	–	–	–––+	+–++	–
916–17	+++–	++++	–	–	+––+	–+++	–
1196	++	–	–	–	–+––	+–––	–
25	++–+	++++	–	–	+––+	+–+–	–
8621	+++–	++++	–	–	–+++	++++	–
949–51	+++–	++––	–	–	+–++	–+––	–
134	–++–	++––	–	–	+–+–	––	–
709	+++–	+––+	–	–	++–+	–++	–
1524	++––	+–++	–	–	––+–	+–––	–
449	+++–	+–++	–	–	++++	++++	–
1613–15	+–++	+––+	–	–	–++–	++––+	–
125	++––	+++–	–	–	+++–	–++	–
1749	+++–	++–+	–	–	+–++	+++	–
2741	++–+	+–++	–	–+++	++++	++++	–

A) Strain number; B) serum factors.

The 14 strains of listerella which we had available were checked

in the agglutination reaction with the adsorbed sera which we had prepared. The results obtained are shown in Table 1.

The data given in Table 1 show that 12 of the strains studied may be included in serotype 1, since they agglutinated sera containing factors I-II, I, AB, and A.

Strains 1196 and 2741 cannot be included in any of the four types, since they have aberrations in their antigenic structure.

Strain 1196 differs from typical cultures of serotype 1 only in the fact that it is not agglutinated by serum containing factor I. For some unknown reason this type 1 strain apparently lacks nonmotile antigen I.

Culture 2741 is agglutinated by sera containing factors I-II, I, V, AB, and A. This strain consequently consists of nonmotile antigens I, II, and V and flagellate antigens AB and thus includes antigens of serotypes 1 and 4.

These data show that it is possible to prepare type-specific listerellosis sera containing factors I-II, I, IV, V, AB, A, and D.

The majority of the listerella strains which we studied belonged to Peterson's serological type 1.

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THE MATERIALS ON PREPARING LISTERIAL TYPOSPECIFIC ADSORBED SERUMS

Burenkova L.A.

By adsorbtion of agglutinins by typical strains serums containing I, II, I, V and AB factors were prepared.

AN ACCELERATED METHOD FOR PRODUCING ADSORBED AGGLUTINATIVE DYSENTERY SERA

Ye.S. Naumova

Adsorbed dysentery agglutinative sera are widely used for the serological identification of the causitive agent of dysentery.

Repeated addition of the appropriate adsorbants is necessary to free the immune serum of all nonspecific agglutinins; after each addition the microbial serum suspension is placed in a heater (at 37°C) for from 2 to 18 hours (depending on the volume of serum being adsorbed) The serum is then tested and centrifuged and the completeness of adsorption is checked in the agglutination reaction on glass.

The laboriousness and duration of this process impelled us to seek an accelerated adsorption method. For this purpose, we carried out adsorption with the aid of centrifuging as well as by the method used in production in accordance with the current instructions (the latter being our control).

Adsorption of sera by centrifuging consists in the following. The basic adsorbant (the one which reacted with the serum in the highest titre in a preliminary agglutination reaction) is added to the serum, which has been diluted with physiological solution (1:2), and the mixture is centrifuged (for 10-15 min. at 3000 rpm). We used a formalin-killed microbial suspension (200-600 billion cells per ml) as the adsorbant. Adsorption occurs on centrifuging (the group antibodies of the immune serum are taken up by the antigens of the adsorbant). The centri

TABLE 1

Time Necessary to Produce a Single Series of Dysentery Serum Using Various Adsorption Methods

Название сыворотки A	№ серии B	Метод C	Количество внесенной адсорбента D	Время, затраченное на адсорбцию E
F Бойд — Новгородской	1	Опытный L	5	75 мин. N
		Контрольн. M	5	10 час. O
Флекснер G	7	Опытный	2	30 мин.
		Контрольн.	2	4 час.
Григорьева — Шига H	4	Опытный	4	60 мин.
		Контрольн.	4	8 час.
Шмитц — Штуцер I	8	Опытный	1	15 мин.
		Контрольн.	1	2 час.
Флекснер, тип „Д" J	11	Опытный	3	45 мин
		Контрольн.	3	6 час.
Флекснер, тип „В" K	13	Опытный	4	60 мин.
		Контрольн.	4	8 час.

A) Serum designation; B) series number; C) adsorption method; D) quantity of adsorbant added; E) time required for adsorption; F) Boyd-Novgorodskoy; G) Flexner; H) Grigor'-yeva-Shiga; I) Schmitz-Shtutser; J) Flexner type D; K) Flexner type B; L) experimental; M) control; N) min; O) hr.

fugate is poured into a sterile dish and the completeness of adsorption is immediately checked in an agglutination reaction on glass. If the serum still enters into reaction with heterogeneous cultures adsorption is continued and another check is made.

This entire process of serum depletion can be carried out within a shorter time than when the method approved by the instructions is used.

We obtained 24 series of adsorbed agglutinative dysentery sera and subjected 19 of them to systematic observation to determine the stability of their properties on storage. 12 of these series were stored

under refrigeration and at room temperature and 7 were stored under refrigeration only.

A volume of 40 ml of each series was prepared. Our observations showed that there was a large difference in the time required to prepare a single series of serum by different adsorption methods. The data obtained are given in Table 1.

It may be seen from the material in Table 1 that the time necessary to produce one series of adsorbed serum by centrifuging was only one-eighth that required when the adsorption method described in the instructions was used.

It was found in our experiments that the quantity of adsorbant in billions of cells required to take up the group agglutinins of a single series of immune serum was the same for both the control (following the instructions) and experimental adsorption methods.

We also tested the simultaneous introduction of 2 or 3 adsorbants and found that the same quantity of microbial suspension is consumed in the two methods when two or three adsorbants are simultaneously added.

The simultaneous use of several adsorbants in serum depletion by centrifuging even further curtails the time required for the process.

We studied the stability of the properties of the agglutinative sera prepared by the different adsorption methods on storage under refrigeration and at room temperature.

The results obtained indicate that sera prepared by centrifuging are not inferior in agglutinative properties to those for which adsorption was carried out by heating.

Of the 19 serum series on the stability of whose properties we made observations 4 lost their ability to enter into the agglutination reaction after 3 months of storage, regardless of the adsorption method used, while 15 retained this capacity for 6 months (the observation

period) both when refrigerated and when kept at room temperature.

As a result of the observations which we made we may draw the following conclusions.

1. The preparation of adsorbed agglutinative dysentery sera may be considerably accelerated if adsorption is carried out by centrifuging.

2. The same quantity of microbial suspension is consumed in adsorption by centrifuging as in adsorption by heating.

3. 2 or 3 adsorbants may be used simultaneously in preparing adsorbed agglutinative sera by centrifuging.

4. Adsorbed dysentery sera produced by centrifuging retain their agglutinative properties for no less than 6 months (our observation period) both under refrigeration and at room temperature.

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QUICKENED METHOD OF RECEIVING ADSORBED AGGLUTINATING DYSENTERIC SERUMS

Naumova E.S.

Adsorbtion with the aid of centrifugation was used and the properties of serums did not become worse.

IMPORTANCE OF MERTHIOLATED ANTIGEN IN THE PRODUCTION OF AGGLUTINATIVE SERA

O.A. Vasil'yeva

Several methods have been proposed for preserving the antigen vaccines used in the production of diagnostic agglutinative sera. These include, for example, heat treatment of the microbial suspension and sterilization with carbolic acid, alcohol, formalin, or ultrasound. However, none of these methods can completely satisfy production requirements, since each of them causes a greater or lesser destruction of the microbial cell.

Organic mercury compounds (monosept, phenylmercuric nitrate, and merthiolate) have recently been suggested for the preservation of certain vaccines. These substances, which are quite bactericidal, are harmless in doses sufficient for the sterilization of bacterial suspensions and, according to the data of certain authors, do not alter the antigenic properties of the bacteria.

There are reports now in publication on the effectiveness of merthiolate in preserving pertussis-diphtheria (M.S. Zakarova) and influenza (A.I. Gorokhovnikova and O.I. Fayn) vaccines. S.A. Usikova believes that the mercury preparations monosept and phenylmercuric nitrate ensure rapid sterilization of suspensions of typhoid-paratyphoid bacteria, the vaccines retaining their ability to cause production of O-, H-, and Vi-agglutinins, and stabilize the immunogenicity of the vaccines (in comparison with formalized vaccines).

We were given the task of testing merthiolate, sodium ethylmercurithiosalicylate, as a preservative for dysentery antigen vaccines. This preservative was used in the production of diagnostic agglutinative sera for Flexner, Schmitz-Shtutser, Sonne, and Boyd-Novgorodskoy dysentery bacteria.

The experiments were performed on rabbits weighing 2.5-3.5 kg, which were divided into uniform groups by weight, breed, sex, and initial antibodies. The results of the experiments were evaluated from the agglutination reaction. The highest dilution at which the agglu-

TABLE 1

Results of Immunization of Rabbits With 4-5 Cycles of Merthiolate-Killed Dysentery Bacteria

A Группы	B Вид иммунизации	C Консервант	D К-во животных	E Титры				F Средний титр
				1:3200	1:6400	1:12800	1:25600	
1	Флекснер G	Формалин I 0,15 %	54	2	17	14	21	1:15800
		Мертиолят 1:.0000 J	56	1	5	22	28	1:18200
2	Шмитц-Штуцер H	Формалин 0,15 %	11	—	3	8		1:11000
		Мертиолят 1:10000	21	—	6	15		1:11000

A) Group; B) type of immunization; C) preservative; D) number of animals; E) titres; F) mean titre; G) Flexner; H) Schmitz-Shtutser; I) formalin; J) merthiolate.

tination reaction went to +++ was taken as the titre. The antigen was prepared from a washing from a day-old culture of the appropriate type of microbe (3-7 strains being used) containing 1-2 billion microbes per ml; one portion of the washing was preserved with formalin vapor and the other with merthiolate in a final dilution of 1:10,000.

Immunization was carried out with intervals of 4-6 days between injections, the antigen being given intravenously in doses of 400-800-1200 million microbes. There were 219 rabbits under observation. The animals were divided into several groups.

Our first task was to investigate the effect of the merthiolated antigen when it was given to rabbits which had previously undergone 4-5 immunization cycles. By an immunization cycle we mean a 1.5-month period during which the rabbit was given three injections of antigen, a subsequent phlebotomy, and a month's rest before the first injection of the next cycle.

This group contained 140 rabbits, which were immunized with antigens

from Flexner and Schmitz-Shtutser dysentery bacteria.

As may be seen from Table 1, the merthiolated Flexner antigen has a certain advantage; the experimental and control sections of the second subgroup yielded identical results. It is possible that the prolonged exploitation of the animals and the fact that they were immunized with the same type of antigen created an unusual immunological stereotype and changing the antigen had no substantial effect on the results of the immunization.

The next stage of the work was to determine the value of the merthiolated antigen for immunizing previously unvaccinated rabbits.

TABLE 2

Results of Immunization of Intact Rabbits With Dysentery Bacteria Killed With Merthiolate

A Группа	B Вид иммунизации	C Консервант	D Кол-во животных	Титры E 1/12800	1/25600	1/51200	1/102400	1/201800	F Средний титр
1	Флекснер G	Пары формалина I	10	—	6	4	—	—	1 35000
		Мертиолят 1:10000 J	10	—	5	5	—	--	1 30000
2	Шмитц-Н Штуцер	Пары формалина	10	4	—	2	4	—	1 50000
		Мертиолят 1:10000	10	—	2	3	2	3	1 90000

A) Group; B) type of immunization; C) preservative; D) number of animals; E) titres; F) mean titre; G) Flexner; H) Schmitz-Shtutser; I) formalin vapor; J) merthiolate.

In this experiment we used 40 rabbits, which were immunized in the same fashion as in the first experiment, with antigens from Flexner and Schmitz-Shtutser dysentery bacteria.

The results are shown in Table 2.

The advantage of the merthiolated antigen (especially with respect to the Schmitz-Shtutser culture) over the antigen treated with

formalin vapor again appeared in this case.

In order to compare the merthiolated antigen with a live culture an experiment was set up involving immunization with antigens from Sonne dysentery bacteria. A group of animals (18 rabbits) was divided into 3 subgroups, the subjects in which were immunized with a suspension of live microbes, the merthiolated antigen, and the formalized antigen respectively. The highest serum titre (1/31,000) was obtained when the rabbits were given the merthiolated antigen, the second highest (1/25,600) was obtained with the live culture, and the third highest (1/12,000) with the formalized vaccine, i.e., in this case the merthiolated antigen yielded a serum titre 2.5 times as great as the antigen treated with formalin vapor and 25% greater than the live vaccine.

How is this to be explained? It seemed to us that the merthiolate served as a nonspecific stimulus in this case.

The following observations were carried out in order to elucidate this hypothesis. Merthiolate diluted to 1:10,000 was added to the ordinary "formalin-vapor-killed" antigen from the Flexner and Boyd-Novgorodskoy strains. The rabbits in the experimental group (9) were immunized with this antigen, while those in the control group (8) received the "formalin-vapor-killed" antigen in the same doses.

As a result, it was found that the mean titre for the experimental rabbits was 1/4620, while that for the control animals was 1/2800, i.e., administration of merthiolate stimulated the development of antibodies and caused an increase of 65% in the serum titre.

CONCLUSIONS

1. It is expedient to use vaccines preserved with merthiolate in the production of dysentery agglutinative sera.

2. Immunization of rabbits with a merthiolated antigen makes it

possible to obtain agglutinative dysentery sera whose titres are 30-200% higher (within the limits of experimental accuracy) than those of agglutinative sera obtained by the ordinary method.

3. Because of their sterility and high antigenic activity merthiolated antigens can be used under conditions which rabbits immunized with a live culture could not withstand.

4. Revaccination with the merthiolated antigen of rabbits previously immunized repeatedly with formalized vaccines does not yield any substantial advantage.

Tomsk Scientific Research Institute for Vaccines and Sera

THE MEANING OF MERTIOLATE ANTIGEN AT PRODUCTION OF AGGLUTINATING SERUMS

Vasilyeva O.A.

The meaning of merthiolate as a conservant of dysenteric antigens used at getting agglutinating serums was investigated. Definite advantage of it before formalin antigen and even living culture has been shown.

FEASIBILITY OF USING WHITE RATS FOR DETERMINING THE QUALITY OF BACTERIAL PREPARATIONS*

B.G. Trukhmanov, Ye.I. Kleytman, L.A. Yegorshina

Guinea pigs are widely used as laboratory animals in the production of various bacterial preparations. However, since these animals are predisposed to a whole series of diseases (pneumonia, paratyphoid, pasteurellosis, etc.) and are not very prolific, there are a number of difficulties associated with breeding them in the nurseries of small institutes.

All this becomes even more complicated in the northeastern portion of the USSR, where guinea pigs are imported from the southwestern area and the change in climate and customary diet have a sharp negative influence. In searching for a replacement for guinea pigs we settled on white rats. First brought into our institute in 1955, the rats became aclimatized very rapidly and, neither falling ill nor requiring any special conditions, multiplied to such an extent that we were forced to check the increase in population artificially. As a result, the cost of a rat was only one-eighth to one-tenth that of a guinea pig under our conditions.

All of the aforementioned led us to the idea of determining how feasible it would be to use white rats in our production process, in which the consumption of guinea pigs ran into many thousands of animals and substitution of another animal (even though for only some of the guinea pigs) would have a marked economic effect.

The literature contains reports on the use of white rats as laboratory animals for immunological purposes. Thus, according to the data of Ye.V. Glotova, rats are sensitive to the toxins of the basic types of gas gangrene. O.Ya. Ostryy and Ye.N. Kryzhanovskiy used white rats to study the mechanism by which tetanus toxin acts; A.Ya. Alymov, D.F. Pletsityy, and L.L. Aver'yanova used these animals to investigate the restoration of immunological reactivity and the treatment of experi-

mentally-induced tetanus. It must be noted that O.Ya. Osipova, who induced an experimental pertussis in white rats, established that these animals are extremely sensitive to pertussis endotoxin; this is very valuable, since it makes it possible for us to determine residual pertussis-vaccine toxicity so small in extent that it cannot be detected by tests on other species of animals.

Nungester, Moore, Mika, and Summers made a comparative study of the resistance of guinea pigs and white rats to infection, using Pneumococcus and Bacillus anthracis. They were able to establish that the sensitivity of white rats to Pneumococcus is approximately 1 million times as great as that of guinea pigs, while their sensitivity to Bacillus anthracis is 1000 times as great.

Thus, white rats are in principal rather sensitive laboratory animals, particularly with respect to anaerobic infections. However, we did not find any suggestions in the literature for the use of white rats in determining the quality of bacterial preparations.

In the work described herein we set ourselves the task of clarifying the the sensitivity of white rats to those microbial products which we were producing and of determining how much they differed in this respect from guinea pigs.

Experimental Method

The experimental rats were kept in small metal cages, 3 or 4 animals in each cage. Their diet consisted of oats, roots, and milk with cod-liver oil added. Rats weighing from 40 to 265 g were used in the experiment. They were weighed on ordinary pan balances, the youngest and most active animals being placed in a previously weighed cardboard box.

Subcutaneous and intracutaneous injections of the preparations were given with the aid of a special device, the skin on the rat's withers being held tightly in a forceps, the other hand being used to lift its tail and one hind leg. The use of a small laboratory table eliminated the need for an assistant.

In setting up the ocular test, after the rat was immobilized in the ordinary manner it was subjected to light ether anesthesia for 1-2 minutes, in order to make scarification of the cornea and inunction

of the detritus easier; for this purpose a piece of cotton slightly moistened with ether was placed in a small graduate and this "mask" was fitted loosely on the animal's head. After the experiment the rats were killed by being placed in a jar with a cover containing cotton copiously wetted with ether. The experiments on guinea pigs were conducted by the usual method.

In the first series of experiments, which was carried out on 145 white rats and 58 guinea pigs, we studied the sensitivity of rats to tetanus toxin. The minimum lethal toxin dose for guinea pigs was first very carefully titrated.

TABLE 1

Sensitivity of Guinea Pigs and White Rats to Tetanus Toxin

A) МЛМ столбняч. токсина	B) Животные: C) вид	B) Животные: D) к-во	E) Не заболели	F) Форма заболевания: G) легкая	F) Форма заболевания: H) средняя	F) Форма заболевания: I) тяжелая	J) Пали с 3-го по 6-й день	K) Сняты с опыта
4	Белые крысы L	10	—	—	—	10	10	—
"	Морск. свинки M	5	—	—	—	5	5	—
2	Белые крысы	10	—	—	—	10	10	—
"	Морск. свинки	6	—	—	—	6	6	—
1	Белые крысы	31	—	—	—	31	29	2
"	Морск. свинки	8	—	—	—	8	8	—
0,5	Белые крысы	17	—	—	—	17	5	12
"	Морск. свинки	10	—	—	—	10	..	10
0,25	Белые крысы	15	—	3	11	1	—	15
"	Морск. свинки	5	2	—	2	1	—	5
0,1	Белые крысы	15	4	5	6	—	—	15
"	Морск. свинки	6	3	—	3	—	—	6

A) MLD of tetanus toxin; B) animals; C) species; D) number; E) not killed; F) form of illness; G) mild; H) moderate; I) severe; J) died between 3rd and 6th days; K) discarded; L) white rats; M) guinea pigs.

In order to check the sensitivity of the white rats the tetanus toxin was given in doses of from 4 to 0.0125 MLD. Guinea pigs served as the control.

Table 1 shows the experimental setup and the results obtained.

In a special experiment rats were given up to 50 MLD of tetanus toxin. All of the animals died during the first day. The experiments showed that 4 and 2 MLD of tetanus toxin caused the experimental rats and guinea pigs to die on the 3rd or 4th day. No special difference was observed in the effect of doses of 1 and 0.5 MLD of tetanus toxin on guinea pigs and white rats.

Small doses of toxin (0.25 MLD) caused severe forms of tetanus in individual guinea pigs and white rats, but did not lead to death. A dose of 0.025 MLD promoted the development of only mild forms of the disease, no special difference being noted between the sensitivity of the guinea pigs and that of the rats in this case. When 0.0125 MLD of the toxin was administered no differences whatsoever were noted between the two species of animals.

Rats of different weights (from 40 to 265 g) were used in the experiment and no special difference was observed in their reactions to the tetanus toxin. The data obtained indicate that white rats are not inferior to guinea pigs in sensitivity to tetanus toxin and may apparently be used for biological monitoring of the strength of tetanus toxin.

The second part of our work was intended to determine the extent of immunity in rats immunized with tetanus toxoid. We used 74 rats in the experiment, immunizing them with different doses of tetanus toxoid (1 and 5 ml). After definite intervals, up to 30 days, the intensity of the immunity induced was checked by administering 100 MLD of tetanus toxin. The control was 85 guinea pigs which received the same doses of the preparations.

Immunization with 1 ml of tetanus toxoid proved to be of little effectiveness and did not set up a full immunity, since 75% of the rats

developed tetanus when given 100 MLD of toxin. Neither the guinea pigs nor the rats developed full immunity sooner than 30 days after administration of 5 ml of toxoid, since earlier administration of toxin (after 17 days) was accompanied by a clinically marked tetanus in the majority of the animals.

One month after immunization with 5 ml of toxoid there was no marked difference in the development of immunity in the guinea pigs and in the rats. While all of the control animals died on the 4th-5th day after receiving 1 MLD of toxin, immunization prevented death in more than 50% of the rats.

Consequently, rats as well as guinea pigs may be used for checking the immunogenic properties of tetanus toxoid.

In a separate experiment on 8 white rats and 5 guinea pigs we investigated the sensitivity of white rats to B. perfringens toxin.

We found that B. perfringens toxin in doses of 3 and 6 guinea-pig MLD did not have any detrimental effect on the rats, but that larger doses, beginning with 15 MLD, produced a pathological effect which leads to death during the first few days. White rats are consequently sufficiently sensitive to this toxin and, when precisely determined, the lethal dose for this species of animal can be used both for checking the strength of the toxin obtained and for verifying the harmlessness of the corresponding toxoid.

In the second series of experiments we investigated the feasibility of using white rats for determining the titre of smallpox vaccine preliminarily tested on guinea pigs and rabbits (see Table 2).

Smallpox vaccine series No. 8 GK-192 was given to nine white rats, being applied to the scarified cornea of the right eye (Gins' method). Only one animal failed to develop a reaction within three days; the others exhibited opacification of the cornea and blepharitis both at

TABLE 2

Sensitivity of Rats to Smallpox-Vaccine Virus

A Вес животного, в г	B Доза оспавакцины (разведение)	C Способ введения	Дни опыта D				
			1	2	3	4	5
185	1:10	по Гинсу	N	N	N	N	N
255	"	" E	N	N	отек G пленка	то же J	бельмо K
212	1:100	"	N	N	N H	помутн.	помутн.
340	"	"	N	N	отек	то же	"
130	1:1000	"	N	N	отек I помутн.	то же	помутн.
110	"	"	N	N	"	"	"
120	"	"	N	N	"	"	"
100	1:10000	"	N	N	"	"	"
120	"	"	N	N	"	"	"
100	1:10 1:10000	по Гроту F	N	N	N	N	N
145	1:10 1:100	"	N	N	N	N	N
145	1:10000 1:1000	"	N	N	N	N	N
135	1:1000 1:10000	"	N	N	N	N	N

A) Weight of animal, in g; B) dose of smallpox vaccine (dilution); C) mode of administration; D) day of experiment; E) Gins' method; F) Grot's method; H) film; I) opacification; J) the same; K) corneal spot.

a detritus dilution of 1:10 and at higher dilutions, 1:100 and 1:10,000. A negative reaction was obtained from the remaining 4 rats, which were given the smallpox vaccine intracutaneously, by Grot's method, in dilutions of from 1:10 to 1:10,000. Control experiments on 10 guinea pigs showed that they responded to a high smallpox-vaccine dilution (1:20,000) with an inflammatory reaction of the cornea, while the limit of sensitivity for the rats was a dilution of 1:10,000. However, at this dilution the rats yielded the same corneal inflammation as the guinea pigs, so that they can obviously be used for checking smallpox detritus by Gins' method when an appropriate coefficient is introduced.

The third series of experiments was performed on 23 rats and 6

control guinea pigs and showed the complete insensitivity of white rats to diphtheria toxin. The rats withstood subcutaneous injections of whole undiluted toxin and did not react to intracutaneous injection of several necrotizing doses of toxin, while the control guinea pigs died 3-4 days after receiving a single dose.

In the fourth and last series of investigations (which were carried out on 15 rats and 15 guinea pigs used as the control) we studied the feasibility of using white rats for determining the harmlessness of sera and toxoids.

For this purpose we used native encephalitis serum (series 131 and 2) and perfringens toxoid rejected in safety-testing, as well as a number of sera (diphtheria, encephalitis, etc.) which were artificially infected by being kept in the laboratory in open ampules.

It was found that the white rats reacted less strongly than the control animals to administration of these preparations. Although infiltrations were formed in both the rats and the guinea pigs, they were small in size, were not accompanied by necrosis of the skin and subcutaneous cellular tissue, and were resorbed on the 6th-7th day in the majority of the animals.

In a more detailed study of this problem it will probably be possible to establish the limit necessary for determining the safety of sera and other preparations by increasing the dose of the preparation under investigation.

Our observations, made on 278 white rats and 179 guinea pigs, indicate that rats may apparently be used for biological evaluation of the quality of anaerobic preparations and certain others.

It is undoubtedly necessary to study a great many subjects to determine the limit of the white rat's sensitivity to various bacterial toxins and other preparations and to determine precisely the corres-

ponding doses, calculating them specifically for this animal. However, the results already obtained are of definite interest and, it seems to us, give grounds for recognizing the feasibility of using white rats for the needs of vaccine and serum production.

ABOUT THE POSSIBILITY OF USING WHITE RATS FOR DEFINING THE QUALITY OF BACTERIAL PREPARATIONS

Trukhmanov B.G., Kleitman H.I., Egorshina L.A.

An attempt was made to substitute in conditions of serum – vaccine production of the institute guinea pigs for simple and easily propagated white rats.

The possibility of using white rats for control of bacterial preparations in the first place of anaeroble group of infections was shown on 179 ducing preparations especially for white rats.

The authors consider it to be necessary to work out the doze of introducing preparations especially for white rats.

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EFFECTIVENESS OF CUTANEOUS VACCINATION WITH BTsZh [BCG] IN TOMSK

A.P. Gerasimenko

We were set the task of collecting and processing data on the effectiveness of cutaneous vaccination against tuberculosis in Tomsk.

Children ranging in age from one to 16 years were vaccinated and revaccinated during the period 1949-1954. A total of 2032 persons were under observation: these consisted of a group of children (749) vaccinated with BTsZh vaccine cutaneously only, a group of persons (303) vaccinated orally with BTsZh during childhood and then revaccinated at various intervals, and a group of children (980) on out-patient treatment from the city tuberculosis dispensary, 261 (or 26.6%) of whom had been vaccinated orally with BTsZh during childhood.

The children's state of health was followed for a number of years after vaccination by studying their developmental histories, histories of illness, and data obtained in medical examinations and roentgenoscopy.

Tuberculosis took a considerably milder course in the children vaccinated with BTsZh than in unvaccinated children and, in the majority of cases, the disease terminated in recovery. The characteristics of the clinical forms of tuberculosis are shown in Table 1.

Focal tuberculosis in the infiltration, decomposition, and dissemination stages was observed ten times as often among the children which did not receive BTsZh, tubercular bronchoadenitis occurred more

than twice as often, tubercular meningitis occurred three times as often, etc.

In 64.4% of the children vaccinated with BTsZh the process was limited to intoxication, while in 15% it reached the primary complex and was eliminated at this point. There were no deaths among vaccinated persons who contracted tuberculosis. Death occurred in 4.3% of all cases as a result of other illnesses (dysentery, pneumonia, etc.). More than 31% of the children vaccinated with BTsZh who contracted tuberculosis came into clear contact with tuberculosis patients in their own families or in a common residence. Of the children not vaccinated with BTsZh who incurred tuberculosis approximately one-third also had come into contact with tuberculosis patients. The mortality from tuberculosis among the unvaccinated children was 0.28%, while that from other diseases was three times as great (0.84%).

Of the total number of persons vaccinated with BTsZh cutaneously alone (521 persons: 346 aged 7 years or more, 77 aged 1 to 3 years, and 98 aged 4 to 6 years) 10, or 1.9%, contracted tuberculosis.

Table 2 shows the intervals after cutaneous vaccination with BTsZh at which tuberculosis developed, the clinical forms of the disease, and whether or not there was contact with tuberculosis patients.

As may be seen, tuberculosis set in in the aforementioned children between the 2nd and 6th year after vaccination and only one child exhibited phylctenulous conjunctivitis after 3 months. There were no deaths in this group.

In the group vaccinated with BTsZh orally during childhood, primarily with liquid vaccine, and then revaccinated cutaneously at different intervals (303 persons; these times frequently exceed the actual intervals before revaccination) 11 individuals, or 3.6%, contracted tuberculosis.

TABLE 1

Clinical Forms of Tuberculosis in Children and Adolescents Under Out-Patient Care at the Tomsk City Tuberculosis Dispensary

A Диагноз	Дети, не вакцинированные БЦЖ (% к общему числу) B	Дети, вакцинированные БЦЖ (% к общему числу) C
D Туберкулезная интоксикация I ст.	24,7	64,4
E Туберкулезная интоксикация II ст.	6,5	4,2
F Туберкулез легких очаговый и в фазе инфильтрации	9,5	—
G Туберкулез легких в фазе распада и обсеменения	2,7	1,2
H Милиарный туберкулез	0,3	—
I Первичный туберкулезный комплекс	11,2	15,3
J Туберкулезный бронхоаденит	27,7	11,0
K Туберкулез лимфоузлов	5,5	0,6
L Туберкулез костей и суставов	0,3	—
M Туберкулез глаз	2,8	—
N Блефаро-конъюнктивиты и кератиты туберкулезной этиологии	1,48	1,22
O Туберкулезный интерлобит	—	0,6
P Плевриты туберкулезной этиологии	4,1	0,6
Q Туберинфицированность	—	11,6

A) Diagnosis; B) children not vaccinated with BTsZh (% of total number); C) children vaccinated with BTsZh (% of total number); D) tubercular intoxication, degree I; E) tubercular intoxication, degree II; F) tuberculosis of the lungs, focal and infiltration phases; G) tuberculosis of the lungs, decomposition and dissemination phases; H) miliary tuberculosis; I) primary tubercular complex; J) tubercular bronchoadenitis; K) tuberculosis of the lymph nodes; L) tuberculosis of the bones and joints; M) tuberculosis of the eyes; N) blepharitis-conjunctivitis and keratitis of tubercular etiology; O) tubercular interlobitis; P) pleuritis of tubercular etiology; Q) tubercular infection.

The time after cutaneous vaccination at which the disease appeared varied from 2 to 3 years. In two cases phylctenulous and scrofulous conjunctivitis set in at intervals of from 10 months to 1 year 2 months.

The clinical form and outcome of the disease, as well as the interval after vaccination at which it appeared are characterized by the following data (Table 3).

In the majority of the persons vaccinated orally only the disease developed during the second year of life (51%). A rather large number of children fell ill during their third year or later (33%). In a

TABLE 2

Data on Intervals After Cutaneous Vaccination With BTsZh at Which Tuberculosis Appeared

A Клинические формы туберкулеза	B Количество случаев	C Контакт с больными туберкулезом	D Срок заболевания от момента вакцинации
E Туберкулез легких	1	+	4 года K
F Туберкулезный бронхоаденит	1	—	6 лет K
G Туберкулезная интоксикация II ст.	2	+	2 года K
H Туберкулезная интоксикация I ст.	2	—	2 г. 8 м. L
I Конъюнктивиты фликтенулезные и скрофулезные	4	—	от 2 мес. до 2 л. 7 м L
J Летальный исход	—	—	— K

M Здоровых — 511 чел.
N Заболевших туберкулезом — 1,9%.

A) Clinical forms of tuberculosis; B) number of cases; C) contact with tuberculosis patients; D) time after vaccination at which illness appeared; E) tuberculosis of the lungs; F) tubercular bronchoadenitis; G) tubercular intoxication, degree II; H) tubercular intoxication, degree I; I) phylctenulous and scrofulous conjunctivitis; J) death; K) years; L) months; M) healthy-511 persons; N) tubercular diseases-1.9%.

preponderant number of cases (53.3%) the tuberculosis took the form of a first-degree tubercular intoxication and had a favorable outcome. Approximately 29% of the children had tubercular bronchoadenitis. In addition, 4.3% of the children in this group died of other diseases.

TABLE 3

Data on Times at Which Tuberculosis Appeared in Children Vaccinated Orally and Revaccinated Cutaneously Against This Disease

A Клинические формы заболевания	B Количество случаев	C Срок заболевания от момента ревакцинации БЦЖ	D Исход заболевания
Первичный туберкулезный комплекс E	1	2 года I	Благоприятный
Туберкулезный бронхоаденит F	2	2 года 8 мес.	" K
Туберкулезная интоксикация I ст. G	3	2—3 года J	"
Конъюнктивиты фликтенулезные и скрофулезные H	5	10 мес. 2 года	"
Процент заболевших L	3.6		

A) Clinical forms of disease; B) number of cases; C) interval after revaccination with BTsZh at which disease appeared; D) outcome of disease; E) Primary tubercular complex; F) tubercular bronchoadenitis; G) tubercular intoxication, degree I; H) phylctenulous and scrofulous conjunctivitus; I) years; J) months; K) favorable; L) percent contracting disease.

In studying the anamneses of children and adolescents born in 1935-1954 who were under out-patient care at the city tuberculosis dispensary (a total of 980 persons) and comparing them with the records on BTsZh vaccination it was established that only 26.6% (261) of these individuals had received such vaccination during infancy and only 3.4% had been revaccinated at various times. Only 6 persons were vaccinated cutaneously at a late age.

Thus, the majority of the children and adolescents who contracted tuberculosis and were under out-patient care (713 individuals) had not been vaccinated with BTsZh during infancy or at a later age. More than 30% of these children had come into contact with tuberculosis patients. The unvaccinated children included adolescents born in 1935-1939, when BTsZh vaccination was not widely carried out in Tomsk. Children born in subsequent years (1940-1949) did not receive revaccination at the times later set up. The official instructions on revaccination were promulgated in 1949.

Local and more severe forms of tuberculosis were frequently encountered in the children and adolescents who had not been vaccinated against this disease and were under out-patient care at the tuberculosis dispensary. Tubercular meningitis occurred in from 4 to 11% of the children 4-14 years of age, tuberculosis of the lungs in from 4 to 9%, miliary tuberculosis in from 2 to 3.6%, and disseminated tuberculosis in from 4 to 23%. The greatest percentage of this latter form occurred in adolescents from 15 to 18 years of age.

CONCLUSIONS

1. Tuberculosis developed in only a small percentage (1.9%) of children and adolescents vaccinated cutaneously with BTsZh, occurring 2-4 years after vaccination. There were no deaths among those who incurred the disease.

2. The clinical course of the tuberculosis was milder in the individuals vaccinated cutaneously with BTsZh.

3. Cases of tuberculosis were recorded in 3.6% of children and adolescents vaccinated during infancy with BTsZh, primarily orally with liquid vaccine, and revaccinated at various intervals. The disease set in from 1 to 2 years or more after revaccination.

4. Tuberculosis also occurred in milder forms among persons vaccinated with BTsZh during infancy only (orally).

5. It was established that 31.4% of the children and adolescents vaccinated with BTsZh came into contact with tuberculosis patients in nidi of the disease.

6. In children and adolescents not vaccinated with BTsZh the tuberculosis took more severe forms (tuberculosis of the lungs with decomposition and dissemination, tubercular meningitis, and pleuritis of tubercular etiology) 2 or 3 times as often as in the vaccinated individuals. Focal tuberculosis of the lungs occurred in 9.5% of all cases. Tubercular bronchoadenitis was encountered 3 times as often in the unvaccinated children. Tuberculosis of the lymph nodes occurred in 5.5% of all cases and tuberculosis of the eyes in 2.8%. It was established that 30.5% of the unvaccinated individuals had come into contact with tuberculosis patients. The mortality from tuberculosis was 0.28%.

7. Following the instructions strictly, children reaching two years of age must be given an initial revaccination against tuberculosis, with a subsequent revaccination after no more than 3-4 years.

8. According to our observations, cutaneous vaccination against tuberculosis is undoubtedly effective.

Tomsk Scientific Research Institute for Vaccines and Sera

THE EFFICIENCY OF CUTANEOUS METHOD OF VACCINATION OF B.C.G. IN TOMSK

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The efficiency of inoculations of B.C.G. by cutaneous method was studied on the group of 2032 children. From 749 persons vaccinated by B.C.G. cutaneously only 1.9 percent of children during the period from two to six years fell ill with tuberculosis after vaccination. Clinical course of tuberculosis in 64.4 percent was limited by tuberculosis intoxication with safe result.

From inoculated by B.C.G. at birth per os and then revaccinated cutaneously during different dates from the moment of initial inoculation 3.6 percent in the period from two to three years fell ill with tuberculosis after the inoculation. The diseases were in mild case. By nonvaccinated by B.C.G. children the tuberculosis was observed more often by way of serious local forms with lethal result in 0.28 percent.

BACTERIOLOGICAL AND PATHOLOGOANATOMICAL CHARACTERISTICS OF EXPERIMENTAL ACUTE RADIATION SICKNESS INDUCED BY BETATRON IRRADIATION AT A POWER OF 25 Mev

G.Ye. Nebolyubova and N.V. Sokolova

A survey article by N.P. Kiselev, published in 1957 (Meditsinskaya radiologiya [Medical Radiology], 1957, No. 5), contains the accurate statement that the most thoroughly studied field of contemporary radiobiology is the problem of the state of natural immunity and the characteristics of the course of infectious processes in animals injured by penetrating radiation. However, all of these numerous and very valuable observations were made in experiments in which the irradiation source was x-rays of ordinary voltage (180-400 kv), while there are almost no works devoted to studies of the influence of the betatron.

We know only of investigations in which the effect of betatron rays and of ordinary x-rays on cultures of bacteria from the gastrointestinal tract were compared. The results of these experiments proved to be contradictory. Thus, C.Dickmann and W. Dittrich (1950) irradiated an agar culture of Bacterium coli in vitro with 6-Mev fast electrons from a betatron and 180 kv x-rays in a dose of 500-4000 r and found that, judging from the inhibition of culture growth, the betatron rays had an efficiency of 2. In other words, the bacterial culture was more sensitive to these rays.

D. Lea, R. Haines, and E. Bretscher (1941) obtained similar results in their work. In J. Lindenmann's investigations (1953) the efficiency of 31-Mev gamma-quanta from a betatron and 180-kv x-rays, as determined from their action on Escherichia coli, proved to be 1. Inhibition of 50% of culture growth on sheet agar was obtained at doses of 2368-2586 r, no matter what the irradiation source.

The investigations which have been carried out to characterize the influence of betatron rays on bacteria in vitro naturally do not enable us to evaluate their action on the autoflora of an irradiated organism. The wide use of the betatron for therapeutic purposes gives practical importance to the study of the state of this autoflora during the development of the reaction to radiation damage.

Our investigation was intended to be a study of the autoflora of the alimentary tract and mouth during acute radiation sickness caused by betatron irradiation.

The experiments were performed on 40 adult guinea pigs of both exes weighing 500-600 g. Massive whole-body irradiation was carried ut with a betatron power of 25 Mev, a dose rate of 30-35 r/min, a istance to focus of 35 cm, and doses of 1800 and 3600 r. The measure- ents were made with an ionization chamber having a volume of 20 cm^3 nd plexiglass walls of uniform thickness.

All of the animals died of typical acute radiation sickness, within -10 days at a dose of 1800 r and within 5-6 days at a dose of 3600 r. he subjects were given careful bacteriological, hematological, and orphological examinations.

Before irradiation both the experimental and control animals were subjected to repeated (2 or 3 times) bacteriological examination of the alimentary tract and mouth. In addition, cultures from the blood, livers, spleens, and brains of the control guinea pigs were investigated. Material was taken from the mouth with a sterile swab and from the rectum with fine rectal tubes. A quantity of 0.1-0.2 g of fecal material was carefully mixed with 1 ml of physiological solution and 0.1 ml of this suspension was cultured in dishes containing Endo's and Levin's media, meat extract, and 5% blood agar. Mucus from the mouth was cultured on sugar bouillon and then transplanted to dishes containing blood agar and ordinary agar. The cultures were incubated in a heater at 37°. After one day the morphology of the colonies is studied and they are counted and recultured on Ressel's medium. The cultures are later subjected to bacteriological analysis.

After the appropriate incubation investigation of smears showed gram-negative bacilli to be present and these were cultured with various carbohydrates to give a color series. The motility of the bacteria and their ability to split proteins to form hydrogen sulfide and indol were then determined. The pathogenicity of the staphylococcus cultures

was determined from the plasma coagulation reaction and the extent of the zone of hemolysis on blood agar, as well as from the necrosis test in rabbits. The blood and organs used for the cultures were removed under sterile conditions when the killed animals were dissected. The cultures were made on sugar and bile bouillon and subsequently transferred to solid nutritive media; the cultures isolated were then identified. A similar method was used for investigating the irradiated animals, from which material was taken during the agonal period.

Bacterium coli, Proteus vulgaris, nonpathogenic Staphylococcus albus, and gram-positive Bacillus sporogenes were obtained from the intestinal contents of all of the guinea pigs before irradiation. The number of colonies varied from 18 to 217 in different animals. Staphylococcus albus, Streptococcus hemolyticus, and diplococci were isolated from the cultures of oral material. The number of colonies here varied from 38 to 218. The blood cultures proved negative for all of the animals, with the exception of one guinea pig, from which diplococci were isolated. The cultures made from organs were sterile in all cases.

The same microbes were obtained from the intestinal contents and mouths of all of the irradiated animals, but in considerably larger quantities; the number of colonies varied from 42 to continuous growth, so that it was occasionally impossible to make even an approximate colony count. The blood and organs of the guinea pigs irradiated in a dose of 3600 r (which lived 5-6 days) proved sterile, except in one case, where pneumococci were detected in the blood culture. Staphylococcus albus and Bacterium coli were isolated from the blood and organs of the majority of the animals irradiated in a dose of 1800 r, which survived for a correspondingly longer time (7-10 days); in one case only Staphylococcus matogenes was observed and in another only Proteus

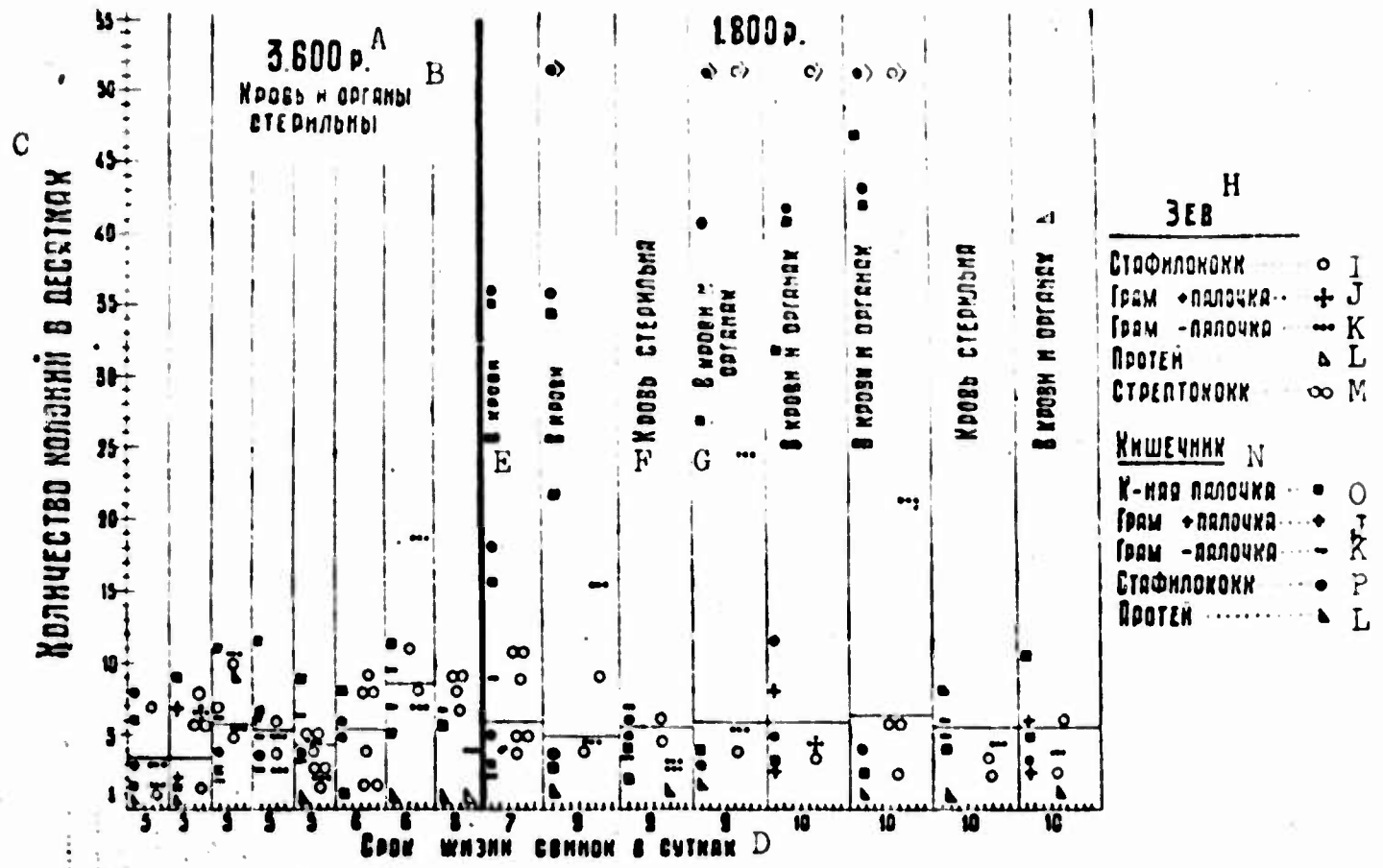

Fig. 1. A) r; B) blood and organs sterile; C) number of colonies, in multiples of ten; D) survival time of guinea pigs, in days; E) in blood; F) blood sterile; G) in blood and organs; H) mouth; I) staphylococci; J) gram-positive bacilli; K) gram-negative bacilli; L) protei; M) streptococci; N) intestine; O) Bacterium coli; P) staphylococci.

vulgaris occurred. All of the cultures from two animals were sterile. Composite data on the results of the bacteriological investigation are shown in Fig. 1, in which the number of colonies obtained is shown along the ordinate in multiples of ten and the survival time of the irradiated animals is shown along the abscissa in days. The symbols below the lines represent the mean number of colonies before irradiation (the colonies from the mouth are shown in white and those from the intestine in black), while the symbols above the lines represent the number of colonies after irradiation.

In order to determine the initial hematological picture the blood of all of the experimental animals was subjected to repeated clinical examination after irradiation; this clearly showed the development of

the acute radiation sickness.

The typical picture of acute radiation sickness was observed on morphological investigation of the animals which died. Signs of hemorrhagic diathesis with preponderant localization of the hemorrhages in the pulmonary parenchyma and the gastrointestinal mucosa were detected macroscopically. A decrease in the size of the liver and spleen, liquefaction of the bone marrow, severe flaccidity of the testicles, and the presence of liquids and a large quantity of gases in the intestine occurred in all cases. The severity of these changes depended on the time for which the animals survived; the picture was clearer when the disease had a more prolonged course. Microscopic examination revealed destruction of the hematogenic tissue and marked signs of destruction in the vascular walls, the latter being accompanied by a more or less substantial plasmorrhagia. The radiation affection was also recorded in wide-spread focal necrobiotic changes in the gonads, gastrointestinal tract, lungs, liver, and neural tissue.

In these investigations we were not particularly interested in the ordinary morphological picture of acute radiation sickness; our basic task was to determine the influence of generalization of an autoinfection on the character of a radiation injury. The data which we obtained in the series of experiments involving irradiation in a dose of 1800 r proved to be extremely favorable for the accomplishment of this task, since we were able to compare morphological data in cases where bacteriemia was present and absent in animals having identical survival times (this being an absolutely obligatory condition for the morphological study of radiation sickness!). By careful investigation of the blood vessels and organs in cases in which the cultures yielded microbes we were also able to find the latter (bacilli and cocci) in the tissues. We were unable to find any regularity in their distribu-

tion; the microbes were localized equally frequently in the cytoplasm of endothelial and reticular cells and were freely distributed at the surfaces of the cells, between the cells, and in the foci of necrosis.

A very scrupulous investigation failed to reveal any tissue reaction in the presence of the microbes; there were no signs of alteration in the areas in which the microbes were localized, no migration of leucocytes, and no proliferative reaction. In other words, we were unable to detect any signs of inflammation; the unusual characteristics which inflammation acquires in the presence of radiation sickness are well known from the data of N.A. Krayevskiy, C. Congdon et al. (1955), and other investigators. More precisely, there was no selective localization and multiplication of microbes in the vicinity of the necrotic foci, which were obviously aseptic and developed solely as a result of the radiation injury. We did not observe any intensified tissue edema in the region where the microbes were located; N.A. Krayevskiy considers this to be the clearest sign of inflammation occurring against a background of tissue areactivity. There were no septic thromboses in the blood vessels.

We were unable to detect inflammation at its favorite places of localization, those with the highest autoflora contents. There was no damage to the oral or pharyngeal mucosa. The changes in the intestine were limited to a necrobiotic process in the epithelium and lymphoid tissue, accompanied by edema of the stroma. Hemorrhaging, formation of unusual erythrocyte crystals, and plasmorrhagia occurred in the lungs in many cases. However, the plasma which copiously impregnated the walls of the alveoli and interstitial tissue of the lungs contained neither fibrin nor colonies of microbes. We consequently had no basis for evaluating these changes as pneumonia; they fell quite within the ordinary picture of a typical radiation injury.

The data cited enable us to assume that intensified autoflora multiplication accompanied by the subsequent development of bacteriemia occurs in acute radiation sickness caused by a single betatron irradiation. The flora in the foci of autoinfection, the blood, and the organs retains its composition after irradiation, but its properties may occasionally be altered. In particular, staphylococci become pathogenic. These observations are in complete accord with the data of R.V. Petrov, N.P. Kiselev, G.A. Chekotilo, L. Gordon et al. (1955), O. Preisler (1952), and many others. Whether or not bacteriemia develops and its severity depend on the duration of the radiation sickness and the individual sensitivity of the animal. Thus, in our observations there were 2 cases in which the radiation sickness took a comparatively long course (7-10 days) and bacteriemia did not develop before the animals died.

We may state with certainty that when the radiation sickness takes this long a course generalization of the autoflora does not affect the character of the morphological manifestations of the illness and does alter its duration.

In addition to the development of bacteriemia, study of the ways in which the intestinal flora infiltrates and disseminates throughout the organism is of great interest in characterizing the state of the autoflora of irradiated animals. Investigations of this type were carried out by Gordon and V.L. Troitskiy, who were able to demonstrate graphically that bacteria, particularly Bacterium coli commune, first enters the lymphatic system from the intestine, then reaching the blood and the internal organs. In precise experiments involving the use of the color reaction on the polysaccharides evolved during the vital activity of the intestinal flora, V.L. Troitskiy traced the fate of the bacteria which disseminated through the lymphatic system from the intes-

tine of an irradiated animal. These experiments were performed on rabbits irradiated with ordinary-voltage x-rays. There naturally arose the question of how high-energy betatron rays affected the rate and modes of propagation of the intestinal flora. We have only recently begun investigations in this area and still cannot make any statements about our results.

A special feature of the material which has been presented is the time at which bacteriemia develops. Judging from the data in the literature [N.P. Kiselev, R.V. Petrov, V.F. Sosova, Bond et al. (1954), and others], bacteriemia is detected on the 4th-5th day in radiation sickness caused by large doses (800-1000 r) of ordinary-voltage (180-200 kv) x-rays. In Congdon's experiments bacteria were isolated from the organs of mice on the second day after irradiation in a dose of 900 r. At lower irradiation doses, 350-600 r, and the correspondingly longer survival times bacteremia develops during the second week of illness [N.P. Kiselev and Ph. Miller et al. (1951)]. In our experimental setup, in the cases where bacteriemia developed the animals received 1800 r of betatron rays, which corresponds to approximately 1000-1200 r of ordinary-voltage x-rays (the mean efficiency coefficient for a betatron is 0.6-0.7). Our data may consequently be compared with the first group of observations, which is characterized by an early development of bacteremia. We observed bacteriemia only when the animals lived for 7-10 days after irradiation.

This delay in the generalization of the autoflora is apparently a result of the lower biological activity of the betatron. Its high-energy gamma-quanta disrupt tissue structure to a lesser extent; this enables the reticuloendothelial system to retain its function longer and this, according to the data of N.P. Kiselev, I.A. Pigalov, V.L. Troitskiy, and C. Hammond et al. (1954), is of basic importance in

maintaining the protective systems of the organism.

It may be supposed that this relative retention of the protective mechanisms of natural immunity makes the development of infectious complications after betatron irradiation less probable and results in this type of irradiation being quite suitable for therapeutic purposes.

CONCLUSIONS

1. In acute radiation sickness caused by exposure to 1800 r of betatron rays multiplication and generalization of the intestinal and nasopharyngeal autoflora occur no sooner than 7-10 days after irradiation.

2. The delay in autoflora generalization results from the low efficiency of betatron rays; this enables the protective systems of the organism to retain their structure and function longer.

Tomsk Scientific Research Institute for Vaccines and Sera

Tomsk Medical Institute

BACTERIOLOGIC AND PATHOLOGIC CHARACTERISTICS OF EXPERIMENTAL SHARP RADIAL DISEASE OCCURED UNDER THE INFLUENCE OF BETATRON IN 25 MEV

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Experimental investigations for finding out the state of autoflora at sharp radial disease occurred under the influence of betatron in 25 MEV were made.

It was established that the state of autoflora by the animals influenced by radiation is defined by the period of course of radial disease. The activization of the flora, the penetration of it into the blood, and then into the organisms increases with the lengthening of the period of animals life on the 7th, 10th day.

The slowness of generalization process of autoflora is the result

of lower efficiency of betatron rays that preserve the structure and the function of protective systems of organism longer.

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CHANGES IN THE ORGANS OF HORSES ENGAGED IN PRODUCING TICK-BORNE ENCAPHALITIS SERUM

A.M. Khlopkov, O.S. Strokina, S.S. Pavlitskaya,
K.K.Gavrilova and L.I. Korochkin

The horse has a rather marked immunological reactivity and, as a result, is widely used as a producer of therapeutic and prophylactic sera. It is well known that all sera, both native and purified or concentrated, used in medical practice (with the exception of measles serum) are blood sera from hyperimmunized horses. The encephalitis and antirabies gamma-globulin which have recently been produced are also prepared from horse serum. Despite the wide utilization of horses as producers of therapeutic and prophylactic sera the pathological changes which develop in their tissues and organs as a result of hyperimmunization have not been sufficiently well studied. To a considerable extent, the available literature deals with the changes observed in horses in the production of diphtheria and dysentery sera. So far as we know from the literature, no investigations of this type have been carried out on horses producing encephalitis serum.

The production of tick-borne encephalitis serum at the Tomsk Institute for Vaccines and Sera was begun in 1954. It is well known that horses do not contract tick-borne encephalitis under natural conditions, although in natural nidi of the disease, particularly those in the inhabited taiga, horses are one of the prime hosts of the adult stage of the basic reservoir and only local vector of the infection, the

wood tick (Ixodes persulcatus). As a result of being bitten by virus-carrying ticks the blood of the horses is found to contain antibodies which neutralize tick-borne encaphalitis virus. Such horses, which have acquired a "ground immunity" under natural conditions, are good producers of antiencephalitis serum. Consequently, when production of this serum was begun at the institute horses from natural nidi of tick-borne encephalitis were used. It was later found that horses from areas where tick-borne encephalitis is not encountered may also yield high-quality antiencaphalitis serum.

The method by which the serum was prepared in the horses, some of which were subjected to the morphological examinations described in this article, consisted of the following.

Immunization was carried out with a 10% suspension of brain tissue from white mice inoculated with strains of tick-borne encaphalitis virus isolated in the Far East(DV), the Ural Mountains (TKR), Alma-Ata (Prokushev), Byelorussia (No. 256), and locally (Kargasokskiy). Certain series of antigen used during 1955-1956 included the strain "Sof'in."

The antigen was administered subcutaneously in increasing doses, with intervals of 6-7 days during the immunization cycle; depending on the length of time for which it had been in service, each horse received from 125 to 495 ml of virus-containing suspension. In a number of cases the horses reacted to injection of the antigen with a rise in temperature to 39-41°. Phlebotomies (2 or 3 per cycle) were begun on the 9th-10th day after the last injection, then being performed at one-day intervals; 10-23 liters of blood were taken per cycle.

During four years of serum production (1954 -1957) a number of the horses developed a disease accompanied by neurological symptoms (paralysis of the facial and cervical muscles and the front or hind legs, tremors, blindness, etc.) which indicated that they had contracted

tick-borne encaphalitis. Total exanguination was consequently performed. These cases developed regardless of the horse's age, the time for which it had been in service, the stage of the immunization cycle (after receiving a regular dose of antigen, between injections of antigen, or during the rest period), and the quantity of antibodies in its blood. The brains of four sick horses were subjected to virological examination and strains of tick-borne encephalitis virus were isolated in two cases. A number of the horses consequently developed tick-borne encephalitis during hyperimmunization with the live virus. Total exanguination was performed for reasons other than neurological symptoms, principally excessive reactivity.

Material from 10 horses, 7 of which had contracted tick-borne encephalitis, was subjected to histopathological investigation. We studied the histological changes in certain branches of the nervous system (the cerebellum and the intramural apparatus of the gastrointestinal tract) and the general histological changes in the heart, liver, and spleen.

The material was fixed in 15% neutral formalin. Further histological treatment was carried out in accordance with the tasks to be performed.

The cerebella of 4 horses were investigated (Pavlitskaya). They were stained with EH, impregnated by the Bil'shovskiy-Gross method, etc.

The 2 horses exanguinated after 10-17 months of service as a result of their marked reactivity exhibited severe changes in the ganglia layer of the cerebellum. The Purkinje cells were in various stages of degeneration. Their bodies had nonuniform contours and their nuclei were hypertrophied and displaced to the periphery. The nuclei contained basophilic granules of abnormal shape, located in an extremely fine linin network. There was a large oxyphilic nucleolus. Some of the gang-

lion cells in the stratum granulosum were surrounded by light-colored slitlike spaces. These cells also exhibited complete tigrolysis. The distended capillaries were surrounded by thickened argentophilic fibers. The general argentophilic framework of the organ was destroyed. The nerve fibers in the molecular layer took a very tortuous course. There were no changes in the glia.

The cerebellum of the horse which had been hyperimmunized for 17 months and then developed neurological symptoms presented an even more severe picture. The majority of the Purkinje cells had died and appeared as decolorized shadows with some visible nuclear residue. The sites of these cells were occupied by lumps of granular detritus. The protoplasm of the surviving cells was markedly oxyphilic, while their nuclei were decolorized, hypertrophied, and almost completely lacking chromatin. The nucleolus was displaced to the periphery and, in individual cells, had passed out of the nucleus. The bodies of the cells in the stratum granulosum had been lyzed and this had caused the layer to thin out and be replaced by glial elements.

The intramural apparatus of the gastrointestinal tract (stomach, duodenum, and large and small intestines) was studied in 6 horses engaged in the production of antiencephalitis serum (Korochkin). We employed silver-impregnation by the Bil'shovskiy-Gross method or the Kampos method as modified by A.S. Shubin, with subsequent gold-impregnation and staining with EH or toluidine blue.

The changes detected in the nerve cells and fibres were both reactive and pathological. The latter were observed only in the type I Dogiel cells of Auerbach's plexus and took the form of displacement of the nucleus to one of the poles of the neuron. Exfoliation of the sharply contoured and, as it were, destroyed nucleus from the highly decolorized uncontoured nucleolus was observed considerably earlier. In

individual cases we encountered neurons exhibiting signs of cytoplasmic vacuolization and fibrillosis. Reactive processes which were expressed morphologically by an increase in the number of dendritic processes, considerable ramification of these processes, and a marked increase in the surface area of the dendritic laminae appeared quite clearly, especially in the type I Dogiel nerve cells of the small intestine. Our attention was also struck by the focal character of the changes, which involved whole groups of neighboring neurons.

The reactive change in the type II Dogiel cells reduced to the formation of course pseudopod-like processes of a dendritic character with a dense, highly-impregnable neurofibrillar network. These processes were capable of giving off collaterals, which curved and returned to the body of the neuron, merging with its cytoplasm to form typical end-cells. The reactive changes in the type II Dogiel cells also took the form of formation of spherical and club-shaped prominences which were fastened to the body of the neuron by a thin pedicle (bridge). As the development of this process continued a neural network (basket-like plexus) formed around the body of the nerve cell. When there was no opening in this network the neurons became reminiscent of the well-known Cajal "cometal cells." Interneuronal bridges were encountered between types I and II Dogiel cells. This gives us reason to suppose that the reactive processes led to the formation of new interneuronal connections and even of protoplasmic anastomoses between nerve fibers.

There were no changes in the cells of Meissner's plexus. Investigation of the nerve fibers showed that there were both pathological and reactive changes. The pathological changes included varicose thickenings, complete impregnation of the varicosity, vacuolization, decomposition of the neurofibrillae into small fragments, and homogenization of these fragments. The reactive processes in the nerve fibers

took the form of excessive growth, the presence of lateral branches, and the development of thickenings which were highly silver-impregnable. Nerve fibers ending in typical bulbs or having a spiral course were clearly seen in a number of cases.

The changes in the cardiac tissue were studied in 10 horses (Khlopkov and Korochkin). The sections were stained by van Gieson's and Mallory's method and with Mallory's solution (without fuchsin) and were impregnated with silver. It must be noted that all of the characteristic structural features of the working muscle tissue of the myocardium (its reticulation, the thick intercalary plates which separate the muscle segments from one another, and the differentiation of these segments by color and structure) appear exceptionally clearly both in normal horses and those with pathological conditions (Khlopkov, 1946). The graphic clarity of these characteristics of myocardial tissue in horses considerably simplified the detection of a number of pathohistological abnormalities in our experiment. The picture described below was observed in the horses exanguinated because of excessive reactivity or because they had contracted tick-borne encephalitis; the extent of the disruptions observed could not always be shown to be a direct function of the number of hyperimmunization cycles.

The changes in the myocardium were characterized primarily by the presence of marked spots (yellow and blue) of different sizes on staining with Mallory's solution alone (without fuchsin), these corresponding to individual muscle segments or groups of segments. In this case the yellow areas exhibited equally marked signs of myolysis, completely losing their structure; the layers of muscle in these areas also displayed a tendency toward fragmentation, first by distension of the intercalary laminae (prefragmentation) and then by complete and marked fragmentation along the lines of these laminae, which were split

in half in a direction transverse to that of the fibre, forming light-colored slits between the segments. The etiology of this fragmentation is still considered to be a riddle.

Prof. M.I. Avdeyev, a well-known expert on the histopathology of the myocardium, is convinced that "fragmentation must be considered to be an agonal phenomenon which develops at the instant when the heart dies." We can hardly agree with his assertion, since this phenomenon is encountered in many experimental procedures, particularly after exanguination or administration of a virus. True fragmentation of the myocardium must consequently rather be considered as an intravitam histopathological phenomenon. However, it must again be emphasized that fragmentation occurs only along the intercalary laminae and that all of the pathological changes described below take place only within segments bounded by these laminae; this convincingly refutes the widely held view that the myocardium has a syncytial structure. In addition, transverse fissures which must be classified as posthumous phenomena, could also be seen in the muscle fibers throughout the preparations.

Besides the phenomena described, throughout the material we found foci of muscle-segment homogenization of the ceraceous-degeneration type, destruction of the muscle fibers, distension of the segments and partial separation into fibers, and foci of complete necrosis. In places we could clearly see considerable foci of granular decomposition of the myofibrillar substance of the muscular trabeculae; these were also limited to the lines followed by the intercalary plates.

Finally, we must make special note of an interesting type of degeneration, which was reminiscent of Zenker's degeneration of somatic muscles and individual affected muscle segments. The latter were greatly distended, taking on a homogeneous or slightly fibrous appearance; they acquired a faint lilac hue when stained with hematoxylin and were sur-

rounded by a delicate basophilic film (the sarcolemma). The blood vessels were occasionally intergrown. In the centers of these sheaths was the residue of the degenerated muscle fibers, which stained brightly with eosin; faint morphological signs of their muscular nature appeared only in places. The interstitial connective tissue, especially that between individual muscle bundles, was affected by being impoverished in both fibrous and cellular components, which, as a result of the edema, were scattered far apart in disorderly fashion in the clear spaces between the muscle elements. The blood vessels, especially the veins and capillaries, were greatly dilated, thin walled, and engorged with formed elements. In places there were tears in the blood vessels accompanied by hemorrhages in the intermuscular spaces.

The changes in the liver were studied in all 10 horses (Strokina). The sections were stained with EH, picrofuchsin, and Sudan III. The extent of the histomorphological changes in the liver depended on the quantity of antigen which the horse received and the time for which it was in service. All of the animals exhibited necrobiotic processes with subsequent necrosis of the hepatic parenchyma and signs of adipose degeneration, the latter occasionally encompassing the entire liver. There were disturbances of both portal and hepatic circulation and occasionally stasis of the blood vessels, disruption of the integrity of the walls of the sinusoids, and hemorrhages within the lobes of the liver. The walls of the interstitial blood vessels and bile ducts were thickened. The cellular and fibrous elements making up the walls of the bile ducts were located at a considerable distance from one another because of the edema. In places the walls of the blood vessels had lost their structure (hyalinosis). The perivascular spaces were rich in cellular elements, primarily polyblasts. For the most part the endothelium of the blood vessels and sinusoids was hypertrophied

and its nuclei were distended, containing individual masses of chromatin arrayed in disorderly fashion. In certain cases the endothelial nuclei were pyknotic and greatly deformed. The interstitial tissue and hepatic capsule were thickened; proliferative processes were observed in them. Connective tissue penetrated from the interstitial tissue and the capsule into the microscopic lobules, replacing portions of the necrotic hepatic fissures, which had a focal character. The protoplasm of the majority of the hepatic cells contained fatty inclusions of varying size, ranging up to complete drops which filled the entire cell.

Of the organs of the reticuloendothelial system, the spleens of 9 horses were also investigated (Gavrilova). On examination of these organs we detected disturbances of circulation, which took the form of individual hemorrhages in both the connective tissue (capsule and trabeculae) and the Milpighian bodies. There was a marked thickening and homogenization of the collagenous fibers and a severe edema in the tissues, especially the Milpighian bodies; as a result, the latter lost their proper appearance. Their reproductive centers were expanded and the ordinary accumulations of lymphocytes at the periphery of the bodies had the appearance of a rim; an occasional Milpighian body appeared to be an accumulation of reticular tissue alone, its mesh containing individual cells of the lymphoblast type with vacuolized protoplasm and pyknotic nuclei. The pulp exhibited intensified erythrolysis and certain other abnormalities (elevated contents of polymorphonuclear histocytes, plasma cells, and Russell bodies). The endothelium of the blood vessels was distended, entering the lumens, and occasionally exhibited signs of proliferation. These histopathological changes in the spleen must be considered to be reversible reactive phenomena and are encountered in many other pathological and experimental processes.

CONCLUSIONS

Histomorphological examination of horses which had been subjected to hyperimmunization with tick-borne encephalitis virus and had been exanguinated because of their high reactivity or because they had contracted tick-borne encaphalitis showed that there were marked changes in a number of organs and systems. In the cerebellum these changes involved primarily the Purkinje ganglion cells. Degeneration processes ranging up to complete disappearance of these cells were encountered. The separation of the cellular elements in the stratum granulosum was increased; the argentophilic membranes became coarse in texture, the nerve fibers were twisted, and the capillaries were dilated. The changes observed in the cerebellum were undoubtedly specific, resulting from administration of large quantities of live tick-borne encephalitis virus to the horses. This theory is reinforced by the experimental investigations of a number of authors (N.S. Yurkevich and Yu.M. Zhabotinskiy), who observed a number of similar changes in experiments on laboratory animals infected with tick-borne encephalitis and equine encephalomyelitis viruses.

Our investigations of the intramural apparatus of the gastrointestinal tracts of horses hyperimmunized with tick-borne encephalitis virus were the first of their kind. These investigations showed that type I Dogiel cells react by increasing the number of their processes and the surface area of their dendritic lamellae and by forming new interneuronal connections, both with type II Dogiel cells and with one another. The changes in the type II Dogiel cells consisted in the formation of rifts, basket-like plexi, and the aforementioned interneuronal connections. There were also slight changes in the nerve fibers. Diverse processes consequently occur in the neural elements of the intramural ganglia of horses after the hyperimmunization described above.

These changes are apparently also specific.

Investigation of the cardiac tissue showed that all of the aforementioned diverse histopathological symptoms are typical of parenchymatous myocarditis. Study of the changes in the liver and spleen indicated that the process which occurs in these organs is the same.

As is well known, these organs exhibit identical changes in horses hyperimmunized with diphtheria and dysentery toxins and toxoids. All of this indicates that the changes in the heart, liver, and spleen cannot be unconditionally assumed to be specific, developing solely as a result of the reaction of the organism to foreign substances such as microbial antigens.

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THE CHANGES IN ORGANS OF HORSES-PRODUCERS OF SERUM AGAINST TICK ENCEPHALITIS

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In the process of horses' hyperimmunization at preparing anti-encephalitis serum some part of them fell ill with typical tick encephalitis.

Histopathologic changes in some organs by these animals; in cerebellum, ganglions of intestines, heart, liver and spleen are given in this article.

CERTAIN DATA ON THE INFLUENCE OF BETATRON RAYS ON THE NATURAL IMMUNITY OF WHITE RATS TO TULAREMIA

Ye.I. Kleytman

Numerous experimental investigations (V.L. Troitskiy et al., M.A. umanyan and A.V. Izvekova, and others) have shown that ionizing radiation has an inhibitory effect on both natural and acquired immunity.

Both external (x-rays) and internal (isotopes of phosphorus and odine) radiation sources were used. As a result of the fact that there re a number of data on the nonuniformity of the pictures of radiation ickness obtained by exposure of the organism to different radiation ources, we undertook a study of the resistance of natural immunity in nimals irradiated with gamma-rays from a betatron.

We selected a virulent strain of Bacterium tularense as the infectious agent. White rats were used as the subjects, since, according to he classificatory data of N.G. Olsuf'yev and T.N. Dunayeva, they are nimals which are susceptible but not very sensitive to tularemia.

Whole-body irradiation was carried out with a betatron having a ower of 25 Mev; the skin-focus distance was 45 cm and the irradiation ime 7.5-8 minutes. Each rat was placed in a cardboard box.

Our investigations were conducted on 61 white rats weighing from 170 to 200 grams. The experimental animals were divided into three groups: two control and one experimental. One group, which consisted of 25 rats, was used to check the immunity of unirradiated animals to the virulent strain (No. 6) of Bacterium tularense (the inoculation control).

Another group (consisting of 21 rats) was used to study the influence of various doses of gamma-rays on experimental animals (the irradiation control). The third, experimental group (13 rats) was infected against a background of preliminary irradiation, in order to determine the changes which occur in the natural immunity of rats during radiation sickness.

TABLE 1

Survival Time of Irradiated Rats After Infection With Bacterium tularense

Облуч. доза, в r (A)	Зараж. доза, в млн. м. к. (B)	Время между облучением и заражением (C)	Длительность жизни в днях (D)
100	10	1 месяц (E)	выжила, забита (G)
200	10	"	"
300	10	"	"
400	10	"	"
1000	500	"	"
1000	500	2 месяца	"
1000	100	4 часа (F)	пала на 4-й день (H)
1000	100	"	"
1000	100	24 часа	пала через 1 месяц (I)
1000	10	48 часов	пала на 4-й день
1200	10	"	"
1400	10	"	"
1400	10	"	"

A) Irradiation dose, in r; B) infecting dose, in millions of microbes; C) time between irradiation and infection; D) survival time; E) month; F) hours; G) survived, was killed; H) died on 4th day; I) died after 1 month.

In all groups the investigation was accompanied by hematological checks (determination of the leucocyte count and calculation of the leucocytic formula) and observations of the animals' weight and general condition. The rats which died were dissected and the macroscopic changes were noted. The animals which survived the experiment were killed after 2-3 months and dissected.

In our experiments the rats were irradiated with ten different doses of gamma-rays from the betatron (100, 200, 300, 400, 500, 600, 800, 1000, 1200, and 1400 r); only the last two doses proved to be ab-

solutely lethal (causing death within 4-7 days). Three of the seven animals irradiated in a dose of 1000 r died within 4-7 days. The remaining rats were killed at the times indicated.

The survival times among the animals which were subjected only to inoculation with Bacterium tularense also depended on the size of the dose administered. Six doses were investigated. Thus, doses of 5 and 25 million microbes did not cause any of the animals to die; a dose of 10 million microbes caused one of the seven rats which received it to die on the fourth day of the experiment. Three of the six rats which received 100 million microbes died; at a dose of 500 million microbes two of 5 rats died. The absolute lethal dose was found to be 1 billion cells.

In the third (experimental) group four animals were inoculated with 10 million microbes of the tularemia strain 1 month after massive whole-body gamma-irradiation in different doses (100, 200, 300, and 400 r) and not one died. After two months they were again inoculated with a large dose of microbes (500 million); this also failed to cause visible changes and the rats were killed two months afterward, still being in good general condition. (Table 1).

Two rats received a large irradiation dose (1000 r) and were inoculated with 500 million microbes of the tularemia strain, one after one month and the other after two months; both survived and were killed.

Thus, a single irradiation undergone 1-2 months before inoculation did not reduce the natural immunity of the rats to tularemia. When the rats were inoculated comparatively soon after irradiation (4, 24, and 48 hours) different results were obtained against the lower leucocytic background. Thus, four rats irradiated in a dose of 1000 r and inoculated with different doses of the tularemia microbes (10 and 100 million cells) all died (three on the fourth and only one on the 30th day).

Their spleens and lymph nodes were biologically tested in white mice, which died on the fifth day exhibiting the typical symptoms of tularemia; this was also confirmed bacterioscopically.

The rats inoculated with 10 million microbes 48 hours after irradiation in doses of 1200 and 1400 r died on the 4th-7th day, just as the control animals.

The literature does not contain a single opinion on the influence of the interval between irradiation and inoculation on mortality; this may possibly be a result of the fact that the authors experimented with different species of microbes and animals and different radiation sources.

Thus, Perkins, who worked with mice irradiated and then inoculated, did not note any relationship between mortality and time of infection. Khemond and his colleagues detected an increase in the susceptibility of mice to infection rather long (the end of the second week) after irradiation.

Contradictory data were obtained by V.L. Troitskiy, who, in experiments on rabbits and mice, detected an increase in resistance to dysentery and typhoid 3-4 weeks after irradiation with x-rays and radioactive isotopes of phosphorus and iodine. In experiments on mice subjected to x-irradiation in a sublethal dose, A.S. Shevelev noted a decrease in resistance to vaccine-induced tularemia if the inoculation was carried out during the first three days after irradiation. The percentage of mice dying with symptoms of tularemia was lower when the inoculation was carried out 7-10 days after irradiation. Our investigations, which showed that inoculation of rats with tularemia microbes at rather long intervals after betatron irradiation (1-2 months) does not cause death, apparently as a result of the restoration of the animals' natural immunity, agrees with the data in the literature (V.L. Troitskiy and A.S.

Shevelev).

TABLE 2

Leucocyte Level in Rats Inoculated With Bacterium tularense After Irradiation

A Доза облуч. в r.	B Зараж. доза, в млн. м. к.	C Время между облуч. и зараж.	Количество лейкоцитов, в тыс. D					Примечание H
			E перед облуч.	F перед зараж.	после заражения, в днях G			
					1	2	3	
1000	100	4 часа I	15,4	—	7,0	4,2	3,6	пала на 4-й день L
"	"	"	14,6	—	13,2	3,8	3,0	"
"	"	24 часа	16,4	9,2	4,2	4,0	5,8	пала через 1 месяц M
"	500	1 месяц J	17,6	13,4	6,0	28,0	29,0	забита через 2 месяца N
300	10	"	7,4	7,2	28,2	29,5	27,5	"
200	10	"	15,4	12,8	25,6	28,6	29,2	"
K нет	100	—	—	14,6	18,6	25,2	39,2	"
нет	"	—	—	15,8	42,4	41,8	38,0	пала на 8-й день O
1000	нет	—	8,4	—	4,2	—	2,6	забита P
"	нет	—	8,2	—	5,4	6,0	2,8	"
1200	нет	—	5,4	—	1,6	—	—	пала на 4-й день L

A) Irradiation dose, in r; B) infecting dose, in millions of microbes; C) time between irradiation and infection; D) number of leucocytes, in thousandths; E) before irradiation; F)before infection; G) after infection, in days; H) notes; I) hours; J) month; K) none; L) died on 4th day; M) died after 1 month; N) killed after 2 months; O) died on 8th day; P) killed.

Determination of the leucocyte level at the instant of inoculation, which is an indirect index of the degree of resistance, may be of some value in understanding this phenomenon. In our experiments the rats were inoculated with Bacterium tularense in a dose of 10 million microbes and larger doses caused a severe neutrophilic leucocytosis, which, in certain rats, reached 42-43 thousand leucocytes per mm^3 of blood after one day, in comparison with the initial 10-15 thousand leucocytes (Table 2).

It is also interesting to note that a primary inoculation (100 million microbes) one day after irradiation in a large dose (1000 r)

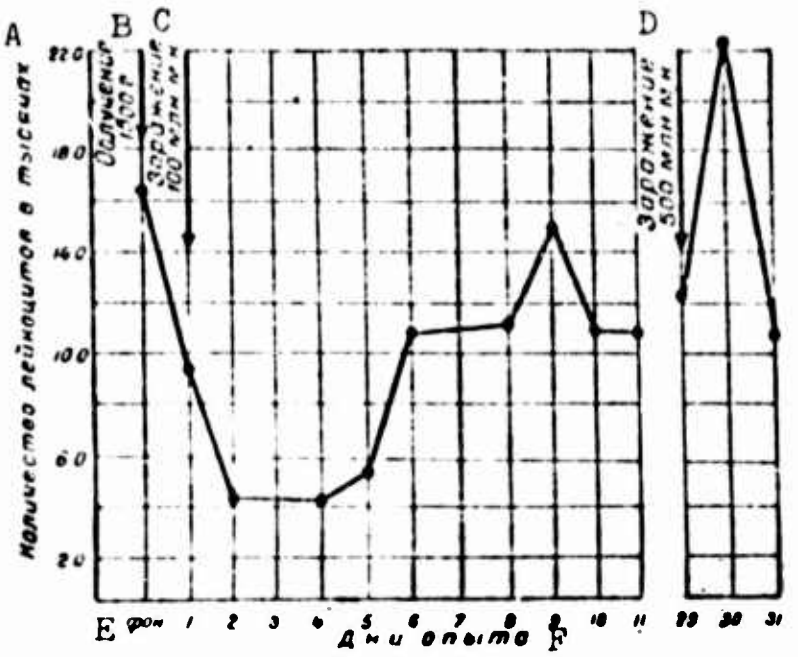

Fig. 1. Leucocytic reaction to administration of infectious agent to a white rat having chronic radiation sickness. A) Number of leucocytes, in thousandths; B) irradiation, 1000 r; C) inoculation, 100 million microbes; D) inoculation, 500 million microbes; E) background; F) days of experiment.

did not cause any rise in the curve representing the leucocyte count, as was indicated above, but that giving the rats which survived a second inoculation after one month, i.e., during the compensatory period, produced a different positive leucocytic response, a rise in the curve; this indicated that the disrupted hematogenic functioning of the bone marrow had been restored (Fig. 1).

We noted the presence of Bacterium tularense arrayed in clumps between the formed elements in blood smears taken before death on the third day of the experiment from the rats irradiated in a dose of 1000r and inoculated 4 hours later with 100 million microbes.

Our experiments showed that inoculation of betatron-irradiated white rats with a virulent culture of Bacterium tularense has different effects, depending on the interval after irradiation at which inoculation is carried out.

Administration of certain doses within 24-48 hours after irradiation

causes all animals to die, while inoculation after 1-2 months is not lethal. This is apparently explained by a reduction in the protective capacities of the organism during the first few days after irradiation in large doses, a phenomenon also indicated by the absence of leucocytes after administration of microbes during this period.

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SOME DATA ABOUT THE BETATRON RAYS ON NATURAL IMMUNITY OF WHITE RATS TO TULAREMIA

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Our experiments have shown that the infection by virulent culture of tularemia microbes of white rats radiated by betatron effects differently depending on the time after radial influence it was produced. The introducing of definite doses during first 24-48 hours after radiation causes the death of all animals while infection after one-two months does not lead to it.

This is explained by lowering the defensive powers of organism in the first days after radiation with large doses of rays, the absence of leucocytes after introducing microbes in that period witnessing about this.