

The Institute of Contemporary Russian Studies

Fordham University
Bronx, N. Y. 10458

Selected Abstracts

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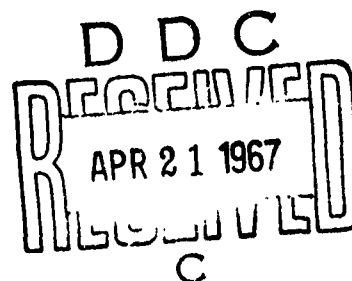
Prepared by

Dr. Robert Pollitzer

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The Moldavian Scientific - Research Institute
of Epidemiology, Microbiology and Hygiene

The Scientific - Methodological Anthrax Center
of the USSR Ministry of Health

A N T H R A X

(Problems of Immunology, Clinique
and Laboratory Diagnosis)

ANTRAKS
(Voprosy immunologii, kliniki
i laboratornoi diagnostiki)

Edited by E.N. Shlyakhov

"Kartia Moldoveniaske" State Press
Kishinev * 1964

Translated by Robert Pollitzer, M.D.
Translation Edited by Walter C. Jaskiewicz, S.J., Ph.D.

Fordham University
The Institute of Contemporary Russian Studies
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The article

Immunobiological Investigation
with Preparations of the
Anthrax Allergen-Anthraxin

by

E. N. Shliakhov
will be concluded
in the next issue
of

Selected Abstracts

Editor's Note

It is unscholarly to say, "It wasn't planned this way; it just happened!" but that is the way it was. Our purpose in initiating the Selected Abstracts in the beginning was to offer a limited audience an idea of what was being done in the field of infectious microbiological diseases in the Soviet Union. In the course of these investigations - for the I.C.R.S. Medical Reports which had a broader scope and for the Selected Abstracts - specialized material began to accumulate. As a result we published in limited offset edition Plague and Plague Control in the Soviet Union (N.Y., I.C.R.S., 1966) and have in press at this time. History and Incidence of Tularemia in the Soviet Union (N.Y., I.C.R.S., 1967).

Thus, in completing the Selected Abstracts, the monograph on Anthrax edited by E. N. Shliakhov came to our attention. Dr. Pollitzer, our chief investigator, judged that some of this information should also be included. As time went on what began as summaries grew into translations of selected articles and these eventually into a translation of the entire monograph.

To me, then, as Editor, fell the decision to adhere to the original idea of summaries or to think in terms of another monograph. The present work is the result of a compromise. Because of the limited means at our disposal it was decided to conclude the Selected Abstracts with Series IV and include all the translations in Nos. 2-4 in such a way that with the addition of the front matter those who wished could bind the three concluding numbers in a more permanent form for themselves. As a result there is

double pagination: the continuation of Selected Abstracts pagination including the serial numbers for the individual articles and separate pagination at the bottom for this Anthrax collection. It is the editor's hope that this will not compound the confusion.

It is appropriate here to acknowledge the excellent and painstaking work of Dr. Robert Pollitzer in preparing the translation which required very little editing and the patience and efficiency of Mrs. Carmela Ottaviano who typed and retyped the manuscript. The final responsibility, of course, is mine.

Walter C. Jaskiewicz, S.J., Ph.D.

Institute of Contemporary
Russian Studies
Fordham University
February 1, 1967

P R E F A C E

The resolutions of the Central Committee of the Communist Party of the Soviet Union and of the USSR Council of Ministers of January 14, 1960 "Measures for Further Improvement of Medical Service and for Protection of Public Health in the USSR" and of January 9, 1963 "Measures for Further Development of Biological Science and Strengthening Its Practical Applications" put serious tasks before medical science and practice.

The problem posed was to liquidate within the next years a number of infectious diseases on the territory of our country or markedly to decrease their incidence; anthrax was included in this list.

Owing to the researches of Soviet scientists the epidemiological and epizootological peculiarities of this disease have been thoroughly investigated, means of disinfection have been devised and the peculiarities of the causative organism have been studied. A great achievement was the production of original uncap-
sulated live anthrax vaccines (STI - Ginsburg, 1942; Shuia-15 - Kolesov, 1949).

The use of these vaccines in the farm work of the USSR fundamentally solved the problem of the specific prophylaxis for animals.

The successful study of the STI vaccine on animals preceded its use for the purposes of specific prophylaxis in man and, for the first time in the world, twenty years ago this vaccine was used in the USSR for medical purposes. At present a chemical anthrax vaccine is under study.

During the last decades new efficacious therapeutic substances (anthrax gamma-globulin, antibiotics) have been approved in our country, new diagnostic preparations have been devised as well as methods and means for the improvement of the laboratory diagnosis and the identification of causative organisms.

All this resulted in a marked decrease of anthrax morbidity - the incidence of this disease in man became fairly rare in our country, the foundations have been laid for the full protection of man against it.

✓

One of the fundamental requirements for the realization of this difficult but meritorious task is a theoretical preparedness of the medical and veterinary workers and their acquaintance with the latest achievements of the Soviet and foreign investigators in the study of the problems of anthrax.

In answer to these tasks and in accordance with the instructions of the USSR Ministry of Health the present collection of articles has been prepared by the Scientific-Methodological Anthrax Center in the Moldavian NIIEMG (Director - E.N. Shliakhov); it includes topical materials dealing with the immunology, clinique, therapy and laboratory diagnosis of anthrax.

A. P. Diskalenko,
Director, Moldavian NIIEMG

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(#85) FURTHER DEVELOPMENT OF THE
KNOWLEDGE OF ANTHRAX

(A contribution to the centennial
of Davaine's discovery)

Professor N. N. Ginsburg
(Original pp. 4-21)

In 1863 the French Academy of Sciences published a communication of Davaine (1,2) reporting the discovery of the causative organism of anthrax. This discovery marked not only the beginning of the scientifically founded fight against this infection, the results of which became soon manifest*, but, as Metelnikov (3) truly pointed out, exerted "a profound and many-sided influence on the development of scientific thought on the concepts of epidemiology, epizootology and microbiology."

During the last century (the lore of) anthrax in man and its epidemiology underwent certain changes. Particularly during the last 20 years our knowledge on the causative organism of anthrax increased almost beyond recognition. The aim of the present article is to present the results of this work.

As is known, in Tsarist Russia anthrax was one of the most prominent diseases of man. This induced Sviatlovskii (4) to remark in 1891 that "in Russia anthrax is constantly present. In its way it is our national disease."

According to statistical data published by N. N. Mari (5) during the period from 1896 to 1913, 268,000 persons fell ill with anthrax (at an average 16,000 per year) and the lethality reached 25%. The incidence of the disease among the agricultural animals was many times in excess of that in man.

*) As is known, already in 1881 Louis Pasteur prepared from the anthrax bacillus the first highly effective vaccine for agricultural animals.

At present, notwithstanding the incomparably higher number of agricultural animals in the Soviet Union, the frequency of anthrax attacks is considerably lower, being sporadic in character, and in the absolute majority of cases the disease is met with in privately owned, not vaccinated animals.

This state of affairs deserves great attention. It shows firstly that in our country immuno-prophylaxis is the fundamental measure in the fight against anthrax (which, in the opinion of the present author, is not the best solution to the problem) and secondly that highly effective vaccines are available for this purpose.

As was to be expected, such a marked decrease of the anthrax incidence in animals led to a decrease of the frequency of the disease in man. Still, no direct relation exists in this respect.

On the one hand an influence is exerted by the transmutation of anthrax from an epizootic into a sporadic infectious disease. On the other hand two new hitherto unimportant factors began to exert an influence. They are the importation of raw animal products from countries where anthrax is still present among the agricultural animals (Iraq, Pakistan, Afghanistan, etc.) and a revival of the contact of man with the soil due to building activities in inhabited regions and the cultivation of virgin territories.

Mention has to be made in this respect of the contact of man with parts of the ground infected with anthrax spores, such as the graves of cattle or places of slaughtering anthrax-infected animals or simply such where, as an inheritance of pre-revolutionary times, deaths of unrecognized anthrax-affected animals took place.

In the first case preventive measures (ascolization [?] of the imported raw animal products and their disinfection at the frontier stations combined with obligatory vaccination of the personnel engaged in receiving and handling the products) permit countermeasures against this new source of the importation of anthrax into the USSR in a comparatively simple and, if properly used, highly effective manner. However, the fight against the second source of infection is incomparably more complicated.

So far any measures taken in this connection are fraught with great difficulties: it is still unknown how to select with the least degree of error the points for taking samples from the soil of places suspect for the presence of B. anthracis; it is still not clear which method suitable for large practice ought to be used for the most rapid demonstration of the anthrax bacilli in the samples; finally there exist no sufficiently convincing data on simple and effective methods of the disinfection of soils contaminated with anthrax spores (which evidently vary under different conditions of the climate and the soil). Still, one must keep in mind that, however subordinate the role of soil contamination in the epidemiology of human anthrax might be in the USSR and however difficult the task of dealing with this source of infection might prove, this problem must be solved - in the first line by the scientific research institutes.

* * * * *

At present it is quite clear that the pathogenicity (virulence) of the anthrax bacilli is closely related to, or is perhaps fully determined by, two factors, the capsule formation and toxin production.

Careful and prolonged studies of the capsule (6-14) have shown that, on the one hand, it impedes the phagocytosis of the bacilli - a process taking place more actively in the body of animals (guinea pigs) than in serum-containing media (13); on the other hand, as shown by the results of investigations made in our laboratory to study the relations between the anthrax bacilli and animal cells in vitro, the capsule promotes the fixation (adherence) of the capsulated organisms to the cells, a property practically lacking in the uncapsulated variants of the anthrax bacillus (14). As will be shown later, such capsulated bacilli, "belaguering" the cells of the animals quickly lead to their destruction.*

*) Reported at the scientific conference of the Tarasevich State Control Institute in 1960, at a conference in the Gamaleia IEM, AMS, USSR and the 3rd Scientific Session of the Moldavian Institute of Epidemiology, Microbiology and Hygiene in 1962.

A study of the basic component of the capsule of the anthrax bacillus - the polypeptide of glutamic acid, made with particular care and in various respects during the last decade (15-25) so far did not solve the problems of its specific peculiarities and its possible importance for the "protective" and "fixating" functions of the capsule.

Special mention must be made of the most important fact that the hereditary loss of the ability of the anthrax bacillus to form capsules turns virulent into avirulent and - as has been shown later - vaccinal strains (26-33).

Reports on the toxin of the anthrax bacillus have been made first by a group of British investigators in Portland in 1953-1955 (34-37). They succeeded in observing in, and isolating from, the plasma of guinea pigs succumbed to anthrax a substance which they called at first the "lethal factor." This substance, administered intradermally to guinea pigs, produced edema and, given intravenously, proved fatal for mice and guinea pigs.

The authors asserted that the edema formation and the lethal effect was related to one and the same substance. Later (1955) they called this the "anthrax toxin" and pointed to a possible relation existing between it and the protective antigen of the anthrax bacillus which had been obtained at that time in vitro by the British worker Gladstone (37). They also expressed the opinion (proved afterwards untenable) that the protective antigen is the "toxoid" form of the lethal factor. They also determined the lethal doses of the toxin (given intravenously 0.5 kill 80% of mice and 6 ml 73% of guinea pigs), described methods of obtaining it in vivo and of testing it. The data obtained confirmed that the anthrax toxin was labile, becoming fully destroyed when heated for 1 1/2 hours at 60°C and inactivated at a pH less than 5 and above 10; it could be fractionated through ultracentrifugation.

Anti-sera obtained from horses hyperimmunized with capsulated as well as with uncapsulated anthrax bacilli Baybridge Strain neutralized the edema-producing and lethal actions of the toxin; this indicated the absence of a direct relation between these factors and the capsule of the anthrax bacillus.

In 1958 the authors in Porton reported (38) that they had succeeded also in obtaining the anthrax toxin in vitro through cultivation of a virulent strain (NPA) and an attenuated one (Baybridge-Sterne) in a medium consisting of a mixture of serum and tryptic meat digest. The properties of this toxin did not differ from those of toxin obtained in vivo, but the lethal doses were somewhat higher. The authors showed that the toxin in the cultures was quickly destroyed by the ferment systems of the bacilli themselves after the populations of the latter had reached a certain critical concentration. According to the authors the addition of serum was of great importance for the toxin formation in vitro.

A different opinion was held by Thorne (39-40) who studied the anthrax toxin together with Strange. He asserted that serum was not necessary for the formation of the anthrax toxin and that it acted on the one hand as a "buffer" maintaining the pH level during cultivation and on the other hand as a "carrier" of the "toxin" passing through the filter during filtration. Thorne and Strange found that the toxin was formed when the anthrax bacillus grew on media containing instead of native protein a compound of the amino-acids of acid casein hydrolysate, on completely synthetic media when 10% gelatine was added, and finally under the conditions necessary for the production of the protective antigen. Still, the filtrates of such toxic cultures were completely atoxic and kept their protective properties (if previously present) at titers near those originally present. The authors maintained that the filtration through glass wool permitted to divide the "anthrax toxin" into two components: the protective substance passing into the filtrate and a "filter factor" becoming adsorbed to the filter from which it could be eluted with an alkaline buffer. Separated from the protective antigen this factor was quite atoxic, and its toxic properties were present only in mixtures with the protective antigen. The addition of serum to the cultures prevented the adsorption of the toxic component to the filter. A study of the "filter factor" with the aid of gel precipitation with an anti-serum raised against the uncapsulated strain Baybridge showed the presence of two lines of precipitation (different from the line produced by the protective antigen). Therefore the authors tried to divide the "filter factor" into its two components. With the aid of fractionation they obtained preparations of each component. One of them was not toxic either per se or in mixture with the protective antigen. The other was not toxic by itself but it became toxic if mixed with the protective antigen and thus, in the opinion of Thorne, was the toxin responsible for the toxicity of the anthrax bacillus. Moreover, administration of this substance to rabbits produced in the latter an immunity against anthrax; this led the authors to the postulation that the anthrax bacillus produced at least two protective antigens.

In conclusion it must be noted that no apparent relation has been established between the degree of virulence of the anthrax strains and their ability to produce the toxin (obtained by the authors from avirulent (vaccinal) as well as from virulent strains) and that the mechanism of the action of the toxin needs further study. Quite recently (1961) Stanley and Smith (41) reported that, studying the chemistry of the first component of the anthrax toxin, they were able to observe a further, third factor (a toxin which kills mice if mixed with the second, but not with the first factor). In their paper these authors published details of the chemical composition of the first component of the anthrax toxin.

The literature quoted above undoubtedly does not render full justice to the problem of the toxic products formed in vivo and in vitro by the anthrax bacillus in its virulent capsulated form. This can be easily gathered from comparative observations in our laboratory on the behavior of virulent and vaccinal strains in tissue cultures (human fibroblasts and guinea pig macrophages) in vitro (14,42). Both withstood well the contact with vaccinal strains but degenerated and perished when in contact with virulent (capsulated organisms).

* * * * *

As stated previously, at present immuno-prophylaxis is one of the essential measures in the fight against anthrax. Widely used in veterinary practice this method also begins to be used on an ever increasing scale in medical practice. At present two methods have become available for the preparation of anti-anthrax immuno-prophylactics - the use of live vaccines and of protective antigens.

Anti-anthrax prophylaxis with live vaccines has been known since the time of L. Pasteur. As mentioned already, anti-anthrax prophylaxis with the specific protective antigen is a recent achievement. Nevertheless it would be a great mistake to claim that in view of the availability of the protective antigen the use of live vaccines, because considered a dangerous as well as an antiquated method, ought to be given up definitively.

First of all it must be stated that the principles underlying the preparation of the modern live anti-anthrax vaccines for man are different from those adopted in the past by

L. Pasteur and his distinguished successors TSenkovskii, Lange and Eichhorn. It is known that their vaccines for agricultural animals consisted of more or less "attenuated" variants (mutants) of capsulated virulent forms of the anthrax bacillus.

In order to avoid untoward results following the use of these vaccines (i. e. deaths of animals due to post-vaccinal anthrax sepsis) it was necessary to resort as a rule to the use of two, three or even four (USA) vaccines attenuated to a different degree, which were administered to the animals successively at varying intervals. In place of these undoubtedly dangerous vaccines which would have been unsuitable for man, now vaccines are available which have been prepared from hereditably changed uncapsulated variants (mutants) of the anthrax bacillus, i. e. forms incapable of forming capsules either in vitro or in vivo or to infect animals with anthrax since the specific pathogenicity (virulence) of the organisms is related to the capsule. At the same time these variants, called "vaccinal anthrax strains," together with other functions determining the appearance of an insusceptibility to anthrax in vaccinated animals, also retained a marked ability to form in vivo that very important substance which by now has been amply studied and is known under the name of the "protective antigen."

The first anthrax vaccine for the immunization of man, prepared according to these principles, the STI vaccine, was recommended in the USSR by the author of the present article in 1942 (31), even though hereditably changed uncapsulated variants of the anthrax bacillus were obtained and described earlier (26-30). Moreover, an anthrax vaccine prepared from an uncapsulated strain for the immunization of agricultural animals was first used in South Africa (Onderstepoort Laboratory) already in 1936 and, as is known, Max Sterne (27) recommended since 1937 for the preparation of such veterinary vaccines his uncapsulated variant 34 F₂, which at present has become widely known and is successfully used abroad (England, USA).

After both careful study of the STI vaccine in all respects in the present author's laboratory and approval after massive use in different species of agricultural animals under various geographical conditions and at different seasons, followed by use in volunteers (N. F. Kopylov, N. N. Ginsburg et al.) and in limited groups of people (N. N. Ginsburg, R. A. Saltykov and A. L. Tamarin), finally in 1944-1951 the vaccine was used for the large-scale immunization of man (A.L. Tamarin, R. A. Saltykov, E. N. Shliakhov et al.).

It must be remembered that this work was preceded by a large-scale theoretical discussion, the scope of which in the opinion of the present author went far beyond the problem of the anthrax vaccine alone and dealt with the live bacterial vaccines in general. The details of this discussion have been dealt with in the materials published at that time (11, 43-49) and at present it seems sufficient to refer only to two principal problems, without a solution of which it would be impossible to make practical use of the STI vaccine. The first of these problems concerns the assertions, founded on general biological concepts, of the principal possibility of a reversion of single uncapsulated (vaccinal) forms of the anthrax bacillus into capsulated (virulent) forms.

The advocates of such a reversion, among them the Academician I. I. Shmalgauzen, warned against the possible danger of such reversions in the course of the practical use of the STI vaccine for man, even though they did not directly deny the possibility of using the vaccine under the condition of a most strict control of each lot.

The advocates of the opposite view, among them the author of the present article, asserted that such reversions cannot take place even under the most favorable conditions, e.g. in the body of the vaccinated. Besides the theoretical considerations, based on general evolutionary concepts (law of the irreversibility of evolution) they referred to the enormous factual material of the results of the use of the STI vaccine in animals which showed that even after complicated vaccinations it had never been possible to find in the body of the vaccinated capsulated (virulent) anthrax bacilli.

Attempts to decide this controversy with the aid of experiments led to discovery of the "phenomenon of survival" (N. N. Ginsburg) which has found acceptance in the microbiological literature and has been subsequently confirmed afterwards in the case of other live vaccines (tularemia - N. N. Ginsburg and R. A. Saltykov [46]; plague- Perry [51]). In this way it became known that the reversion of single vaccinal organisms into the original virulent form, if actually occurring as some biologists assert, to the extent presently accepted for the microbial populations (one individual per millions of organisms), involves no danger for the vaccinated and therefore has no practical importance.

This conclusion has been confirmed at present by the experiences of 20 years on the use of the STI vaccine for man and agricultural animals (of which latter more than 50 million are immunized per year). From the reaction of the animals to the administration of the STI vaccine and from the results of these vaccinations studied for 20 years it is possible to pass judgement upon the condition and behavior of the vaccinal strain STI-I and upon the efficacy of the vaccine prepared from it for prophylactic and emergency immunizations - experiences which it is fully justified to apply to man.

The use of the STI vaccine in groups of people was delayed by the at that time unelucidated problem of complications met with particularly when administering the vaccine to young sheep and goats, specially the latter. Notwithstanding the low percentage of these complications, they developed and could not be disregarded. From the site of subcutaneous vaccinations a jelly-like edema spread in the subcutaneous tissues which afterwards led to the partial shedding of the affected tissues and death of the animals from secondary causes. Cultivations from the edema as a rule either remained sterile or yielded growths of organisms not directly related to the vaccine, such as cocci, pasteurellae, etc.

A careful study of the "complications" by the present author showed that they were the result of a hyperergic reaction of the animals to the vaccine and were the consequence of a disturbance of the normal physiological conditions of the animals due to different causes, e. g. exposure to a cold temperature, overheating, harborage of the organisms of various infections, etc. In the light of the present knowledge on pathergies these complications have to be classed in the category of para-allergic reactions with an exudative process as the basic clinico-anatomical substrate and, in the chronic infections of the vaccinated animals, with the development of a corresponding "microbial output" (Nicolle).

If one can give credence to the postulations of the existence of an inapparent form of anthrax (52-54)*, some of these complications are possibly the result of a specific sensibilization.

*) Asymptomatic anthrax infections have been described first in the USSR by G. IA. Sinai in 1933.

It has to be added that similar "complications" have never been observed in man even though the STI vaccine is administered subcutaneously, cutaneously and in the form of aerosols (55-59). Moreover, in the case of all these modes of administration of the vaccine to man the reactivity was rather low.

Nevertheless, the above mentioned observations on the nature of the post-vaccinal complications met with after immunization with the STI vaccine, confirmed by experiences with live anthrax vaccines prepared from uncapsulated mutants (Baybridge-Sterne, Kolesova and others), render it advisable to be cautious in the case of the vaccination of physiologically impaired persons and to use in such cases a vaccine prepared from the protective antigen or, considering the mechanism of the post-vaccinal hypergic reactions, to administer the STI vaccine through skin scarifications. The materials published by American authors in April 1962 show that the vaccine prepared from the protective antigen according to Wright cannot be recommended for the above mentioned purpose.

Finally it must not be forgotten that from the viewpoint of the post-vaccinal reactions the vaccinations with live vaccines and with the protective antigen (P. A.) are by no means equivalent. This can be easily seen when one analyses the mechanism of the immunogenesis at work in these two methods of vaccination, paying attention not only to the modern theoretical concepts but also to the practical results.

Studying the recent statements of the anglo-American authors regarding the examination of the sera of persons vaccinated against anthrax with the protective antigen and of not vaccinated controls (60-61) one must agree that the immunity resulting from the use of the protective antigen is of an "antitoxic type," leading to the formation of specific antibodies. This means that the response to the administration of the protective antigen develops in the animal body according to the type of plasmocytary reactions, characteristic for the parenteral administration of the majority of protein antigens, being thus accompanied by a proliferation of the plasmatic cells and a synthesis of specific antibodies during the process of histogenesis.

If the vaccinations with the protective antigen are made under optimal conditions, i.e. repeated three times with an obligatory re-vaccination after 6 months and an obligatory

use of depots, then the reaction will not remain limited to the regional areas but will be of a diffuse character (including the lymphoid agglomerations remote from the site of administration of the vaccine). This explains the higher degree of protection conferred to animals vaccinated with the protective antigen according to the American scheme.

Still, one must recognize at the same time that this topography of the plasmocytic reaction to the P.A. is bound to be less diffuse (extensive) than is the case in immunization with live anthrax vaccine because a dissemination of the live vaccinal organisms in the animal body and their persistence in the body for a definite period has been proved, which results in the creation of a large number of long acting foci of immunization with the P.A. in the body. (As shown by investigations in our laboratory, the vaccinal strain STI-I is an excellent producer of the P. A. in vivo as well as in vitro.) Still more important is the fact that the immunity engendered by the live anthrax vaccine and its maintenance differ in principle from the immunity produced by the protective antigen in view of the supplementary involvement of the macrophage elements of the reticulo-endothelial system. As a result it comes to a change in the reactivity of this system, i. e. in the rapidity of the response to the infect with the whole sum of the reactions concerned: an accelerated formation and multiplication of the macrophages, an increase of their phagocytic activity, conversion of the phagocytosis into completion, appearance of forms resistant to the specific toxic factors, etc.

Practical consequences of these most important peculiarities of the immunogenesis in the case of the live anthrax vaccine are a response to the administration of single doses, the quite early appearance of the insusceptibility (which renders the STI vaccine suitable not only for the prevention but also for the suppression of anthrax outbreaks) and the length and intensity of the immunity engendered (2 years in experiments on sheep).

The above gives reason to assert that immunization with the live anthrax vaccine produces an incomparably more extensive, one might say general, transformation of the cells, tissues and systems of the body than is the case in immunization with the protective antigen alone and that the latter is so to say merely a quite important and useful element of the immunization with live vaccines (50).

Thus there is reason to assert that, wherever possible, it is better to use the live vaccine instead of the protective antigen for immunization against anthrax.

In view of its large-scale use for the immunization of man it is necessary briefly to refer to the "scarification" method of administration of the STI vaccine.

First of all attention has to be paid to the principal aspects of the method. In contrast to the subcutaneous method where a strictly defined, easily dosable amount of live vaccinal organisms is introduced, in the case of scarification an inconstant and to a considerable degree accidental amount is applied to the uppermost layer of the tissues. If, in the case of the presence in the vaccinated of some degree of resistance to anthrax (as a result of previous vaccinations or a previous inapparent anthrax infection or finally of an increased natural resistance to the infection) the number of organisms introduced in this manner is not large, their elimination may result and the vaccinations or re-vaccinations do not produce the necessary effect.

This circumstance induces us to assert that (with possible rare exceptions) the application of any bacterial vaccine with the aid of the scarification method cannot give 100% positive results. We are inclined to make exceptions only in the case of vaccines prepared from microbial species capable of penetrating into the cells and of multiplying there (e. g. in case of the tularemia vaccine) (62). As shown by investigations in our laboratory (14), except in the case of an active phagocytosis the anthrax bacilli in either their virulent or their vaccinal form do not penetrate into the cells of man; moreover, in contact with the cells of the human mesenchyma they become destroyed with comparative ease and thus, if their numbers are small, they may not find the conditions necessary for the development of the vaccinal process at the site of scarification.

This is the reason why the vaccine administrations with the aid of scarifications must be made with a sufficiently concentrated STI vaccine, if possible twice with an interval of two weeks and, if indicated, must be repeated every year.

In confirmation of the above mentioned considerations it must be stated that, according to several authors who used the anthrax allergen anthraxin of E. N. Shliakov for an assessment of the efficacy of the administration of the STI vaccine by scarification, positive results were obtained in 20-80%, depending upon the number of vaccinations and the care taken to administer them (58,59,63-65, etc.).

* * * * *

Anthrax

Selected Abstracts-IV/73

The production of the anthrax protective antigen (66) in vitro and of an effective vaccine from it are undoubtedly among the most interesting and important achievements of experimental microbiology in the present century.

At the beginning of World War II studies on the vaccine prophylaxis of anthrax were commenced in various laboratories of the world.

The authors went back to the forgotten work of Bail (1904-67) on the sterile anthrax edema fluid which produced an immunity in vaccinated animals, of Preisz (1909-68) on the anthracocidal substance liberated by the tissues and to the later investigations of Okuda (1923;64), Matsumoto (1924;70), Hruska (1926;71), Urbain and Rossi (1926, 1927;72-73), Stamatin and Stamatina (1936;74), Ivanovich (1938;75-77) and others which during a period of almost 40 years confirmed the observations of Bail. Persistent studies were commenced to search for the "active principle" in the edema fluid and attempts were made to isolate and purify it and to elucidate its nature.

In France work in this direction was done by Staub and Grabar (1943-1944;78-81) who first found two polysaccharides in the edema fluid and then isolated these. Still, as was found later, in the absence of the protein of the edema fluid these polysaccharides were not endowed with protective properties.

In the USA at Fort Detrick (where such investigations were made on a particularly large scale and where the scope of the work with the anthrax bacillus exceeded all that was done in this direction at other times and elsewhere) these problems were studied by a group of investigators under the direction of Cromartie (82-83), Watson (84-85) and Bloom (86-87).

The main results of the work of this group were the isolation from the edema fluid (of rabbits) and the detailed study of two substances: the "factor of inflammation," producing an affection of the tissues of the experimental animals and of the P.A., producing in the latter an immune reaction. In electrophoresis the P. A. migrated between the beta- and gamma-globulins. It was destroyed by heating at 57°C for 30 minutes and digested by trypsin, which pointed to its protein nature; it was not decomposed by formol in a concentration of up to 1%, was stable within a pH range from 5.5 to 11.0 and stable for a long time in a lyophilized state. The immunizing properties of the P.A. were studied in different animal species. The best results were

obtained in rabbits, less satisfactory results in mice. The authors were not able to obtain the P.A. in a purified and concentrated form from the edema fluid. Further work related to the production of the P.A. in vitro by Gladstone was discontinued.

Gladstone (66) reported that he had determined the conditions under which it is possible to obtain the P.A. in vitro and found that this product was as active in experiments with rabbits, sheep and monkeys as the P.A. obtained in vivo. He reported that the conditions determining an agglomeration of the P.A. in the cultures are as follows: presence of serum protein in the media ("non-dialysable factor") and of some "dialysable factor" of the plasma or serum (which can be replaced by sodium bicarbonate)* and a pH of more than 6.5. At the same time he showed that the production of the P.A. is not related to the virulence or the capsule of the anthrax strains or their ability to form spores.

In a later article (1948-88) Gladstone described an original method of producing the P.A. in cellophane bags immersed in the media under constant aeration, recorded more precise formulas for the preparation of the serum-containing media and the necessary periods of incubation of the cultures. Using the uncapsulated anthrax strain Baybridge as producer, the author obtained with the aid of the method devised by him the P.A. in a 25 times higher concentration than in 1946. Resorting to 3 times repeated immunizations at weekly intervals he was able to protect rabbits with a dose of 0.04 ml against 100 DCL of a spore-bearing anthrax culture. Notwithstanding the extraordinarily intricate character of Gladstone's method, his work solved in principle the problem of producing the P.A. in vitro.

This opened the road to the production of bacilli-free anthrax vaccines.

In 1949 Heckly and Goldwasser (39, Fort Detrick, USA) confirmed on the whole the statements of Gladstone but recommended, because of their work, the use of in the media instead

*) Recently (1963) Puziss and Howard showed that sodium bicarbonate, increasing the permeability of the producing cells, promotes the liberation of the P.A. into the media.

of whole serum the purified serum albumin, which freed the final product (P.A.) from the ballast of other serum proteins. An important result of the work of the above mentioned authors was their conclusion that the time of agglomeration of the P.A. in the cultures depended upon the strains selected as producers of the P.A.

Additional and more substantial reports on the P.A. were published only after some years. Wright, Hedberg and Feinberg (1951;90), Wright, Hedberg and Slein (1954;91) and Puziss and Wright (1954;92), because of their investigations, proposed for the production of the P.A. in vitro a "protein-free" (as they called it) synthetic medium (No.528), containing crystalline albumin (obtained from cattle or horses), an ultrafiltrate of ox blood, 17 amino-acids, adenine, guanine, uracil, thiamine, glucose, salt, etc. --altogether 28 separate components. Quite close to this is found the medium 599, proposed by Puziss and Wright (92). Afterwards Wright, Puziss and others proposed a medium with a refined content of amino-acids (No. 963) with which they were able to obtain a 4-6 times higher yield of the protective antigen than on the medium No. 599 (114). They described a new, more simple and practicable method of producing the P.A., its partial purification, its preservation (merthiolate) and testing; finally they recommended its precipitation with alum for the preparation of the anti-anthrax vaccine.

In their subsequent publication Wright, Green and Kanode (93) recorded the results of a study of the reactogenicity and immunizing activity of the alum-precipitated P.A.: the reactogenicity was studied on guinea pigs and mice, the immunizing activity on rabbits and monkeys. The animals were vaccinated subcutaneously, the monkeys twice (with doses of 0.5 and 1.0 ml or with 1.0 and 1.0 ml), the rabbits 5 times.

Challenge of the animals, including the monkeys, with a virulent anthrax culture through the respiratory routes showed that as a result of the immunization there developed a reliable and fairly long lasting immunity (persisting for one year in monkeys).

The authors were the first to test the innocuousness of the vaccine prepared from the alum-precipitated P.A. for man by administering this subcutaneously to 55 volunteers (doses of 0.5 ml given two or 3 times with intervals of two weeks and with the first booster dose after 6 months) and noted that these administrations led to general reactions in 0.7%, to considerable local reactions in 2.4% and pointed to the allergic nature of the latter.

Almost simultaneously with the articles of Wright et al. appeared the papers of Belton and Strange (94-95) in which the authors described their original method of obtaining the P.A. and of a vaccine from it: the producing strains (among them the vaccinal strain "Bayridge"), the nutrient media (synthetic and casein media), the conditions and times of cultivation, the filtration, the concentration and preservation, the lyophilization and precipitation of the P.A. with alum and also the tests to assess the innocuousness and immunogenicity of the proposed vaccine for laboratory animals.

A study of the P.A. obtained by these workers showed that it was a protein endowed with a protective action: 25 mg reliably protected rabbits against 250 lethal doses of virulent anthrax spores. Still, the authors did not obtain at that time reliable proofs for the homogeneity of the P.A. through analysis with the aid of sedimentation. At the end of one of their publications the authors dealt with the technology of the production of the P.A. for the large-scale manufacture of the anthrax vaccine.

The results of a control of the vaccine prepared in accordance with this technology were published in 1956 by Darlow, Belton and Henderson (96). The authors tested the vaccine on 273 persons twice immunized subcutaneously with an interval of 10 days with 1 ml doses and receiving a booster dose of the same size after one year, and also on monkeys (Macacus rhesus) vaccinated twice. The authors recorded the reactions to the vaccination in the immunized persons (with a special code) and determined with the aid of the method of toxin neutralization tests in rabbits intracutaneously injected with mixtures of the serum of the vaccinated and anthrax toxin (Belton and Henderson, 97) the level of "antibodies" in the blood of the vaccinated, i.e. the efficacy of the vaccination. In the monkeys this was determined directly through administration of 10-15 LD 50 doses of the spores of a virulent anthrax strain into the respiratory tract.

The results obtained were as follows: the reactogenicity of the vaccine was low (0.19% of febrile reactions, 78% without marked local reactions); full confirmation was obtained for the allergic (anaphylactic) character of the local reaction which became more frequent in repeated vaccinations; the intracutaneous test (in rabbits) showed that in the vaccinated persons an insufficient immunological transformation had taken place as a result of two vaccinations (at an interval of 10 days) and that an increase of the immunological response became manifest after the administration of booster doses.

It is important to note that the authors were unable to establish a parallelism between the manifestation of the sensibilization following the repeated administrations of the vaccine, or the P.A. and the toxin neutralization tests with the sera of the vaccinated persons on rabbits, apart from a general tendency to increased response after the administration of the booster doses. The challenge tests in monkeys gave results permitting the assertion of a high intensity of the immunity obtained by the administration of the vaccine prepared according to the method of Belton and Strange - all vaccinated monkeys, challenged at different times during a year with virulent anthrax cultures in the above mentioned doses remained healthy.

At the end of 1955 Boor and Tresselt (99), resuming the work of Heckly and Goldwasser in order to work out a more practical method of producing large quantities of a highly effective P.A. with a minimal sensitizing action, proposed a method of manufacture of their own (medium 243: serum albumin, yeast extract, dextrose, salt; strain "5-B," cultivation and apparatus for its growth, stabilization of the filtrate with formol, concentration with the aid of fractionation).

The authors succeeded in demonstrating firstly that through sedimentation with ethanol at low temperatures or fractionation with ammonium sulfate the yield of the P.A. becomes 4.9 times larger or, when these two methods are used in combination, 12 times larger than that obtained by Heckly and Goldwasser and secondly that the P. A. can be characterized as a protein similar to alpha-globulin.

To a certain degree the acme of the investigations on the P.A. during the last decade has been reached through later complex Anglo-American work (Porton-Fort Detrick). Making use of Thorne and Belton's (1956;61,100) method of determining the activity (titer) of the P.A. with the aid of gel precipitation with anti-serum* and also the neutralization reaction for the determination of the antibody titer in the serum of the vaccinated, Strange and Thorne (101) made a thorough and comprehensive study of the P.A. obtained with the somewhat modified method of Belton and Strange.

*) The authors noted "an excellent correlation between the P.A. titer and its immunizing activity in rabbits."

The results of this work were as follows: From 9.3 liters of the original material with an activity of 8 units (or rarely 10 units) per ml the authors obtained through fractionation with ammonium sulfate followed by solution in a buffer 300 ml of raw antigen (AS) with an activity of 200 units per ml. The AS gave three lines in gel precipitation.

Through subsequent purification of a more than double portion of the AS (799 ml) the authors succeeded in obtaining four fractions (20 ml of each) with an activity ranging from 80 to 5,000 units per ml. From the most active fraction which gave two lines in gel precipitation, the authors separated with the aid of ultracentrifugation two fractions with the highest activity, each of which gave one line in gel precipitation tests: "C₁" with an activity of 3,480 units per ml and "C₂" with an activity of 5,120 units per ml. These two fractions were studied in greatest detail.

The nature of the maximally active fraction "C₂" has been described by the authors thus: its solutions give positive biuret and phenol reactions; the spectrum of ultraviolet absorption is typical for protein free from nucleic acid; after acid hydrolysis paper chromatography shows a majority of amino-acids; the dialyzed and lyophilized product contains 14.3% nitrogen, 0.12% phosphorus and 0.5% carbohydrate (calculated as glucose). The prepare is most stable at a pH from 8 to 9. Serum stabilizes it. The fraction "C₁" differs little from "C₂" but is less stable because it is contaminated to a higher degree with peptidase; the authors consider it as a product of degradation of "C₂."

The immunizing dose of the fraction "C₂" for rabbits (vaccinated five times at intervals of one day with fractionated doses) is 2.5 mg, i.e. 10 times less than that of the P.A. previously obtained by Belton and Strange. In mixture with 1% horse serum which stabilizes it, "C₂" protects rabbits in still smaller doses: 0.4 mg saved 50% of the infected animals.

The yield of the most active fractions of the P.A. -- "C₁" and "C₂" -- calculated in units of activity according to the method used by the authors amounted to about 25% of the initial amount in the native filtrate.

The vaccines prepared from the P.A. with the above described methods and precipitated with alum have been widely investigated in the USA already for a number of years. They were studied first on agricultural animals (sheep, pigs, cattle)

by Schlingmann, Devlin et al. (102-103). Challenging the vaccinated animals with virulent cultures, the authors confirmed in principle the immunizing activity of the P.A. but pointed firstly to the necessity of repeated vaccinations for the production of a fully solid immunity and secondly to the limited duration of the immunity: a vaccine prepared from the P. A. and concentrated 20 times, when used twice with an interval of one month was found unable in tests made after 7 months to protect five out of eight immunized animals.

In the medical practice of the USA tests with the proposed vaccines are made in establishments handling raw animal products imported from countries where anthrax is present in agricultural animals (Iraq, Iran, Pakistan, India).

So far publications have been made of evaluations of the vaccine of Wright and his co-authors in which ample use was made of serological tests according to the method of Thorne and Belton to demonstrate the presence of antibodies in the human sera. It is interesting that in this way the authors were first able to establish the possibility of inapparent anthrax infections in persons working in rooms where raw animal products were handled and in the air of which anthrax spores were constantly present in definite concentrations. As a rule vaccinations are administered to man subcutaneously three times at intervals of two weeks in doses of 0.5 ml. Schemes providing for a varying number of booster doses (reinforcing doses) at longer intervals are under study.*

The published materials (52-54, 104,105) permit to conclude that the initial immunization with this vaccine gave serum titers not exceeding 1:2 and 1:4 (Thorne and Belton) and that maximal titers equal to 1:32 and 1:64 could be obtained through the administration of booster doses given 2-3 times. For a long time the data assembled under these conditions did not render it possible for the authors to come to final conclusions regarding the efficacy of the vaccine prepared from the P. A. according to Wright for the immunization of man. Such conclusions were recorded only in 1962 after the completion of tests commenced in the USA more than four years ago (106).

*) Quite recently (1963) tests were made in man with a P.A. prepared by Puziss and Wright from organisms grown under anaerobic conditions.

These tests were made simultaneously in four factories engaged in the processing of goat hair contaminated with anthrax spores with an annual morbidity rate among the workers ranging from 0.6 to 1.8 per 100 persons. Use was made of the vaccine of Wright: P.A. precipitated with alum (0.1%). The P.A. consisted of a sterile filtrate of cultures of strain R₁-NP (an unencapsulated not proteolytic mutant of the strain Vollum) grown on the medium No. 599.

The tests were made on 1,249 persons of whom 495 received the vaccine, 414 a placebo (a 0.1% solution of alum) and 340 were not vaccinated. The vaccine and the placebo were administered subcutaneously into the region of the deltoid muscle, always in a dose of 0.5 ml.

The initial vaccination consisted of three injections given at intervals of two weeks. After the first, second and third half-year periods booster doses were administered and finally, one year after the last half-yearly vaccination a seventh vaccination was made. In the group receiving the placebo 17 persons fell ill (two with the pulmonary form of anthrax with one death and 15 with the cutaneous form); in the unvaccinated group 6 persons fell ill (three with the pulmonary form leading to death); in the vaccinated group 3 persons fell ill: one of them had received the three initial vaccinations and fell ill five months afterwards without having got a booster dose; one had received two initial vaccinations and the third one. All suffered from the cutaneous form of anthrax.

A statistical evaluation of these results gave the authors reason to postulate (a) the efficacy of the vaccine used by them for the protection against the cutaneous form of anthrax; (b) the insufficient duration (5 months) of the immunity induced by the initial vaccination (3 injections) and (c) related to this the necessity of booster doses, ensuring a protection for an additional six months.

Of extraordinary interest are the findings made in regard to the reactivity of P.A. The number of persons showing a heightened reaction to the vaccine grew with each new (repeated) injection, reaching 35% of the total vaccinated after the fifth vaccine administration including 7.8% with severe reactions. The number of reactions was found to be lower after the sixth and seventh vaccination.

The reactions were of a pronounced allergic character. At the site of the vaccination one could note after 24 hours reddening and infiltration, in more severe cases slight edema. The reddening and infiltration which had in typical cases a diameter of 1-2 cm, disappeared after 48 hours. In the case of increased reactions the reddening and infiltration had a larger diameter (3-4 cm) and persisted; after 48 hours fever appeared. In the case of still more severe reactions a marked edema appeared; the infiltration and the reddening reached larger dimensions. In three instances a widespread edema was noted from the region of the deltoid muscle to the middle of the forearm and in one case to the wrist with an effusion into the elbow joint. Such are the last published data. They offer much food for thought, primarily with regard to the imperfection of the preparation under test.

One must postulate that the next stage in the further development of the preparation will be an attempt to produce a vaccine from the P.A. which is maximally free from "ballast." In this connection consideration has to be given also to the work of Klein et al. (115) who recommend that in view of the short duration of the immunity engendered by the P.A. the vaccination with the protective antigen ought to be combined with the subsequent administration of live vaccine. Such a combination permits the immunization guinea pigs, animals highly sensitive in respect to their reactivity to vaccinal anthrax strains, without any complications and increased more than 100,000,000 times their resistance to infection with virulent anthrax cultures.

In the Soviet Union during the last years the production of the P.A. for the preparation of anthrax vaccines has attracted the attention of a number of authors - N. I. Aleksandrov and his co-workers (107, 110), M. V. Revo and G. V. Dunaev (108-109). The authors use for this purpose the vaccinal anthrax strain STI-1 which in comparative tests always gave the best results in respect to the production and agglomeration of the P.A. in the culture fluids. They recommended for this purpose their original medium (a milk medium with glucose and sodium bicarbonate) and concentration of the P.A. with ethanol (110).

* * * * *

In the conclusion of this article it has to be noted once more that, since so far the infected soil, that constant natural reservoir of anthrax, is not the subject of the most intense attention of the institutions engaged in the eradication of anthrax, immuno-prophylaxis remains as before the most active means of fighting this infection.

Regrettably so far no methods have become available permitting to verify the efficacy of anti-anthrax vaccination in man. Its evaluation has been based on indirect evidence obtained with the aid of the infection of laboratory animals and of mass vaccination of agricultural animals. These two methods will not lose their decisive importance in the future as well. Still, at present in addition to them three methods have been devised offering the possibility of assessing the results of the vaccinations directly in the immunized persons. These are: (1) the method of detecting antibodies against the protective antigen in the serum of the vaccinated proposed by Thorne and Belton (61) in the form of inhibition tests of the specific gel precipitation; (2) the method of neutralization of the anthrax toxin with the serum of the vaccinated in the skin of rabbits according to Belton and Henderson (97); (3) the method of detecting a state of specific sensibilization of the vaccinated with the aid of the allergen anthraxin according to E. N. Shliakhov (111-113).

At the present it would be premature to speak of the preferability of any of these methods since no comparative tests, indispensable for this purpose, have been made. However, now already one may say in regard to Shliakhov's method, proposed by him particularly for an evaluation of the cutaneous vaccination of man with the STI vaccine, that with its aid it will be possible apparently to answer with a high degree of reliability two questions of great importance for an assessment of this method of vaccination: (1) whether the spores of the STI vaccine deposited on the skin which is then scarified have penetrated into the interior of the body and (2) whether the organisms have sufficiently multiplied there to produce the transformation necessary for the production of a reaction to the specific antigenic complex contained in the anthraxin. If this reaction is still insufficient for a direct solution of the problem of the specific immunological state of the vaccinated, nevertheless it furnishes an excellent answer to the principal problem of the suitability of the chosen method of vaccination (by scarification or other methods).

These are briefly the results of the progress in the study of anthrax during the last two decades.

In this study it was naturally not possible to deal with other important aspects of the problem: new methods of treating the anthrax patients; early diagnosis of the disease; accelerated methods of detecting the anthrax bacillus in the environment, etc., even though recently considerable progress has made also in these fields, particularly in the Soviet Union. These problems deserve to be dealt with in special articles, some of which are embodied in the present compilation.

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ANTHRAX OF MAN

(Pathogenesis. Clinique. Differential Diagnosis.
Treatment.)

Professor G. P. Rudnev
Member AMS USSR
(Original pp. 22-28)

The causative organism of anthrax can penetrate into the body by various routes, the localization of the portal of entry being of great importance for the development of the various clinical forms of the disease: cutaneous, pulmonary, intestinal and septicemic which pathogenetically may be subdivided into primary and secondary (i. e. metastatic) affections. According to the opinion of A.M. Besredka the skin alone possesses a susceptibility for anthrax infection. According to the presently accepted views the infection is capable of entering through the mucous membranes (of the gastro-intestinal tract or the respiratory passages) only in the presence of lesions (even slight) of their integrity.

In the opinion of some authors the intestinal form develops secondarily, metastatically, being due to an invasion through the blood stream. Other authors propound the same view also in respect of the pathogenesis of the pulmonary form. According to N. K. Rosenberg the appearance of this form is related to an obligatory primary sensibilization of the epithelium of the respiratory tract due to an inhalation of anthrax spores. A secondary septicemia is considered as complication, possible in any clinical variant of the disease. The primary septicemic form develops in the presence of a massive infection, a high virulence of the causative organisms and a simultaneous insufficiency of the protective mechanisms of the macro-organism.

Of great importance for the pathogenesis of the primary pulmonary form of anthrax in man is the inhalation of dust containing besides anthrax spores a mass of various prickly small particles. This irritating admixture serves, so to say, as a peculiar conductor for the penetration of the anthrax infection. Consequently the theoretical conceptions expressed in the contemporary publications on the pathogenesis of anthrax in man speak of the undoubted importance of the introduction of the infection by vehicles like dust, droplets and food and confirm (the value of) the practical system of the necessary prophylactic measures.

The statements made in the literature regarding the frequency of the various clinical forms of anthrax in man are in agreement with the statements made above. Thus most frequent is the cutaneous form of anthrax, observed in 95% of the cases, whereas visceral (internal) affections occur in 5%; among the latter the intestinal form occurs in 48%, the pulmonary form in 23%, while the other internal organs are affected in 29% (1-5).

Pathologo-anatomical findings. Autopsies show a marked hyperemia of the organs. The blood is of dark-red color, does not coagulate and abundantly fills the veins. In the cavities of the heart one finds also fluid dark blood containing many anthrax bacilli. The heart muscles have a dark-brown color; in the lungs one finds a marked edema; the pleural cavities also contain an abundance of sero-hemorrhagic fluid. On the mucosa of the respiratory tract one finds characteristic spots of hemorrhages and sometimes pustules. In the lungs one finds consolidated parts and in places confluent conglomerations. An affection of whole lobes of the lungs is possible. The mucosa of the stomach and parts of the intestines is edematous with characteristic hemorrhages (6). The tracheo-bronchial and mesenteric lymph are enlarged and may also show hemorrhages. In the peritoneal cavity one finds a serohemorrhagic fluid. The spleen is of a dark-purple color, enlarged and rich in blood. The liver and kidneys are hyperemic. In the meninges one finds edema and hemorrhages. The brain substance is hyperemic; in the turbid cerebrospinal fluid one finds an abundance of anthrax bacilli.

The incubation period lasts usually 2-3 days but may be extended to 6-8 days or shortened to a few hours (5).

Clinically typical for the cutaneous form of anthrax are successively a macula, a papula, a vesicle, a pustule and an ulcer. At the portal of entry of the infection there appears first reddish macula which very rapidly becomes transformed into a papula of copper-red color, sometimes with a purple tint, prominent above the level of the skin. Local itching with a sensation of slight burning appears and increases.

After some hours there forms in the place of the papula a small vesicle with a diameter of 2-3 mm; its contents are at first serous, then become dark, hemorrhagic, sometimes purple-violet (Pustula maligna). Because of the intense itching the patients often tear open the papula when scratching; more rarely the pustule bursts spontaneously. There forms a scab which darkens and increases in size (Figures 1 and 2).

Of diagnostic value is a characteristic efflorescence of secondary pustules arranged in the form of peculiar "halo," formed as it were by pearls, round the central part occupied by the pustule. The scab may reach considerable dimensions and shows the appearance of a hard, often charred crust which frequently shows tuberosities. Under and round the scab one sees an infiltrate in the shape of a crimson swelling which rises markedly above the level of the normal skin.

Moreover as a rule there forms an edema which in some of the patients is widely spread. The edema is most marked in areas with a loose subcutaneous tissue, particularly on the face. In one of our patients the edema covered besides the head (on which the ulcer was situated in the region of the ear) in the form of a half-cape both sides of the upper part of the shoulder girdle. When the edematous area is struck with a percussion hammer, one notes not rarely a vibration similar to that producible in jelly (sign of Stefanskii). Particularly dangerous is the presence of anthrax affections on the mucosa of the lip which often indicates the development of an extraordinarily marked edema which, afterwards spreading to the upper respiratory passages, may cause asphyxia and even death. The development of a marked edema may lead to the appearance of considerable local necroses.

For differential-diagnostic reasons it is important to note that characteristic for the affected parts is anesthesia: pressure is felt but weakly and even injections cause no pain. This local anesthesia is of particular value for a differentiation from the cutaneous forms of plague and tularemia which sometimes show a close outward resemblance to anthrax. It must be remembered that in the case of tularemia the sensitivity of the skin is intact and that a local hyperesthesia is characteristic for plague.

According to our observations characteristic for the cutaneous form of anthrax is also the presence of three zones of coloration: in the center the black scab which is surrounded by a narrow yellowish-purulent rim and then by a wide zone of the purple swelling. In the cutaneous form of plague we never saw a yellowish-purulent rim. The eventual development of a regional lymphadenitis is not rare in the cutaneous form of anthrax but this is less conspicuous than in plague and tularemia. *Pari passu* with an increased severity of the anthrax attacks the affections of the lymph nodes become more marked and are accompanied by a distinct lymphangitis which as a rule is absent in plague.

During the first hours of illness the general condition of the patients is still impaired (general malaise, pains in the bones and headache); at the end of the first day or on the second day the temperature rises considerably (to 39°- 40°C); this rise of the temperature is accompanied by a deterioration of the general state and by tachycardia. The fever remains high for 5-6 days; in benign cases it then drops, not rarely critically; this is accompanied by a marked improvement of the general manifestations as well as of the local signs. The edema gradually decreases, the lymphadenitis and lymphangitis disappear, the scab falls off (about the end of 2-3 weeks) and the wound thus laid open gradually heals and becomes covered with epithelium, but more often with the formation of a scar (Figure 4)

In other, specially in more severely affected patients the process becomes worse as a result of the development of a secondary septicemia. This is manifested by a new rise of the temperature, repeated chills, a marked increase of the pulse rate



Figure 1. Cutaneous form of anthrax on the eyelid of the left eye (formation of scab + acute edema).



Figure 2. Cutaneous form of anthrax on the face. Beginning formation of the scab.



Figure 3. Cutaneous form of anthrax on the hand.
Formation of secondary pustules and edema.



Figure 4. Cutaneous form of anthrax (stage of healing).

and severe headache. In a part of the patients one notes also vomiting of blood, hematuria and an exhausting fetid diarrhoea, the excretions containing anthrax bacilli. Secondary pustules and other exanthemata of a hematogenic-metastatic nature may appear on the skin; endocarditis may be observed. The blood contains many anthrax bacilli; a hemorrhagic syndrome becomes prominent. The condition of the patient worsens progressively, lung edema leading to the appearance of a bloody sputum appears, followed by sopor and later by coma terminating in death. However, sometimes the sensorium of the patients remain free up to the end.

In some of the sufferers the skin covering the edematous zone round the ulcer may become intensively hyperemic, showing an appearance resembling erysipelas. However, there is no reason to speak of a special erysipelas-like form of anthrax, as some authors are inclined to do.

The development of the gastro-intestinal form of anthrax leads in some patients to the prevalence of local symptoms on the part of the gastro-intestinal tract while in others general signs of an intoxication are prominent. The illness begins often suddenly with acute severe pains in the abdomen while in the case of other patients one observes before the chills, headache, giddiness, pains in the small of the back and general progressive debility. The symptoms become accompanied soon by the vomiting of blood with bile, a sensation of tension and sharp pains in the abdomen and afterwards by bloody diarrhea. It may come to a paresis of the intestines, the clinical appearances resembling those of an intestinal occlusion; sometimes such patients have been operated under a wrong diagnosis. The anthrax affection of the intestines leads to a marked peritoneal irritation, a hemorrhagic effusion and subsequently to perforations followed by peritonitis. For the purposes of a differential diagnosis it is necessary to keep in mind that in some patients the pains are located mainly below the stomach, sometimes in the region of the appendix or of the gall bladder.

In the gastro-intestinal form of anthrax the temperature is at first for a short time subfebrile, then it rises quickly to 39° - 40° and remains high until the terminal period when it may become suddenly subnormal. The pulse is markedly accelerated, arrhythmic, weak and afterwards threadlike. In the lungs one notes signs of congestion with an abundance of moist rales. On the skin one finds often secondary pustules and various hemorrhagic eruptions. The duration of illness is short in the intestinal form of anthrax and usually after 3-4 days or sometimes even earlier the patients die with sign of an acutely progressive heart failure. Recoveries are rare.

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The clinique of the primary pulmonary form of anthrax is characterized by extreme severity. After a short incubation period with signs of marked depression and increasing chest oppression there appear rhinitis, cough, lacrimation and fever with a temperature of 39°-40°C and severe chills. The pneumonia which develops is more of the type of an acute lung edema. Percussion reveals rarely foci of dullness, auscultation an abundance of dry and particularly of moist rales including large-bubbling rales. Coughing which becomes increasingly frequent, soon results in the expectoration of an abundant fluid and frothy sputum, not rarely with an admixture of blood; the sputum often becomes coagulated, showing the appearance of raspberry jelly. Microscopically one finds in the sputum an abundance of anthrax bacilli. The appearance of a hemorrhagic pleural exudate is not rare. The course of the disease is of an extremely severe character and with catastrophically increasing disturbances on the part of the cardio-vascular system ends fatally after 2-3 days.

Characteristic for the primary septicemic form of anthrax is a generalization of the process without a previous appearance of local foci; this form develops in the case of a slight resistance of the macro-organisms and of an extraordinary virulence of the causative organisms invading the body in a high dose, more frequently through the mucous membranes. The process is characterized by a rapid and extremely severe course with an abundance of hemorrhagic manifestations and usually with the presence of an enormous number of anthrax bacilli in the blood and the cerebrospinal fluid. A complex of meningeal symptoms developing in some patients and producing the so-called meningeal form of anthrax is essentially only a complication of one of the variants of the septicemic or any other form of the disease.

Diagnosis. In the list of diseases of potential differential-diagnostic importance one must include tularemia, glanders, plague, melioidosis, ordinary furunculosis and the formation of carbuncles, ordinary pneumonia, sepsis, meningitis, erysipelas, severe influenza, hemorrhagic fevers and sometimes intestinal obstruction and even appendicitis.

Characteristic for glanders are abscesses in the muscles and arthralgia; the skin manifestations differ from the anthrax carbuncle through their painfulness and multiplicity. Typical for furuncles and carbuncles of a banal etiology is their local painfulness, for staphylococcal carbuncles the excretion of pus from several openings. Erysipelas is characterized by the presence of a painful swelling at the periphery of

the zone of redness with jagged edges ("geographical map"), local sensitivity and painfulness in the inflamed parts, and often by a favorable course of the illness. The differentiation of anthrax from plague and tularemia has been dealt with partly above; however, in localities with epidemics it requires a complete bacteriological investigation.

Treatment. The modern method of treatment of all clinical forms of anthrax is in principle complex and carried out in a planned consecutive manner, comprising general and partly (in the cutaneous form) local procedures. In respect of the specific therapy a distinction can be made of three main consecutive stages: serotherapy, chemotherapy (salvarsan) and treatment with antibiotics.

Schematically the modern therapeutic procedures in anthrax can be subdivided into specific and unspecific methods. Serotherapy was undoubtedly of importance in the past and is still of some importance. As is known, the anti-anthrax serum exerts an antitoxic action. It is used in all forms of the disease - the earlier, the better. After preliminary heating to 350-370C the serum is administered (according to Besredka) subcutaneously or intramuscularly or in very serious cases partly intravenously (in single doses of 100 ml for adults). Depending upon the seriousness of the illness the serum administration is continued at intervals of 1-2 days until the crisis. In the absence of medical anti-anthrax serum (destined for the treatment of patients) it is permissible to use the analogous serum prepared for the treatment of animals. The dosage is not changed in this case but care has to be taken not to shake the ampoules or flasks. The effect of serotherapy is manifested by an improvement of the subjective condition of the patients and a decrease of the manifestations of intoxication. If it is indispensable to use large doses, a part of the serum (40-50 ml) is given intramuscularly or intravenously and the remaining amount subcutaneously.

At present the use of therapeutic anti-anthrax serum has been replaced by that of anthrax gamma-globulin which is used prophylactically in the following doses: for children 5-8 ml, for adolescents 12 ml and for adults 20-25 ml.

For therapeutic purposes one gives doses of 30-35 ml of this preparation after the sensitivity of the patients to its protein has been tested. It is recommended to combine the serotherapy with the use of novarsenol (intravenously in an amount of 0.01 per kg of the body weight). Though novarsenol, cannot be considered as a specific agent for anthrax, still its beneficial action has been quite clearly demonstrated (N. K. Rosenberg, 7). Nevertheless it was difficult to render the serious clinical forms (pulmonary, intestinal, septicemic) amenable to treatment and recoveries were rare in these cases.

In the modern therapy an increasingly ample use is made of the administration of antibiotics, particularly of penicillin, aureomycin (biomycin), levomycetin, etc. Since 1945 we observed a good therapeutic action of penicillin, particularly often if treatment was started in good time. The daily doses of penicillin, administered intramuscularly, amounted to 500,000-800,000 units or more, depending upon the severity of the attacks. It is possible to combine penicillin treatment with the administration of anti-anthrax serum and novarsenol. Of importance is the systematic use of symptomatic drugs among which the cardiaca deserve special attention. Sometimes one gives the patients anti-anthrax serum in accordance with the epidemiological indications and prophylactically in doses of 25-50 ml or more (depending upon the epidemiological and other conditions and particularly in the case of massive infections) or one administers antibiotics. Turning to the statements of the literature one notes the efficacy of the treatment with penicillin, leading to a rapid disappearance of the causative organisms from the anthrax affections, has been dealt with in a number of publications: Marff (1944), Ellington (1946), Mann (1947), Griffin (1948), I skov (1949) A.I. Kolobkova (1950), Maiat (1952), Severov (1953) and others (quoted by Rudnev, 4).

La Bocceta (4) cured 36 patients with penicillin alone, Mann used it successfully in 25 patients, Ellington in 22 out of 26 patients and combined it with sulfidin in the other three. A mitigation of the toxicosis, a disappearance of the edema a drop of the temperature took place during the first 2-3 days. According to La Bocceta the anthrax bacilli disappeared within 24 hours after the commencement of the treatment in 30%, within 72 hours in 90% and only in some of patients the organisms were found on the 7th-8th day of treatment. But the healing of the tissues took place more slowly and in severe forms of the disease it is better to combine penicillin treatment with the administration of serum.

According to the severity of the attacks one administers penicillin usually in doses of 50,000 to 100,000 units every 3 hours and gives up to 5-12 million units during the whole course of the treatment. One may combine this therapy with local applications to the affected parts (300,000 units of penicillin in a 0.5% solution of novocaine).

Kindler (1952), Shanahan (1947) and others reported on the cure of patients with anthrax meningitis with the aid of a combined administration of anti-anthrax serum (500 ml), sulfadiazine (28.0), penicillin (4 million units intramuscularly and 110,000 units into the cerebrospinal canal).

Boger (1953) classified the antibiotics according to their therapeutic efficacy in a decreasing order as follows: aureomycin, terramycin, levomycetin (chloramphenicol), penicillin, streptomycin, neomycin. According to G.P. Belikov and his co-workers (8) penicillin used in experiments saved more than half of anthrax-infected mice, synthomycin one fourth; treatment with biomycin saved all mice.

Biomycin or aureomycin are given first in doses of 0.5 g every 4 hours, after improvement in the same dose every 6 hours and totally in an average dose of 8 to 16 g.

Chloramphenicol is administered in one gram doses 4 times and then in 0.5 gram doses 4 times; treatment is continued for 1-2 weeks, altogether in an amount of 12-30 g. Clinical improvement takes place already on the second day, the bacteriological analysis becomes negative but the anthrax bacilli disappear from the blood and the pustules at an average on the third day; the scab falls off on the 10th-14th day of treatment. It is recommended also to use locally dressings with ointments containing penicillin, biomycin or aureomycin.

Terramycin is given during the first 3-4 days in a daily dose of 3 g (0.75 g 4 times), then in 0.5 g doses every 6 hours, the total dose given in the course of treatment (6-8 days) amounting to 16-18 g.

At an average the therapeutic efficacy of aureomycin, chloramphenicol and terramycin in the treatment of anthrax is approximately identical.

In order to accelerate the healing of the ulcers, carbuncles and pustules one uses locally dressings with some ointment (boric, dermatol, zinc ointment or others). It is obligatory to hospitalize the patients in separate rooms and to care for them in the manner used for all seriously ill patients, assigning to them individual crockery, nursing implements and robes. Patients suffering from the pulmonary, intestinal or septicemic form of anthrax must be attended by a special personnel. Their rooms are subjected to moist disinfection (solution of sublimate 1:500 + a 3% solution of phenol). The crockery used by the patients is kept for 5-10 minutes in boiling water containing 2% of soda and is then boiled for a long time. The dressing material used for the patients is burnt. Their sputum, urine and stools are disinfected in loco by pouring over them a 5% solution of carbolic acid or a freshly prepared 10% solution of chlorinated lime left to act for 2-3 hours; then the material is discharged into the canalization. Rubber gloves must be used whenever handling the patients and special gowns must be worn. In the case of the pulmonary form it is indicated to use goggles, gauze face masks, etc. as worn in the case of plague.

Patients recovered from cutaneous anthrax are discharged after the scabs have fallen off completely and the ulcers have become completely covered with epithelium and cicatrized. Convalescents from septicemic, pulmonary and intestinal anthrax are discharged after clinical recovery and after minimally two bacteriological examinations made at an interval of 5 days have given negative results. (Depending upon the form of the disease the blood, sputum, stools and urine are examined.) Persons who have been contact with anthrax patients are kept under medical observation for a period of 8 days starting from the time when the contact has ended.

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ANTHRAXIN AND ITS REACTIONS

(#87) IMMUNOBIOLOGICAL INVESTIGATIONS WITH PREPARATIONS OF THE ANTHRAX ALLERGEN- ANTHRAXIN

E. N. Shliakhov

(Moldavian Institute of Epidemiology, Microbiology and Hygiene)
(Original pp. 29-80)

In the survey of N. N. Ginsburg (p. 5 [Russian text. Ed.] have been shown the most important achievements in the study of the antigenic and immunogenic properties of the anthrax bacillus, among them the biochemical and immuno-chemical peculiarities of the antigenic fractions of variants of the organisms or of the products of their metabolism in vivo and in vitro.

The investigations made during the last century by Soviet and foreign authors were aimed in principle at the production of immunogenic preparations, live vaccines or protective antigens, indispensable for the specific protection of animals and man. One must admit that these endeavors, in the one direction (live vaccines) as well as in the other direction (protective antigens) led to the detection of new data of theoretical as well as of practical importance.

One cannot fail to note also another fact which at first glance appears to be paradoxical. By this is meant that the practical achievements leading to the production of highly effective vaccines conferring a solid immunity considerably outstripped the theoretical consideration of the postvaccinal immunogenesis and the development of methods for the detection of the reactions creating the immunity in the vaccinated. Strange as it seems, at the present time as well as 70-80 years ago at the time of Pasteur and TSenkovskii, verifications of the post-vaccinal immunity are made in animals through infection with virulent anthrax bacilli and in man by taking account of the morbidity among the immunized (but not necessarily "immune") persons.

The method of control infection of the immunized animals has to be evaluated in general as crude, tedious and rigid. The necessity of following a definite regime substantially limits the practical use of the method. Therefore, when any anthrax vaccine is issued, its last guided control is made within the establishment where the vaccine has been produced. In other words, the practical workers receiving the vaccine use it almost blindly, having no possibility to test its immunogenicity under field conditions.

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The epidemiological control of the vaccination in man is still more arbitrary and haphazard - possibly because in the limited professional groups subjected to vaccination during the last 20 years (and in the USA only recently) the incidence of anthrax is very rare - apparently just because the groups have been vaccinated.

If it is possible to assert, in analogy with the findings made for instance in sheep, that the vaccinated persons are really immune against anthrax, it is quite impossible to pass judgement even approximately upon the intensity and duration of the immunity or, more simply, upon its presence during a definite period of time after the vaccination. Not less difficult is it to ascertain whether the vaccinal organisms penetrated into the human body after application of the vaccine with the aid of scarification or another method of extrinsic vaccine application, whether the vaccinal organisms have multiplied in the body and the latter has responded with the development of an immunity.

Practically this means that one cannot speak with confidence of times for re-vaccination, of optimal doses for vaccination or re-vaccination or of an optimal method of vaccination (by scarification, subcutaneous administration or with aerosols).

To put it more briefly, until recently no adequate laboratory tests were available which permitted a judgement upon the actual protection afforded to the vaccinated persons or animals, i. e. no theoretically sound and practically acceptable method had been devised.

Further it must be noted that for the same reasons it is quite impossible to make a retrospective diagnosis of anthrax. In the absence of immunological tests there exist also quite limited possibilities for an objective confirmation of doubtful or "frustrane" (including bacteriologically negative) cases of anthrax.

Why is it that the immunity against anthrax undoubtedly created through vaccination or a clinically manifest attack not suppressed at the onset with antibiotics cannot be detected in the living body with the aid of objective laboratory methods?

A fundamental explanation may be found in the character of the anti-anthrax immunity, features of which are a scantiness and a certain primitiveness of the reactions produced in answer to spontaneous infection or vaccination. It would seem that the postulation of an historical "youthfulness" of anthrax infection (1) has found confirmation in the appearance of only

the most primitive immune reactions (2,3). According to these statements the insusceptibility to anthrax depends to a considerable degree on phagocytic reactions which, as is known since the times of Mechnikov (4), play the leading role in the natural protection against this infection.

Side by side with the assumption of the fundamental role of a cellular immunity a search has been made and is being continued for humoral antibodies which supposedly may appear in response to single applications of a wild anthrax strain (spontaneous infection) or of a vaccinal strain (initial immunization). Still, as will be shown below, humoral antibodies are present in the serum to an insufficient degree, in quantities extremely difficult to demonstrate, irregularly and not in a adequate relation to the actual protection of the body.

Side by side with this it is generally known that through repeated immunization of animals (17-25 times) one may obtain in the case of anthrax also a highly active serum saturated with antibodies which can be used practically as the precipitinose-containing component in the reaction with Ascoli's precipitinogen. Still, this "enforced" production of antibodies rather confirms the fundamental rule - namely the slight production of antibodies. But this does not fully explain the peculiarities of the humoral anthrax antibodies and their functions.

Thus it is known that the capsule of virulent anthrax bacilli, conditioning their fundamental pathogenic properties - aggressiveness and invasiveness - demonstrated experimentally well marked antigenic properties bringing about a good production of anti-capsular antibodies. Still these have no protective functions for the majority of susceptible organisms (with the exception of mice).

On the other hand a search for antibodies as generally accepted in the therapeutic anti-anthrax serum (passively protecting the animals injected with it) remained fruitless. This serum, obtained through hyperimmunization with virulent anthrax strains, does not react with the homologous corpuscular antigen or hapten (5,6).

Still, it has been further shown (see the article by Ginsburg) that the "therapeutic" serum is capable of neutralizing a peculiar substance, called "the lethal factor" by the authors who observed it in the plasma of guinea pigs succumbed to anthrax and formed in the course of the growth of virulent organisms.

And not long ago in the growth products of virulent as well as of attenuated (uncapsulated) anthrax strains, inoculated into animals or cultivated in certain media an "anthrax toxin" has been found which later on could be fractionated (7-10).

Other investigations described in the literature dealt with a study of the sensitizing (anaphylactogenic and allergenic) properties of the anthrax bacillus and its fractions.

In the twenties of the present century it has been established that the intracutaneous or subcutaneous administration of a live or formal-killed anthrax culture to animals produces a local inflammation with a gross infiltration of the skin (11). Somewhat later (12) the sensitizing properties of virulent anthrax bacilli and their products have been studied with the aid of 9 times repeated daily intracutaneous administrations to normal guinea pigs of 0.1-0.2 ml of (a) a cell-free autolysate of B. anthracis; (b) a soluble polysaccharide obtained according to the method of Schockaert (13) and (c) suspensions of one day old growths of attenuated anthrax bacilli.

As a result of these tests a local reaction, in the form of a cutaneous papule, was noted only after introduction of the product "c", the intensity of the local inflammation increasing up to the administration of 6-7 injections and then decreasing.

M. V. Revo (14), subcutaneously sensitizing guinea pigs with the product of sedimentation of a 48 hour old anthrax culture with 10% trichloroacetic acid obtained a general anaphylactic reaction. Still, he did not obtain such a reaction in analogous tests with lipid and somatic polysaccharide fractions.

In 1927 Hruska (15), in order to obtain a highly active therapeutic serum, hyperimmunized horses by the simultaneous administration of the cell-free edematous fluid of anthrax-infected animals and suspensions of the vegetative form of virulent anthrax bacilli. At the beginning of the cycle of hyperimmunization there formed at the site of administration of the mixture an enormous edema which, hand in hand with an increase of the dose of virulent bacilli, gradually decreased and was not observed even after administration of enormous doses of the microbes. However, subcutaneously administering to the hyperimmune animals 10 ml of the sterile edematous fluid, the author noted at the site of administration the appearance of an edema persisting 3-5 days. This phenomenon, called by Hruska "the anthrax status," could not be produced through subcutaneous administration to the

same animals of even large doses of virulent bacilli alone. The author postulated the presence in the edematous fluid of a particular substance, "anthrax plasma-reagin," to which the immune organism constantly responded with a typical local reaction. This substance was thermostable, passed bacterial filters, was not neutralized by highly active immune sera obtained in part with the edematous fluid and could be observed in very small quantities in extracts of the spleen of animals succumbed to anthrax.

One must remember also the skin tests (made by Darlow et al. [16]) in persons vaccinated and re-vaccinated with the protective antigen in order to obtain reactions of an allergic type. Growth media, purified antigen and alum-precipitated antigen, were used as allergen.

Marked skin reactions were obtained mainly with the purified antigen, less marked reactions with the precipitated antigen; the media produced no reactions.

Quantitatively expressed distinct skin reactions were obtained in 3 out of 11 persons only after the first re-vaccination made one year after twice repeated initial vaccinations; 2 skin reactions were obtained in 9 persons tested after the second re-vaccination made one year later. The other persons either did not react or their reaction consisted of a passing tenderness at the site of injection.

Characteristic was that the reactions took place in the main immediately, only a few being of a delayed allergic type. The authors considered the immediate reactions as manifestations of an idiosyncrasy against the protective antigen in the vaccinated.

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In view of the above mentioned trends and results of investigation it is legitimate also to pose and answer the following question: Have endeavors been made on this basis to devise practical methods (immunity reactions) which ensure an elucidation of the immunological response of the body to the administration of anthrax vaccines or permit a diagnosis of anthrax attacks?

In other words the intention is to deal briefly with the materials concerning the various methods recommended in the literature for the detection of the immunological reactions of the body to natural infection or specific vaccination.

Early attention has been given to an evaluation of the agglutination method (17-20). Apart from the fact that an indispensable component of this reaction is a homogeneous suspension of virulent bacilli it has been shown that the agglutination reaction is unsuitable as a method for detecting immune bodies. Thus the sera of normal rats, rabbits, guinea pigs and horses agglutinate the bacilli in dilutions from 1:10 to 1:50 and normal human sera in dilutions of up to 1:500 (20). In other tests the agglutination titer of the serum of immunized horses did not rise in a parallel manner with an increase of the protective properties of these sera (34).

Recently Levi et al. (21) recorded promising results of investigations of the passive hemagglutination and antibody neutralization tests. For these the authors used anthrax precipitating serum as source of the antibodies. The question of the use for the reactions of sera of initially vaccinated persons (animals) or those from individuals recovered from anthrax remained open.

Already in 1910 Sobernheim (17), using the complement fixation test (RCF) with therapeutic anthrax sera obtained inconstant results.

Afterwards evidence has been adduced concerning the absence of a specific activity of the majority of immune sera in the RCF (22). On the one hand normal sheep sera produced a well marked complement fixation; on the other hand extracts of anthrax bacilli (antigens) did not react in such tests with the sera received from various species of immune animals (17, 23, 24). No success was obtained with complement fixation tests used for the demonstration of antibodies in the sera of rabbits four times immunized with the edematous fluid as well as with the sera of control animals killed with anthrax bacilli (25). Paradoxically the highest titers of the RCF were found in control tests with the sera of unprotected animals.

In 1961 were recorded the results of the use of the RCF for the investigation of the sera of rabbits immunized with preparations of protective antigen obtained on synthetic media (26). The authors were unable to obtain complement fixation

with the sera of animals recovered from anthrax or inoculated with live vaccines (controls), because the reaction of these sera with the not protective thermostable part of the antigen masked the reactions with the thermolabile fraction. On the contrary, specific complement fixation was obtained with the sera of rabbits immunized only with the protective antigen and in relation to the homologous antigen (antigens). Hand in hand with this the RCF proved unsuitable if the D-glutamin polypeptide and the somatic polysaccharide were used as antigens (27).

The practical value of these results was explored in an epidemiological study by American authors (28). Studied were the sera of non-immunized persons in parallel tests with the sera of individuals immunized 1-9 times with the aid of the protective antigen precipitated with alum according to the method of Wright (29).

The sera of non-immunized as well as of once immunized persons gave negative results in complement fixation tests. The sera of repeatedly vaccinated persons gave positive reactions at titers not over 1:120; at the same time negative results were obtained in persons vaccinated 3-6 times. In this connection the American authors preferred afterwards a more sensitive method - gel precipitation recommended by Thorne and Belton (10).

Reference has been made already to the property of the lethal factor (toxin) to become neutralized by the serum of animals immunized with live vaccines or the protective antigen. The intracutaneous administration of the lethal factor alone produced local hyperemia, infiltration and hemorrhages; after neutralization with the serum these manifestations remained absent. On the basis of this phenomenon Belton and Henderson (30) proposed in 1956 a method of titration of the immune sera in albino rabbits of a definite weight.

For the titration of the antibodies the standard toxin was mixed in various dilutions with the serum under test in a volume of 0.2 ml and the various mixtures were administered intracutaneously to 2 rabbits. After 18-20 hours determination was made of the highest dilution which inhibited the appearance of a toxic skin reaction. This dilution was considered as the titer of the positive toxin neutralization test.

In experiments made according to this method tests were made with the sera of a group of monkeys immunized with the protective antigen. One week after the blood samples for the titration tests had been taken, the monkeys were infected with 16 DCL; they survived while the controls died. Results

are given of the titration of the serum of one monkey on 4 rabbits. The skin reaction was inhibited only when the toxin was mixed with undiluted serum; a serum dilution of 1:3 was already incapable of inhibiting the toxic skin reaction.

The method of toxin inhibition was studied afterwards in an epidemiological investigation and also in a group of monkeys inoculated with the protective antigen of Belton and Strange (31). The results obtained were as follows: 1) Of the sera of 32 persons who a year before had been given subcutaneously two doses of the protective antigen of 1.25 ml each with an interval of 10 days (cycle of primary vaccination) only one serum inactivated the toxin; 2) of the sera of 28 people, observed for 12 months after their first re-vaccination (made one year after the initial vaccination) 2 sera did not give a positive reaction; 3) the sera of 20 persons tested before and immediately after the first re-vaccination gave positive reactions in 18 instances; 4) in a group of 30 people, tested after the second yearly re-vaccination, only one serum failed to inhibit the toxin; 5) the serum of one person gave a positive reaction after the 4th and 5th re-vaccination; 6) in 17 instances the serum titer of the vaccinated persons became lowered by half in the course of 12 months.

The authors came to the conclusion that the cycle of primary vaccinations was incapable of causing a constant production of antibodies; re-vaccination resulted in a more definite response but the titers became lowered by half in the course of the following year.

In monkeys at the end of the first year after the primary vaccination the serum neutralized the toxin in a dilution of 1:3 or at higher dilutions. After 18 months only the concentrated serum of the same monkeys neutralized the toxin; 2 years after the vaccination antibodies were absent altogether.

The tediousness of the method of toxin inhibition in rabbits and its comparatively slight correlation with the true degree of protection of the vaccinated animals led to a search for other more simple and more sensitive methods of antibody titration.

As an alternative the diffusion method of antibody titration was recommended (10).

For this purpose use was made of a 1% buffered agar (pH 7.3) to which were added 0.01% sodium merthiolate, standard antigen (filtrate of a culture of the vaccinal strain "Baybridge," dilutions of which gave on agar with horse anti-serum a precipitation up to a titer of 1:19.2) and various dilutions of the serum under test.

For the titration of antibodies determinations were made of the ability of the various dilutions of the serum under test to combine in mixtures with the standard antigen (upper row of holes) and to inhibit the appearances of lines of precipitation with the standard anti-serum (lower row of holes). The combination with the antigen was considered as a positive reaction.

In connection with a major outbreak of anthrax (5 attacks of the pulmonary form in the course of 10 weeks) among the workers of a factory processing goat hair in Manchester, New Hampshire (USA), taking place at the end of 1957 (28,32), the antibody level in different groups of the workers (persons formerly suffering from the cutaneous form of anthrax, those vaccinated with the protective antigen and those not vaccinated) was determined with the aid of agar gel precipitation tests (10).

This method was tested in 242 not vaccinated workers of Fort Detrick (a military center for microbiological studies in the USA) who had had no contact with anthrax materials; their sera did not combine with the standard antigen and the reactions were considered as negative.

In the vaccinated and re-vaccinated persons in the factory processing goat hair in 15 (of 33) cases positive reactions were noted (with titers of 1:2 - 1:4 in the vaccinated and titers of up to 1:32 in repeatedly and recently re-vaccinated). In two of 11 tests positive results were obtained with the sera of persons who had suffered from anthrax (higher titers - up to 1:32 - were noted in persons who had been recently affected). Still, the sera of 11 of 72 not vaccinated workers of the factory also gave positive results.

The authors pointed out that the 18 negative reactions observed in 33 vaccinated persons were obtained with blood samples taken at the end of a 6 month period after re-vaccination; they were inclined to ascribe the 11 positive reactions in 72 not vaccinated persons to past sub-clinical infections.

However, it remained unexplained why positive serological reactions were noted only in two persons who had suffered from anthrax two or more years previously; moreover in 4 (out of 5) former anthrax patients 3 months after recovery no antibodies could be detected in the sera with the aid of gel precipitation tests.

This led the authors to the general assumption of a passing character of the "serological positivity" appearing after anthrax infection or artificial immunization.

Evidently even the best method used by the American investigators -antibody titration with the aid of gel precipitation tests - is not sufficiently reliable and sensitive.

The basis for this assertion is the study by Klein et al. (33) published in 1962. Studying the immunity levels in guinea pigs vaccinated separately with the protective antigen and live vaccines or at first with the protective antigen and then with live vaccine, these workers used an old method for the assessment of the immunity - infection of the animals with virulent anthrax bacilli.

The last mentioned variant of immunization (protective antigen and then live vaccine) produced in the guinea pigs a most marked degree of immunity, 1-100 million times exceeding that obtainable with separate vaccinations.

In concluding the present survey one must remark that the opsono-phagocytic reaction has not been worked out practically and that the attempts to use the "opsonic index" (20, 34) as an indicator of the immunobiological status of the organism in anthrax did not receive actual attention.

Former observers showed that the bactericidal properties of the sera animals immunized against anthrax differed but little from those of the normal sera of the same species (18,23). It is noteworthy in this connection that the normal sera of horses and rabbits (6) show a high bactericidal activity while this is lacking entirely in the case of the normal sera of guinea pigs, sheep, cattle, and dogs. The bactericidal properties of the sera are not related to the natural resistance of the animals against anthrax (34).

In a study (35) it has been shown that normal rabbit serum protects 2/3 to one half of the test mice against experimental anthrax infection (inoculation of a vaccinal strain).

These observations materially lower the reliability of tests to determine the degree of protection afforded to the various animal species (or man) with the aid of investigations of the bactericidal (preventive) properties of their sera.

Only one study (36) has been published dealing with the production of a preparation and a method with the aid of which the allergic state (sensitization) to the specific anthrax antigen could be assessed.

This work was based on the observations made by Hruška (15) in regard to the sterile edematous fluid of animals infected with virulent capsule-forming growths of B. anthracis and succumbed to anthrax sepsis. The preparation was used for intracutaneous tests and caused marked and in part extensive local reactions in the shape of an allergic inflammation in anthrax patients and in persons who had suffered from the disease in the past. At the same time, according to the statements of the authors, in 35% of the cases para-allergic reactions occurred. The authors pointed also to untoward tissue-destroying properties of the preparation which they called the "edema-created principle." It has never been tested in vaccinated persons.

* * * * *

Thus the new researches in the realm of immunity and the elaboration of immunological tests in vivo and in vitro failed to furnish in the aggregate sufficiently reliable and practically acceptable methods of investigation. This evaluation refers in the first line to the methods of toxin neutralization in the gel precipitation tests of Thorne and Belton (10) and of complement fixation (26). Concerning the method of toxin inhibition in the rabbit skin according to Belton and Henderson (30) it must be noted that the data furnished by the authors themselves leave no doubt of the imperfection of this kind of immunological tests, the practical use of which was given up by them.

These findings induced us to continue the investigations concerning the production of an anthrax allergen (anthraxin), commenced in 1956, with the aid of which under amply practicable conditions it would be possible to demonstrate

through the results of allergic skin tests the state of immunological reactivity to the anthrax antigen in patients, in persons recovered from the infection or in immunized individuals.

In the present article are recorded some results of immunological studies made during the period from 1956 to 1963 with the aid of various anthrax in preparations in experimental animals as well as the results of the administration of anthraxin preparations to persons vaccinated or re-vaccinated against anthrax and also to patients and persons recovered from the disease.

I. Brief Characterization of the Anthrax Allergen-

Anthraxin Preparations

The various preparations of the anthrax allergen obtained during the above mentioned period of investigation can be divided into 3 groups:

The first anthrax allergic preparation was proposed by us in 1957 (Avtorskoe svidetelstvo SSSR No. 113 171 with priority of April 10, 1957 [37]) and was used for the performance of special experimental laboratory work. This was the native anthrax allergen prepared according to the technique described in previous publications (37-39). Obtainable from the edematous fluid of guinea pigs and rabbits inoculated with the vaccinal strains STI-1 (N.N. Ginsburg) and TSenkovskii No. 2.

Afterwards the technique of producing the anthrax allergen was improved. As a result of these investigations it was possible to pass from the native allergen, obtainable in small quantities only from the edema fluid, to the so-called tissue anthraxin (or simply anthraxin) obtainable through extraction of the active principle from the edematous tissues with the aid of fluid extracting agents. The new technique rendered it possible to use the anthraxin for public health purposes and to commence the commercial production of the prepare (Avtorskoe svid. SSSR No. 131 859 with priority of 28 November 1959 [40]).

The anthraxin which had been the subject of a large scale epidemiological study by a commission in 1958-1959 (investigated were people vaccinated with the STI vaccine cutaneously, subcutaneously and with aerosols) was recommended as

an officinal allergic preparate (allergen) for mass production by the Vaccine and Serum Committee of the USSR Ministry of Health and the Tarasevich State Control Institute in February 1960.

Altogether during the period from June 1960-May 1962 more than 40 series of anthraxin were issued to various anti-epidemic and curative establishments of the country for practical use in the current and retrospective diagnostic of the disease (41-44) as well as for the determination of the immuno-allergic reactivity of persons vaccinated and re-vaccinated against anthrax (45-50).

Further investigations made jointly with S.A. Shvarts indicated the possibility of obtaining a more active preparation of the anthrax allergen. As a result of investigations made in 1961 a third preparation of anthraxin was recommended, called the chemical (tissue-free) anthraxin (Avtorskoe svid. SSR with priority of August 18, 1961). The chemical anthraxin* was subjected to a preliminary investigation through laboratory experiments as well as through epidemiological investigations and, as a result of a favorable evaluation, was recommended by the Vaccine and Serum Committee of the USSR Ministry of Health and the Tarasevich State Control Institute for mass production in June 1962. At present the chemical anthraxin is used by the scientific-practical establishments of the USSR for the same purposes as the tissue anthraxin.

Inasmuch as the fundamental physical, biological, biochemical and immuno-chemical properties of the chemical anthraxin are dealt with in a separate article (see p. 97 [Russian text. Ed.] of the present volume), it is at present only necessary to deal with some elements of the characteristics of the tissue anthraxin.

a) Physico-chemical properties. The tissue anthraxin has the appearance of a transparent fluid with a golden hue. The preparate is thermostable; heating at 90-99°C for 30 minutes did not lower the activity of its active principle.

In its ready state the tissue anthraxin contains at an average about 225 mg % of nitrogen (mainly because of the protein of the animal tissues); the share of residual nitrogen amounted to 46 mg%, that of the protein nitrogen to about 180 mg %.

*) The authors recognize the relative validity of the name "chemical anthraxin."

An analysis of the protein content of the tissue anthraxin with the aid of electrophoresis in the apparatus of Tiselius* (1 hour, veronal buffer of 0.12 M, pH 8.6, temperature + 20°C, current 15 mA) gave the following results of the mean percentage of the albumins, alpha-, beta- and gamma-globulins: albumin = 55.95%, globulins: alpha = 10.8%, beta = 17.4%, gamma = 15.85%. Similar results were obtained with the aid of paper electrophoresis of the tissue anthraxin. Distinguishing features of these results are some lowering of the alpha-globulin coefficient, equalling 1.27, and mainly a marked increase of the specific proportion of the beta-globulin fraction, exceeding the norm 1.5 times.

It is noteworthy that the parameters of the electrophoretic analysis are somewhat different from the sizes of the protein fractions determined with the same method in the blood of the immunized rabbits from the tissues of which the anthraxin was prepared.

The preparations of tissue anthraxin gave positive biuret, ninhydrine and precipitation reactions for protein; they contained cyclic amino-acids (xanthoprotein, Million's and Adamkiewicz's reactions positive), serum-containing proteins, histidine and arginine (diazo-reaction) and also the carbohydrate components of proteins (reaction of Molisch).

The content in reducing substances, determined according to the method of Hagedorn and Jensen, varied from 20 to 40 mg % in different series of the preparation.

The preparations of the tissue anthraxin showed a neutral or weakly alkaline reaction (pH 7.1-7.3); the pH underwent practically no change during prolonged storage; likewise the biological properties of the anthraxin remained unchanged during storage at a cold temperature (+4°C and +10°C) throughout a year (limit of observation).

b) Serological properties. The tissue anthraxin gave a precipitation reaction with commercially produced anthrax precipitating serum (agar gel tests). One noted one line of precipitation in the case of dilutions of the anthraxin of up to 1:40 or at higher dilutions. At the same time the standard antigen for Ascoli's reaction (control) precipitated the serum in dilutions not exceeding 1:4-1:8.

*) This investigation was made in the Moscow Institute of Epidemiology and Microbiology by N. V. Kholchev to whom the thanks of the author are expressed.

In double agar gel precipitation tests the tissue anthraxin was not precipitated by commercially produced therapeutic anthrax serum or its gamma-globulin fraction (kindly furnished by A. V. Mashkov and V. P. Bodisko) and also not by the therapeutic anthrax gamma-globulin of the Orlov Biofabrika.

c) Biological properties. The biological properties of the tissue anthraxin were tested on guinea pigs immunized (or hyperimmunized) against anthrax. The preparation was administered strictly into the skin on the flank of the animals, which had been depilated on the day before. Diagnostic account of the reaction was taken after 24 hours by measuring two diameters of the hyperemic zone of the skin at the site of injection (in mm) and determination of the thickening of the cutaneous layer (with a cutimeter) in comparison with the thickness of the unaltered skin. The allergic test was considered as minimally positive if the diameter of the hyperemic zone exceeded 5 mm and the thickness of the cutaneous layer at the site of injection was double that of a normal portion of the skin.

These parameters were also used for the standardization of serially produced preparations of the tissue anthraxin.

After administration of the tissue anthraxin to anthrax patients, persons who had suffered from the disease or had been immunized against the infection (in doses of 0.05-0.1 ml) one noted already within 6-10 hours the onset of a local allergic inflammation which became maximal after 20-22 hours and gradually disappeared within 48-50 hours without the appearance of a local necrosis.

Accounts of the reaction were taken according to a specially prepared scale.

II. Tests on Experimental Animals

Materials and Methods

Preparations of the anthrax allergen-anthraxin. The following preparations of the anthrax allergen were used in the animal experiments:

1) Native anthrax allergen of 25 experimental series obtained through inoculation of guinea pigs or rabbits with STI anthrax vaccine for animals prepared from the strain STI-1 or with the 2nd vaccine of TSenkovskii in accordance with the technique described by Shliakov (37, 38) and designated by the numbers I-XXV.

2) Tissue anthraxin. Tested were preparations of 7 experimental series (Nos. XXVI-XXXII) obtained by processing the tissues of rabbits (K) or guinea pigs (M) inoculated with the STI vaccine (S) or the second vaccine of TSenkovskii (TS) and also 48 commercially produced series of tissue anthraxin (Nos. 1-48) obtained - as noted above - from the tissue of rabbits immunized with STI vaccine (Strain STI-1).

In earlier publications the experimental native anthrax allergen and the tissue anthraxin were given the common name of the "MIEMG allergen" (of the Moldavian Institute of Epidemiology, Microbiology and Hygiene).

3) Chemical (tissue-free) anthraxin. In the biological tests described below were used preparations of 10 experimental and ten commercially produced series of the chemical anthraxin.

Experimental Animals

- 1) Guinea pigs of mixed breeding, weighing 300-600 g.
- 2) Rabbits weighing 1600 $\pm$ 500 g.
- 3) Sheep: 4-5 month old female lambs and female sheep more than one year old kept in a herd on pasturage.
- 4) Macacus rhesus monkeys (tests made by N. S. Garin)

Immunization of the Animals against Anthrax

1) Guinea pigs were immunized subcutaneously

a) Once with STI vaccine for animals (Strain STI-1) or by administration of 1, 10, 25 and 40 million of spores of Strain STI-1 in normal saline. The suspension of the vaccinal Strain STI-1 had been received from V. R. Arkhipova of the Tarasevich State Control Institute.

b) A bacilli-free vaccine (antigen-filtrate prepared from Strain STI-1 on the synthetic medium No. 25 by G. V. Dunaev (1962) and precipitated with potassium aluminate. This vaccine was administered thrice in doses of 1, 2 and 3 ml with intervals of 7 days (50).

Aerosol immunization of guinea pigs was made with dry fluid aerosol STI vaccines, as described by N. I. Aleksandrov et al. (45,46).

Hyperimmunization of guinea pigs, effected in order to increase the reliability of the immunity created by primary vaccination, was performed by the supplementary administration to animals initially immunized with STI vaccine (40 million spores) 14-25 days after the first immunization doses of 1-1.2 million spores of TSenkovskii's second vaccine. Not immune control animals succumbed after administration of this dose of the TSenkovskii vaccine within 72 hours after inoculation.

2) Rabbits were immunized with the bacilli-free vaccine (antigen-filtrate) of G.V. Dunaev twice in doses of 2 and 3 ml with an interval of 7 days (50).

3) The sheep were immunized subcutaneously:

a) With the STI vaccine for animals once - the lambs in a dose of 0.25 ml, the adult sheep in a dose of 0.3 ml; b) once with the vaccine of the State Control Institute of Veterinary Preparations in a dose of 0.3 ml for both lambs and sheep.

4) The monkeys were immunized once with dry STI aerosol vaccine in a dose of 25 million spores and tested with anthraxin by N. S. Garin (51).

Treatment of Guinea Pigs with Streptomycin
in order to Study the Influence of
Antibiotics on the Immunogenesis.

Chlorhydric streptomycin in a watery solution was given to guinea pigs in a dose of 5,000 units per 100 g weight in two portions @ 2,500 units daily in the morning and at midday.

Performance of Tests with the Anthraxin Preparations
and Evaluation of the Reactions

The preparations of anthraxin were administered to guinea pigs, sheep and monkeys intracutaneously in 0.1 ml doses. Into the skin of an opposite site (the flank of guinea pigs, the layer under the tail in sheep 0.1 ml of a test-control fluid or normal saline were administered. Rabbits received intracutaneous doses of 0.2 ml.

Readings were taken 24 hours (in some cases also 48 hours) after administration of the allergen. In the case of the native and tissue anthraxins a coincidence of the minimal signs served for the recognition of positive reactions: a hyperemic zone at the site of injection with a diameter of more than 5 mm and thickening of the skin amounting to not less than 2 times the thickness of the normal skin. In the case of the chemical anthraxin the minimal diameter of the hyperemic zone had to reach 8 mm while the criterium of the thickening of the cutaneous layer was identical with that noted above. Doubtful reactions were considered as negative.

In a number of tests the positive reactions were grouped according to their intensity into five categories designated by plus signs from + to +++++ in accordance with increases of the degree of the fundamental signs of reaction, the hyperemia and the thickening of the skin.

In the case of tests in groups of animals, besides an evaluation of the dimensions of the positive (or negative) reactions, account was taken also of their intensity (in %) according to a formula proposed by us (47).

The trustworthiness of the various statistical data was ascertained with the aid of calculations of the magnitude t according to the formula

$$t = \frac{P_1 - P_2}{\sqrt{m^2 + m^2}}$$

in which P_1 = Percentage of positive reactions; P_2 = Percentage of negative reactions; m = Mean deviation of percentual indices determined according to the formula

$$m = \frac{\sqrt{P_1 \times P_2}}{n} \%$$

in which n = Number of observations (minus 1 in the case of small samples).

The significance of the differences of the percentual indicators in two tests was ascertained also with the aid of magnitude t according to the formula

$$t = \frac{P_1 - P_2}{\sqrt{m_1^2 + m_2^2}}$$

in which P_1 = the major indicator, P_2 - the minor indicator, m_1 and m_2 the mean deviation of the corresponding percentual indicators.

The calculated magnitudes t were compared with their tabulated (theoretical) value according to the assessment of the level of significance of indicators by Student (52-54).

Control Infection of Experimental Animals

Investigations of the intensity of the immunity against anthrax were made: 1) Through administration to animals of spore suspensions of the TSenkovskii Vaccine No. 2 (commercially produced) or of suspensions of the variant 71/12 of this vaccine obtained from V. R. Arkhipova. Counts of the spores were made either according to the turbidity standard of the GKI (Standard No. 10 = 80-83 million spores per ml) or with the aid of direct counts in the chamber of Goriaev (55). The DCL of the inocula was titrated preliminarily in guinea pigs weighing 400-600 g; 2) Control infection of animals with the spores of virulent anthrax cultures, the DCL of which had been determined preliminarily in the same manner.

Results of the Investigations

Tests in Guinea Pigs

The majority of the biological tests with the anthraxin preparations was made in guinea pigs. This animal is highly susceptible to anthrax though somewhat less so than sheep (56). Moreover, according to the generally accepted opinion, reached by numerous studies of the tuberculin, brucellin, tularin, pestin, pestallergen, etc. The guinea pig is the "animal of choice" for the specific aim of our work - the study of the allergic transformation of the body.

The general laws underlying the appearance of allergic reactions to anthrax preparations in relation to the times and methods of immunization and to the kind of vaccines used has been studied with the aid of serial and continuous tests on guinea pigs. Moreover, studies were made also on these animals regarding the sensitizing properties of the anthrax preparations, the correlation between the alternative indicators of the allergic reactions and the actual degree of protection (or the lack of protection) of the animals against lethal doses of anthrax bacilli, the influence of antibiotics on the post-vaccinal immunogenesis and the influence exerted by this factor on the indicators of the allergic reactions.

Immuno-Allergic Reactivity of Guinea Pigs Inoculated with
Different Vaccines and with the Aid of Different
Methods of Immunization

In Table 1 are shown the results of a study of the native anthrax allergen in guinea pigs immunized once with the STI vaccine (Strain STI-1). Studied were the native allergen obtained from guinea pigs (M) or rabbits (K) immunized with the STI vaccine (S) or the TSenkovskii Vaccine No. 2 (TS).

Table 1

Study of the Native Anthrax Allergen in Guinea Pigs Once
Immunized with the STI Vaccine

<u>Kind of Allergen</u>	<u>Days after Vaccination</u>				<u>Total</u>
	<u>10-13</u>	<u>14-30</u>	<u>31-60</u>	<u>61-180</u>	
M TS	-	3/4	8/8	11/15	22/27
M S	4/5	4/4	22/24	13/14	43/47
K S	-	9/10	13/15	6/6	28/31
Totals	4/5	16/18	43/47	30/35	93/105

Remark: Numerator = Number of positive reactions;
Denominator = Number of tests.

As shown by the data of this table, the allergen obtained with the aid of STI Vaccine (K S, M S) gave better results after longer intervals after the vaccination of the guinea pigs (later than 60 days) than the allergen prepared with the aid of the TSenkovskii Vaccine No. 2 (M TS). On the whole, after all intervals after the vaccination (10 to 180 days) the use of the native anthrax allergen yielded positive results in 93 out of 105 tests (88.5%).

Table 2

Study of the Native Anthrax Allergen in Guinea Pigs
Subcutaneously Hyperimmunized with the
TSenkovskii Vaccine No. 2

<u>Kind of Allergen</u>	<u>Days after Vaccination</u>					<u>Total</u>
	<u>10-13</u>	<u>14-30</u>	<u>31-60</u>	<u>181-240</u>	<u>241 and more</u>	
M S	1/2	14/16	2/2	-	-	17/20
K S	2/2	10/10	-	4/4	2/3	18/19
Totals	3/4	24/26	2/2	4/4	2/3	35/39

Remark: See Table 1.

Repeating the investigations with native anthrax antigen on hyperimmunized guinea pigs (Table 2) results were obtained pointing to an increased activity of the allergic preparations manufactured from the edematous fluid of rabbits (18 positives in 19 tests, including those made even 8 months after the hyperimmunization). On the whole the allergic tests on hyperimmunized guinea pigs were positive after intervals from 10 to 241 or more days after hyperimmunization in 35 out of 39 instances (90%).

Series of tests with experimental tissue anthraxin showed its high biological activity, bringing about within 8 months after the hyperimmunization a large number of positive reactions (Table 3).

Table 3

Study of the Experimental Tissue Anthraxin in Guinea
Pigs Subcutaneously Hyperimmunized with the
TSenkovskii Vaccine No. 2

<u>Kind of Allergen</u>	<u>Days after Hyperimmunization</u>			<u>Total</u>
	<u>10-180</u>	<u>181-240</u>	<u>241 and more</u>	
M S	12/12	-	-	12/12
K S	4/4	5/5	2/3	11/12
Totals	16/16	5/5	2/3	23/24

Remark: See Table 1.

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For a final choice of the animals and the kind of vaccine necessary for the serial production of the tissue vaccine of substantial importance were tests purporting to verify the specificity of various experimental series of the native allergen and the tissue anthraxin made in normal (not immune, fresh) guinea pigs.

Table 4

Study of Experimental Series of the Native Anthrax
Allergen and the Tissue Anthraxin in Normal
(Not Immune) Guinea Pigs

<u>Kind of Allergen</u> <u>(Anthraxin)</u>	<u>No. of Animals</u> <u>Tested</u>	<u>Results</u>		<u>Index of Posi-</u> <u>tive Reactions</u>
		<u>Negative</u>	<u>Positive</u>	
Native - M TS	20	18	2	1:10
Native - M S	73	72	1	0.13: 10
Native - K S	58	58	0	0
Tissue - M S	12	12	0	0
Tissue - K S	26	26	0	0
Total	189			

These tests showed that under otherwise equal conditions the highest specificity was shown by the preparations obtained with the aid of STI Vaccine (Strain STI-1). Concerning the animals for the production a consideration of the figures (and consequently of the yield of the raw material) as well as the satisfactory indices of the specificity led us to the choice of rabbits. Thus the experimental tissue vaccine "K S" became the prototype of the serially produced tissue anthraxins.

The specificity of the native anthrax allergen has been studied formerly in series of guinea pigs immunized with BCG, brucellosis, tularemia and STI vaccines and has been found practically high (38).

Studies on the aerosol vaccination of guinea pigs with STI vaccine, conducted in another laboratory by N. I. Aleksandrov, N. E. Gefen, N. S. Garin and K. G. Gapochko showed that with the aid of the tissue anthraxin one could observe a definite activity of the allergic response in vaccinated animals and could evaluate the comparative immuno-allergic reactivity of the animals to the administration of dry and fluid anthrax aerosol vaccines.

Table 5

Study of Tissue Anthraxin in Guinea Pigs Immunized
with STI Aerosol Vaccine

<u>Doses of the Aerosol Vaccine (Million Live Spores and Kind of Vaccine</u>		<u>Days after Immunization</u>					<u>Total</u>
		<u>10</u>	<u>15</u>	<u>30</u>	<u>60</u>	<u>90</u>	
0.47	(dry)	3/6	-	2/5	1/4	2/5	8/20
2.12	(dry)	4/4	-	3/5	2/4	2/6	11/19
20.0	(dry)	4/4	-	10/15	2/4	2/6	18/29
0.088	(fluid)	-	5/5	3/3	-	-	8/8
0.128	(fluid)	-	5/5	3/3	-	-	8/8
Totals		11/14	10/10	21/3	5/12	6/17	53/84

Remark: See Table 1.

The native anthrax allergen, prepared according to our method, was studied by G. B. Dunaev in a group of guinea pigs immunized with experimental bacilli-free anthrax Vaccine No. 25.

Tested were 16 guinea pigs, 8 immunized with the bacilli-free vaccine and 8 not immunized animals (controls). The allergic tests were made 75 days after the vaccination. Results were as follows:

Table 6

Allergic Reactions in Guinea Pigs Immunized with
Bacilli-free Vaccine

<u>Group of Animals</u>	<u>Negative</u>	<u>Weakly Positive</u>	<u>Positive</u>	<u>Total</u>
Immunized	0	0	8	8
Controls	8	0	0	8

As shown by this table, one observed in the immunized animals clear results of the cutaneous allergic tests with native anthrax allergen in contrast to the control animals in which the results were negative.

In another series of tests serially produced preparations of tissue anthraxin were used. These products were tested on hyperimmune guinea pigs according to the "Temporary Instruction of the Control of the Anthrax Allergen 'Anthraxin' and Its Control Fluid," confirmed by the Tarasevich State Control Institute (GKI) on February 20, 1960.

Inserted below is a table summarizing the results of tests with 48 series of this preparation on guinea pigs, including the results of experiments conducted in April 1961 by a commission in the GKI

Table 7

Results of Tests with the Series 1-48 of Tissue Anthraxin on Hyperimmunized Guinea Pigs and Not Immunized Animals

	<u>Days after Hyperimmunization</u>				<u>Total</u>
	<u>4-13</u>	<u>14-30</u>	<u>31-60</u>	<u>61-90</u>	
Examined in the Moldavian IEMG:					
Vaccinated	32/36	77/78	33/33	20/20	162/167
Controls			0/169		
Examined by a commission in the GKI:					
Vaccinated	-	39/39	-	-	39/39
Controls			0/32		
Total (407 tests):					
Vaccinated	32/36	116/117	33/33	20/20	201/206
Percentage of positive reactions	89.0	99.1	100.0	100.0	97.5
Controls			0/201		

Remark: See Table 1.

The results of these test can be considered as fully satisfactory, permitting a high evaluation of the biological activity of the tissue anthraxin administered to hyperimmunized guinea pigs at different intervals after hyperimmunization.

In 1962 the issue of serially produced chemical anthraxin was started. Preliminary tests, made during the period of developing the technique of the serial production, showed, besides the possibility of a maximal simplification of the process of preparation, good biological properties of this new variant of anthraxin (see p. 97 [Russian text-Ed.] of the present publication).

These investigations as well as parallel control tests made with experimental samples of the chemical anthraxin in the Department of Specially Dangerous Infections of the Tarasevich GKI (R. A. Slatykov and V. R. Arkhipova) indicated the possibility of giving up the more tedious method of hyper-immunizing the test animals. It was found quite possible to test the biological activity of the series of the chemical anthraxin solely in initially (once) immunized animals vaccinated with the STI -1 strain.

Tests were made in separate groups of once vaccinated guinea pigs to which at different intervals after the immunization serially produced chemical anthraxin was administered.

Table 8

Results of kin Tests with Series of the Chemical Anthraxin in Guinea Pigs Immunized Once with STI Vaccine (10-40 million Spores) and in Not Immune Animals

<u>Groups of Animals</u>	<u>Days after Immunization</u>						
	<u>2</u>	<u>5</u>	<u>7</u>	<u>9</u>	<u>14-18</u>	<u>30</u>	<u>60</u>
Immunized	1/46	7/24	27/50	10/12	121/124	13/17	9/10
Percentage of positive reactions	2.2	29.2	54.0	83.3	97.5	76.4	90.0
Level of significance of the indices	<0.01	<0.01	>0.1	<0.01	<0.01	<0.01	<0.01
<u>Not immunized</u>	0/85						
Indices of positive reactions at a level of significance of 0.05	0-4%						

Remark: See Table 1

The results of these tests, showing a definite dynamic of the appearance of positive allergic reactions in relation to the intervals after immunization, are similar in principle to the data obtained in once vaccinated animals tested with the aid of the native anthrax allergen (Table 1).

Dependence of the indices of the skin tests upon the size of the immunizing dose of the vaccine. Separate groups of guinea pigs were immunized once with two doses of the STI vaccine - 40 million and 1 million spores. At different intervals after the immunization separate groups of each series were tests with serially produced chemical anthraxin of one and the same series.

The data of Table 9 (the indices of Group 1 are partly the same as in Table 8) are in favor of a substantial difference between the indices of positive anthraxin tests in relation to the vaccine doses. At the same time one can note a marked positive relation between the size of the vaccine dose and the intensity of the positive reactions to the anthraxin in each group and also in the dynamic of the process.

Relation between the results of skin tests with anthraxin preparations and the specific protection. It was of undoubted interest to determine the "biological trustworthiness" of positive skin tests with anthraxin (commercially produces series of tissue and chemical anthraxin) in guinea pigs vaccinated once subcutaneously with the STI vaccine or hyperimmunized additionally with the 2nd vaccine of TSenkovskii and infected at the time of reading the results with anthrax bacilli (Table 10).

Table 9

Indices of the Skin Tests with Chemical Anthraxin in Guinea Pigs Once Vaccinated with Different Doses of the Live STI Vaccine

<u>Group of Animals</u>	<u>2</u>	<u>5</u>	<u>Days after Vaccination</u>		<u>30</u>	<u>60</u>	<u>115</u>
			<u>7</u>	<u>14-18</u>			
Immunized with 40 million spores	1/46	7/24	27/50	88/91	13/17	9/10	7/18
Percentage of positive reactions	2.2	29.2	54.0	96.7	76.4	90.0	39.0
Level of significance of the indices	< 0.01	< 0.01	> 0.1	< 0.01	< 0.01	< 0.01	< 0.1

(Table continued...)

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<u>Group of Animals</u>	<u>2</u>	<u>5</u>	<u>Days After Vaccination</u> <u>7</u>	<u>14-18</u>	<u>30</u>	<u>60</u>	<u>115</u>
Immunized with 1 million spores	0/42	0/24	3/45	5/22	3/14	3/11	1/12
Percentage of positive reactions	0	0	6.7	22.7	21.5	27.2	8.3
Level of significance of the indices	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Analyzing the data of Table 10 one can note that out of 75 vaccinated animals tested positive skin-allergic tests were given by 69 (92 %)

Out of the positively reacting guinea pigs 61 or 88.5% survived control infection. Still, the data of the table show also that after the use of infecting doses ranging from 1 to 35 DCL the percentage of surviving positively reacting animals increased to 98 (1 death among 49 vaccinated and positively reacting animals), whereas after infection with 50-150 DCL out of 20 positively reacting animals only 13 survived. Thus one may conclude that the positive result of skin tests with anthraxin can serve as a criterium of the protection probably afforded to the animals after infection with doses of the anthrax bacillus within the range of the certainly lethal doses (DCL).

Table 10

Results of Skin Tests with Anthraxin and Survival
Rate of Guinea Pigs after Infection with
Lethal Doses of Anthrax Bacilli

<u>No. of Group</u>	<u>Times of Vaccination</u>	<u>Results of Anthraxin Tests</u> <u>Immunized</u>	<u>Controls</u>	<u>Infection Dose (DCL)</u>	<u>Survived</u> <u>Immunized</u>	<u>Controls</u>
1	Once	15/17	0/21	1	14	0
2	Hyperimmun.	7/8	0/8	5	7	0
3	Hyperimmun.	6/6	0/6	10	6	0
4	Hyperimmun.	6/7	0/7	20	6	0
5	Once	8/9	0/6	35	8	0
6	Hyperimmun.	7/7	0/6	35	7	0
7	Hyperimmun.	5/6	0/6	50	3	0
8	Hyperimmun.	12/12	0/13	75	10	0
9	Hyperimmun.	3/3	0/3	150	0	0

Remark: Numerator = Positively reacting; Denominator = No. tested.

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<u>Succumbed</u>		<u>Time of Death of Succumbed Animals (Hours)</u>				
<u>Immunized</u>	<u>Controls</u>	<u>36</u>	<u>37-48</u>	<u>49-72</u>	<u>73-96</u>	<u>5-7 Days</u>
1 2* + 1**	21	10	8	2	2* + 1	1**
2 1*	8	-	6	1*+ 2	-	-
3 0	6	4	2	-	-	-
4 1*	7	1*+7	-	-	-	-
5 1*	6	4	2	-	1*	-
6 0	6	6	-	-	-	-
7 1*+2**	6	6	1*+2**	-	-	-
8 2**	13	1**+13	-	-	1**	-
9 3**	3	3	-	1**	1**	1**

*) = Vaccinated animals giving negative results in anthraxin tests.

**) = Vaccinated animals giving positive results in anthraxin tests.

Table 11

Anthraxin Tests

<u>Groups of Animals</u>	<u>Negative or</u>		<u>Infection</u>		<u>Remarks</u>
	<u>Doubtful</u>	<u>Positive</u>	<u>Survived</u>	<u>Died</u>	
1. Streptomycin given 24 hours before vaccination	9	0	0	9	The positively reacting animals survived
2. Streptomycin given simultaneously with the vaccine	5	3	3	5	
3. Streptomycin given one day after the vaccination	3	6	6	3	
4. Streptomycin given 48 hours after the vaccination	2	7	8	1	Dto. All positively reacting and one negatively reacting guinea pigs survived
5. Streptomycin given 120 hours after the vaccination	2	7	8	1	One doubtfully reacting and all positively reacting animals survived
6. Vaccinated but not given streptomycin	2	7	8	1	All positively reacting and one doubtfully reacting animal survived
7. Not vaccinated	6	0	0	6	

Sensitizing properties of the anthrax allergens. Since the fundamental tests concerning the biological action of the anthrax preparations were made in guinea pigs, it was important to study their sensitizing properties in the case of this species of animals.

For this purpose series of tests were made in normal not immunized guinea pigs (for the detection of unspecific manifestations of skin allergy) and in immunized animals (for the presence of anaphylactic manifestations).

Influence of a specific antibiotic on the indices of the allergic reactions with anthraxin. Chlorhydric streptomycin was administered to guinea pigs one day before subcutaneous immunization with the STI vaccine, simultaneously with it and 24, 48 and 120 hours after the vaccination. One group was vaccinated without additional administration of streptomycin (control of the anthraxin), another was not immunized (control of the infecting dose).

On the 9th day after the administration of the STI vaccine skin tests with tissue anthraxin were made on all guinea pigs under test and 24 hours after reading of the results all animals were injected subcutaneously with 35 DCL of TSenkovskii Vaccine No. 2.

Results of these investigations (see Table 11) indicate that administration of streptomycin before or simultaneously with the vaccine leads to an interruption of the immunogenesis or its marked weakening. This is shown quite clearly by the anthraxin tests and the biological tests to ascertain the survival rate after lethal infection.

Streptomycin administration following the vaccination exerts a diminishing negative influence on the immunogenesis in proportion to the length of the interval after the immunization; this is also clearly shown by the results of the anthraxin tests and of the animal experiments to determine the resistance to infection with virulent anthrax bacilli.

AnthraxSelected Abstracts-IV/129Table 12

<u>Group</u>	<u>1st Series(Start)</u>				<u>2nd Series</u>		
	<u>No. of Guinea Pigs</u>	<u>Product Given</u>	<u>Result</u>	<u>Interval (Days)</u>	<u>Product Given</u>	<u>Result</u>	<u>Interval (Days)</u>
1	695	KS	-	10	...	...	11
	694	KS	-	10	MS	++++	11
	696	KS	-	10	MS	++++	11
	693	KS	-	10	...	...	11
	687	KS	-	10	MS	+++	11
	686	KS	-	10	..	...	11
	691	KS	-	10	MS	++	11
	698	KS	-	10	MS	++	11
	704	KS	-	10	MS	++	11
	701	KS	-	10	MS	+++	11
Total			0/10			7/7	

3rd Series

	<u>Product Given</u>	<u>Result</u>	<u>Product Given</u>	<u>Result</u>	<u>Product Given</u>	<u>Result</u>	<u>Interval (Days)</u>
695	...	...	...	...	...	...	12
694	...	...	...	...	...	...	12
696	KS	+++	NK	++	NS	-	12
693	...	...	...	...	...	...	12
687	KS	+++++	NK	+++	NS	-	12
686	...	...	...	...	...	...	12
691	...	...	...	...	...	...	12
698	KS	+++	NK	++	NS	-	12
704	KS	++++	NK	+++	NS	-	12
701	KS	++++	NK	++	NS	-	12
Total		5/5		5/5		0/5	

Table 12 cont.

<u>4th Series</u>							
		<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Product</u> <u>Given</u>	<u>Result</u>		
695		...	...	...	...		
694		...	...	...	...		
696		MS	+	NM	+		
693		...	...	...	...		
687		...	...	...	...		
686		MS	++	NM	-		
691		MS	+	NM	-		
698		...	...	...	...		
704		...	...	...	...		
701		MS	+	NM	+		
Total			4/4		2/4		

<u>1st Series (Start)</u>				<u>2nd Series</u>			
<u>Group</u>	<u>No. of</u> <u>Guinea</u> <u>Pigs</u>	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Interval</u> <u>(Days)</u>	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Interval</u> <u>(Days)</u>
2	703	MS	-	10	MS	-	11
	697	MS	-	10	MS	+++	11
	689	MS	-	10	MS	++	11
	699	MS	-	10	MS	+++	11
	692	MS	-	10	MS	-	11
	685	MS	-	10	MS	++	11
	702	MS	-	10	MS	-	11
	700	MS	-	10	MS	+	11
	688	MS	-	10	MS	++	11
	690	MS	-	10	MS	+++	11
Total			0/10			7/10	

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Table 12 cont.

3rd Series

	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Interval</u> <u>(Days)</u>
703	...	...	...	...	...	...	12
697	MS	++	NM	-	NS	-	12
689	MS	++	NM	-	NS	-	12
699	...	...	...	...	...	...	12
692	MS	-	NM	-	NS	-	12
685	...	...	...	...	...	...	12
702	...	...	...	...	...	...	12
700	...	...	...	...	...	...	12
688	MS	++	NM	-	NS	-	12
690	MS	+++	NM	-	NS	-	12
Total		4/5		0/5		0/5	

4th Series

	<u>Product</u> <u>Given</u>	<u>Result</u>	<u>Product</u> <u>Given</u>	<u>Result</u>
703	...	...	...	...
697	MS	-	NM	-
689	MS	+	NM	+
699	MS	++	NM	-
692	MS	-	NM	-
685	MS	-	NM	-
702	...	...	...	...
700	MS	±	NM	-
688	MS	+	NM	-
690	...	...	...	...
Total		3/7		1/7

Table 12 concluded

Explanation of symbols:

MS = Anthrax antigen from the edematous fluid of guinea pigs, inoculated with the strain STI-1

KS = Dto. from rabbits

NK = Normal rabbit serum

NM = Normal guinea pig serum

NS = Normal saline

Numerator = Number of positive local reactions

Denominator = Number of tests made

... = Animals not examined

- = Test negative.

The investigations of the allergizing properties (sensitizing the skin) of the native anthrax allergen were made in 20 normal guinea pigs, out of which 10 were given a native allergen of the type "KS" (prepared from the edematous fluid of rabbits inoculated with a vaccine prepared from Strain STI-1) and the other 10 a native allergen of the type "MS" (edematous fluid from guinea pigs inoculated with Strain STI-1). After 10 days both groups of animals were given the native allergen "MS"; after 11 further days the animals of the first group were given once more the allergen "KS", those of the second group the allergen "MS" and after the following 12 days the native allergen "MS" was used for both groups. The reactions were read 24 hours after the administration of the allergen and were evaluated according to a one to five plus scale (see the section Materials and Methods). At the same time in the 3rd and 4th series of tests the animals were given additionally intracutaneous injections of normal rabbit and guinea pig sera as well as normal saline in the same doses (0.1 ml) as the allergens. In all cases the repeated injections were made at new sites.

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13 ABSTRACT This is a straight translation of the book on Anthrax by E. N. Shliakhov of the Moldavian Scientific Research Institute of Epidemiology, Microbiology and Hygiene. It contains several articles on various aspects of the Immunology, Clinique and Diagnosis of Anthrax under two main headings after a brief synopsis of recent progress in understanding the disease: Anthraxin and Its Reactions, and Methods of Laboratory Diagnosis and Identification of the Causative Organisms. The illustrations in the book are also included here. <i>This volume contains the following articles: Is this a new Man; and Immune-biological</i> *Copies of this report without the Contract Number may also be obtained directly from the Institute of Russian Studies, Fordham University, Bronx, N. Y. 10458 at \$5.00 for the set of three. <i>Investigation with Preparation of the set in Allergen - Anthraxin.</i>			

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