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SELECTED ARTICLES FROM ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII

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DATA ON IMMUNIZATION OF HUMANS WITH LIVE
E STRAIN TYPHUS VACCINE

[Following is a translation of an article by N. N. Urakov, V. A. Pshenikov, V. P. Shchetinin and M. O. Tereshchenko in the Russian-language periodical Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, 1963, pages 40-45.]

The first observations in the Soviet Union on the immunization of humans with live typhus vaccine (E strain of *Rickettsia prowazekii*), published by Pshenichnov et al in 1959, confirmed and supplemented the data of American investigators (Everitt et al, 1954; Fox, 1956; Fox et al), and they also determined the desirability of further research in this direction. The present report contains additional data on vaccination of humans with the E strain.

We obtained the E strain of *Rickettsia prowazekii* from a laboratory headed by Zdrodovskiy, where it had been submitted to comprehensive experimental investigation (Zdrodovskiy, 1958; Zdrodovskiy et al, 1958; Kokorin, 1959; Yablonskaya, 1959). For immunization of humans we prepared and tested three series of live vaccine, in the form of lyophilized 1% suspension of chick embryo yolk sacs infected with strain E *Rickettsia*. Skim milk (series No 5) or skim milk with 10% saccharose (series No 8 and 9) was used as a drying medium. Otherwise the method of preparing the various series of vaccine was the same. The biological activity of these dry vaccine preparations with titration on chick embryos ranged from $10^{8.12}$ to $10^{8.84}$ EID₅₀, and with titration on guinea pigs (intraperitoneal injection) ranged from $10^{6.25}$ to $10^{7.25}$ EID₅₀ per gram of initial weight of the yolk sacs, i.e., it was about the same in all of the tested series. When tested on guinea pigs we were unable to detect pronounced clinical disease even with introduction of the vaccine in a 10^{-3} dilution, although complement-fixing antibodies were found in the animals' serum and the guinea-pigs, in most instances, were found to be immune to subsequent infection by virulent strains of *Rickettsia prowazekii*.

The subjects received subcutaneous injections of 0.25 milliliters in the subscapular region, and in some instances the vaccine was administered intranasally (it was imbedded in the mucous membrane of the nose). The inoculated individuals were observed clinically with daily temperature readings for a period of 18 to 20 days. Those who demonstrated a pronounced reaction to the vaccine were hospitalized and as a rule symptomatic treatment was instituted.

The first series of observations presented in this report pertains to an evaluation of the effectiveness of various dosages of live vaccine. For this purpose, two groups of people were inoculated with series No 5 vaccine. The first group, consisting of 24 people, were given subcutaneous injections in dilutions of 10^{-9} to 10^{-5} , the vaccine was administered intranasally in dilutions of 10^{-9} to 10^{-6} (four people were vaccinated). The results of inoculation of this group showed that all of the tested dosages of vaccine, including those that are infectious for chick embryos, were nonreactogenic, but neither did they elicit a sero-immune response in the inoculated individuals. In only one instance, where the vaccine had been administered subcutaneously in a 10^{-5} dilution was the production of a specific complement-fixing antibody titer of 1:32 observed.

For immunization of the other group vaccine of this same series was used in dilutions of 10^{-4} , 10^{-3} and 10^{-2} . A total of 17 individuals were inoculated, of them, 12 -- subcutaneously and five -- intranasally. Six of the people in this group had not been inoculated previously, while eleven, including all those that were vaccinated intranasally, had been immunized with killed typhoid vaccine. A number of individuals in this group demonstrated early and delayed reactions to administration of the vaccine. The early reaction was expressed objectively by a temperature rise of 37.2° -- 37.5° within the first 48--72 hours. There was no local reaction. A delayed reaction was observed in seven cases, also manifested by a one or two-day temperature elevation to 37.2° -- 37.5° , eleven to fourteen days after vaccination. In two cases, who had received subcutaneous injection of the vaccine in dilutions of 10^{-3} and 10^{-4} , the temperature rose to 38.5° -- 39° and remained at this level for one or two days. The individuals who had a fever demonstrated weakness and headaches. No essential difference was observed in the nature of reactions to intranasal and subcutaneous introduction of the vaccine. Previous immunization (one to two years earlier) with killed vaccine did not prevent the appearance of vaccine reactions.

Serological examination of the people in this group was performed 25 days after inoculation. In eleven out of the twelve cases, subcutaneous injection of the vaccine was accompanied by production of specific complement-fixing antibodies to *Rickettsia prowazekii* in titers of 1:4 to 1:128. In one case, in spite of a strong vaccine reaction, we were unable to demonstrate any antibodies even after 60 days. Apparently this is due to the effect of biomyacin which this female subject had taken on the first day of appearance of delayed reaction. Of the five individuals who had

been given the vaccine intranasally, in one case antibodies with a titer of 1:4 appeared in the serum, while in three cases the amount of antibodies doubled as compared to the initial quantity and attained a titer of 1:64 in two instances and 1:8 in one.

On the basis of a study of the effectiveness of various doses of strain E rickettsia the dosage for subsequent subcutaneous injection was selected: $10^{4.5}$ -- 10^5 EID₅₀ (0.25 milliliters of a 10^{-3} dilution). Series 8 and 9 vaccine was used for the immunization of 218 individuals. Of these 74 were inoculated with live vaccine for the first time, 25 had received an injection of 0.5 milliliters of killed deposited vaccine 10 to 30 days prior to administration of the live vaccine, 16 had been inoculated twice with killed vaccine one to twelve months prior to administration of live vaccine, and the rest were given the live vaccine one to five years after immunization with killed vaccine.

The greatest number of vaccine reactions (both early and delayed) was observed in individuals inoculated with E strain without prior administration of killed vaccine (table 1). In this group early reactions were observed in 12 cases, delayed ones -- in six (16.2 and 8.1%). At the same time, there was almost total absence of reaction to the vaccine in individuals inoculated with live vaccine 10 to 30 days after single injections of killed vaccine as well as one to twelve months after completion of a full course of immunization with killed vaccine. When the interval between administration of the killed vaccine and of the live one was greater (in our observations up to five years), the number of vaccine reactions again rose and represented a total of 9.7%. It must be noted that the delayed reaction was absent in the majority of those that had demonstrated a pronounced early reaction to the vaccine (15 out of 19 people), however in four cases both early and delayed reactions were observed.

The early reaction to introduction of series 8 and 9, as well as 5, live vaccine consisted of a temperature rise, weakness and headaches (table 2). In a number of cases there was a marked local reaction and lymphadenitis. The early reaction symptoms usually appeared 24 to 48 hours after the vaccination and persisted for one to two days. In one case only we observed a pyretic reaction that lasted for three days. Daily clinical examination failed to reveal any changes of the internal organs. Arterial pressure also remained within normal limits. Analysis of the blood and urine failed to demonstrate any deviation from the physiological normal. As a rule the number of early reactions was the same in older individuals as in young ones; in the age group under 40 years old the pyretic reaction during the early period was even somewhat more pronounced. In contrast to the quantity of early reactions, that of delayed reactions and their severity depended on the age of the subjects. While among those who were under 30 years of age there was only one case of delayed reaction, which constituted about 1% of the total number of those inoculated, among those under and over 40 years of age the number

of delayed reactions constituted 8.5 and 11.1% respectively. The main delayed reaction symptoms were: elevated temperature, headaches and muscle aches, excitability, hyperemia of the face and eyes. In six cases we observed elements of roseolus rash primarily on the lateral surfaces of the body and upper extremities. In two patients the rash was quite pronounced and individual petechiae were noted on the skin. The liver and spleen were not enlarged. No significant disturbances of the central nervous and cardiovascular systems were observed. Arterial pressure and the electrocardiogram remained within normal range. Blood examination and urinalysis failed to demonstrate any changes. The leukocyte count was near the upper limit of normal (7,500 to 8,000 per cubic millimeter). In only one case was slight leukopenia observed and in two -- accelerated erythrocyte sedimentation.

The delayed reaction appeared on the tenth to thirteenth days after administration of live vaccine. To curb it symptomatic treatment was usually instituted and in only two cases were antibiotics prescribed. The duration of inability to work due to delayed reactions ranged from two to five days in six patients, seven to eight days in two and eleven to seventeen days in four cases.

As an example we present a description of the most severe vaccine reactions in two elderly individuals.

1. In a male, 52 years of age, the reaction appeared on the tenth day after immunization; he complained of weakness, chills, headaches and muscular aches, loss of appetite and poor sleep. For five days a fever of 38.5 to 39.3° persisted. On the fourth day there appeared a roseolus rash on the skin of the upper extremities, the shoulder region and on the lateral surfaces of the body. On the body there were individual petechiae. The face and eyes were hyperemic. No disturbances of the internal organs were demonstrated. On the third day biomyacin treatment was instituted and found to be effective. The total duration of the reaction and subsequent treatment was 17 days.

2. A 52 year-old male came to the doctor complaining of severe headache, weakness, loss of appetite. The illness began on the tenth day after administration of E strain live vaccine. Examination revealed hyperemia of the face, injected scleral vessels, increased excitability. For three days the temperature fluctuated between 38 and 38.5°. On the thirddday a roseolus rash with individual petechiae appeared on the body. No changes of the internal organs were demonstrated. Biomyacin treatment was instituted on the third day. Twenty-four hours later the temperature returned to normal. The reaction lasted for eleven days.

The final part of the investigations pertained to characterizing the sero-immunity changes after administration of E strain live typhus vaccine. To supplement the previously published data on the complement-fixing antibody titers in vaccinated individuals determined 20 to 30 days after immunization (Pshenichnov et al, 1959) in the present work we determined the antibody level at later dates. We examined the serum of 69 individuals who had been inoculated with strain E without preliminary introduction of killed vaccine. The serum was obtained one to three months, three to twelve months, and one to three years after immunization. The number of positive sera in these groups represented, respectively, 18, 11 and 15, i.e. 86, 61 and 50% (table 3).

The complement-fixing antibody titer in the serum examined one to three months after inoculation reached 1:40 to 1:160, while in the serum examined at later dates it did not exceed 1:5 to 1:10 in most instances.

The 19 sera obtained one to two months after immunization were tested concurrently for complement fixation and Rickettsial agglutination. We were unable to demonstrate any essential differences as a result of testing the serum by these methods (table 4).

In conclusion, it must be noted that the data presented in this report on the reactogenic and immunogenic properties of live E strain typhus vaccine confirm the earlier findings (Pshenichnov et al) as well as those of American authors (Fox and others). Subcutaneous injection of the vaccine in a 10^{-3} dilution, i.e. $10^{4.5}$ to 10^5 EID₅₀, consistently elicited the production of specific complement-fixing antibodies to Rickettsia Prowazekia. On the whole the reaction to vaccination was benign, although in individual instances severe vaccine reactions of a typhoid nature were observed. The observed relationship between the severity of delayed reactions and the subjects' age is in complete agreement with the data of Fox et al (1955). At the same time the observations made earlier, to the effect that single injections of killed vaccine ten days prior to administration of live vaccine almost completely prevents the appearance of vaccine reactions, were confirmed. All of the foregoing indicates the desirability of broader study of this method of immunization.

The first observations on intranasal immunization of humans with live typhus vaccine indicate the possibility, in principle, of vaccination via the respiratory tract. However the effectivity of intranasal inoculation for humans was found to be considerably lower than for guinea-pigs (Urakov, 1961). This is perhaps due to the small amount of material (0.25 milliliters) that is administered; most of it remained on the mucous membrane of the upper respiratory tract and did not reach the proper receptive zones.

Conclusions

1. Live E strain vaccine, in doses of $10^{4.5}$ to 10^5 EID₅₀, was used

for the subcutaneous immunization of 230 people. Among the 74 individuals for whom this was the first immunization with live vaccine twelve (16.2%) demonstrated early vaccine reactions and six (8.1%) -- delayed reactions. The number and severity of delayed reactions depended essentially on the age of the individuals. In elderly people the delayed reactions were severe in a number of instances and caused inability to work for ten or more days.

2. Single injections of killed vaccine ten to thirty days prior to administration of live vaccine, as well as administration of live vaccine one to twelve months after two injections of killed vaccine prevented almost entirely the appearance of vaccine reactions. The results obtained demonstrate the advisability of broader study of the method of combined immunization with killed and live vaccine against typhus fever.

3. The first preliminary observations on intranasal immunization of humans with E strain indicate the possibility of introducing live vaccine through the respiratory tract, however the reactogenic and immunogenic properties of the vaccine with intranasal application requires further study.

4. The sensitivity of complement fixation and Rickettsial agglutination as methods of demonstrating specific antibodies in the serum in the earlier stages after immunization was found to be approximately the same.

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Table 1.

Reactogenic properties of live E strain typhus vaccine (series 8 and 9)

a) Группа привитых	b) Число привитых	c) Число реакций			
		d) ранних	e) отдаленных	f) всего	
g) Первично привитые живой вакциной	74	12 (16,2%)	6 (8,1%)	16 (21,6%)	
h) Привитые живой вакциной после предварительного введения убитой вакцины	i) через 10-30 дней . . .	25	1 (4%)	0	1 (4)
	j) через 1-12 месяцев . .	16	0	0	0
	k) спустя свыше 1 года . .	103	6 (5,8%)	6 (5,8%)	10 (9,7%)
f) Всего . . .	218	19 (8,7%)	12 (5,5%)	27 (12,4%)	

[Legend:] a) Immunized group b) Quantity immunized c) Number of reactions d) early e) delayed f) total g) first vaccinated with live vaccine h) Vaccinated with live vaccine after preliminary introduction of killed vaccine i) 10--30 days later j) 1--12 months later k) over a year later.

Table 2.

The nature of vaccine reactions to administration of live E strain typhus vaccine (series No 8 and 9)

Возраст (в годах)	Ранняя реакция						Отдаленная реакция												
	Число реакций	местная реакция	до 37,5°	37,6—38,5°	и выше	и выше	Число реакций	до 37,5°	37,6—38,5°	и выше	и выше	Головная боль	недомогание	возбуждение	мышечная боль	гиперемия лица и глаз	нарушение сна	смерть	
20—30	95	9 (9,5%)	3	2	2	2	1 (1,1%)	1	1	1	1	1	1	—	—	—	—	—	—
30—40	96	8 (8,4%)	5	2	6	0	8 (8,4%)	2	5	1	1	8	7	3	7	8	3	4	—
40—55	27	2 (7,4%)	1	0	1	0	3 (11,1%)	1	1	1	1	3	3	2	3	2	2	2	—
Всего...	218	19	9	5	9	2	12	4	6	2	2	12	11	5	10	10	5	6	—

[Legend:] a) age (in years) b) quantity immunized c) early reaction d) number of reactions e) local reaction f) fever g) up to 37.5 h) 38.6 and higher i) headache j) weakness k) lymphadenitis l) delayed reaction m) excitability n) muscular aches o) hyperemia of the face and eyes p) disturbed sleep q) rash r) total.

Table 3

Complement-fixing antibody level in the serum of individuals immunized with live E strain typhus vaccine (series No 8 and 9)

a) Срок обследования после прививки	b) Число сывороток	c) Титр антител						
		1:5	1:5	1:10	1:20	1:40	1:80	1:160
d) 1-3 месяца	21	3	4	1	4	5	3	1
e) 3-12 месяцев	18	7	3	5	3			
f) + 3 года	30	15	4	7	2	2		

[Legend:] a) Check-up term following immunization b) Number of sera c) antibody titer d) 1--3 months e) 3--12 months f) 1--3 years.

Table 4

Comparative characteristics of complement-fixing antibody and agglutinins titers in the serum of immunized individuals

a) Метод исследования	b) Число сывороток	c) Титр антител						
		1:5	1:5	1:10	1:20	1:40	1:80	1:160
d) Реакция связывания компонента	19	3	2	1	5	4	3	1
Реакция агглютинации риккетсий	19	1	2	7	8	1		

[Legend:] a) Method b) Number of sera c) Complement-fixation reaction d) Rickettsial agglutination reaction.

Comment

In setting up the complement fixation reaction, four units of antigen and 1.5 complete units of complement were used, fixation was effected at 4--6 for 18 hours. The Rickettsia agglutination reaction was tested by the method described by Zdrovskiy and Golinevich (1956).

EVALUATION OF THE IMMUNOGENIC PROPERTIES OF PENTA-TOXOID
ON EXPERIMENTAL ANIMALS OF VARIOUS SPECIES

[Following is a translation of an article by A. A. Vorob'yev and A. P. Labinskiy in the Russian-language periodical Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, 1963, pages 45-50.]

In the practice of testing the immunogenic properties of prophylactic bacterial preparations, including botulinus toxoids, experimental animals are used of the species that demonstrate best the quality of the particular preparation. The variety of methods used for this purpose (number of inoculations, antigen dosage, interval of determining immunity, variety of immunological tests) make it difficult to evaluate the results objectively.

The purpose of our work, which was conducted on albino mice, guinea-pigs and rabbits, was to determine experimentally the most suitable species of laboratory animals for the study of the immunogenic properties of botulinus penta-toxoid and its components so that a method might be standardized as much as possible. ([Note]: The characteristics of botulinus toxoids are described in the works of A. A. Vorob'yev et al, Zhurnal mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), Communications I, II, III and IV, 1960.)

The method of investigation consisted of a comparative study of the immunological effectiveness of penta-toxoid and its components used in equal antigenic (according to EC [Fixation unit?]) doses for inoculation of the animals. We selected the method of single immunization of the animals, inasmuch as this is the only method which permits demonstration of the immunizing activity of the antigen (Khalyapina and Roshkovskaya, 1950). With two inoculations the effect of immunization is determined to a greater extent by increased reactivity of the organism than by the quality of the antibody.

For the purpose of immunization the antigens were injected to

albino mice intraperitoneally, to guinea-pigs and rabbits -- subcutaneously. The immunogenicity of the preparations was evaluated in albino mice on the basis of the degree of immunity to toxins, in rabbits -- on the basis of antitoxin production, and in guinea-pigs -- both indices.

The degree of immunity in albino mice immunized with penta-toxoid and its components was tested on the sixteenth day after inoculation. Specific toxins were injected intravenously in 0.5 milliliters of saline NaCl. The mice were observed for four days and a record kept of those that died.

The data obtained (table 1) indicated that the resistance to specific toxins of albino mice that had been immunized with either penta- or mono-toxoids was essentially the same.

In rabbits, the antitoxin content was determined 15, 30 and 60 days after inoculation. The antitoxins were titrated in a serum mixture from each group of animals in whose blood no natural antitoxins had been found prior to inoculation. In view of the important role of an adsorbent for the production of botulinus antitoxins we used penta-toxoids and the corresponding mono-toxoids with equal amounts of adsorbent.

The rabbits were given small doses of penta- and mono-toxoids in order to evaluate the quality of the preparations by a more precise method.

The experiments showed (table 2) that in rabbits the antitoxin titers after immunization with penta-toxoid were considerably lower than after immunization with the same amounts of mono-toxoids with equal adsorbent content in the preparations.

In guinea-pigs, immunized with penta- and mono-toxoids, the antitoxin content of the blood was tested 25 days after inoculation and the immunity to specific toxins was tested 30 days after inoculation.

In guinea-pigs, immunized with penta-toxoids, the antitoxin titers were also lower than in the animals that had been immunized with mono-toxoids, particularly types B, C and E (table 3). However, in spite of the lower antitoxin titers, the guinea-pigs immunized with penta-toxoid demonstrated greater resistance to toxins than those immunized with mono-anatoxins. Analogous inconsistencies between antitoxin level in the blood and resistance to toxins were noted with immunization of guinea-pigs with mono-toxoids with increased adsorbent content in the inoculum dose (table 4).

Thus, the comparative study of the immunogenic properties of botulinus penta-toxoid and its components on three species of laboratory animals demonstrated the possibility of using them for the laboratory evaluation of these preparations. However, as specific immunity develops in response to the introduction of mono-toxoids and their complexes there

were several peculiarities related to both the animal species and to the immunity indicator used. Thus, in the experiments with guinea-pigs and rabbits, the immunogenic properties of penta-toxoid according to the antitoxin titer in the blood were found to be weaker than those of the corresponding mono-toxoids. And conversely, the resistance to botulinus toxins of guinea-pigs inoculated with penta-toxoid was appreciably higher than in those receiving equal doses of mono-toxoid. In the experiments with albino mice, there was the same immunological response to administration of both mono- and penta-toxoids.

Consequently the data we obtained demonstrate first of all the impossibility of judging accurately the interrelationships between antigens in polyvalent preparations on the basis of an immunological study on only one species of laboratory animals and using only one immunity indicator. Furthermore, from the described investigations it is quite obvious that the botulinus antitoxin titers of the blood do not reflect fully the true resistance of the organism to toxins. Consequently accurate conclusions as to the effectivity of the compound preparation as compared to its separate components can be made only on the basis of a study of the organism's resistance to the toxin (or to infection).

In view of the foregoing, we recommend that the immunogenic properties of botulinus penta-toxoid be tested on albino mice by the degree of immunity to the toxin. The advantages of this method, in addition to those mentioned above, are also its inexpensiveness and convenience.

Conclusions

1. The resistance to specific toxins of albino mice immunized with penta-toxoid and its components, was essentially the same.
2. The antitoxin titers in the blood of rabbits immunized with penta-toxoid were considerably lower than in animals immunized with mono-toxoids contained in the compound preparation.
3. In guinea-pigs, immunized with penta-toxoid, the antitoxin titer in the blood was lower than in animals immunized with mono-toxoids, however the resistance to specific toxins of the guinea-pigs immunized with penta-toxoid was higher than that of the animals immunized with mono-toxoids.
4. It is desirable to check the immunogenic properties of botulinus penta-toxoid on albino mice, since they react the same to both mono-toxoids and penta-toxoid.

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Table 1.

Comparative immunogenic properties of penta- and mono-toxoids in experiments on albino mice

Тип антигена	Иммуногенность пентаанатоксинов				Иммуногенность моноанатоксинов			
	№ образца	количество ЕС при иммунизации	Dim токсина при заражении	результат	№ образца	количество ЕС при иммунизации	Dim токсина при заражении	результат
A	21	20	25	9/1	114	20	25	8/2
B		20	50	9/1	35	20	50	7/3
C		20	50	8/2	79	20	50	8/2
D		20	10	8/2	187	20	10	8/2
E		5	25	9/1	166	5	25	8/2
A	25	20	25	9/1	108	20	25	9/1
B		20	50	6/4	93	20	50	3/7
C		20	50	8/2	223	20	50	8/2
D		20	10	5/5	184	20	10	8/2
E		5	25	6/4	172	5	25	7/3

[Legend:] a) Type of antigen b) immunogenic properties of penta-toxoids c) Sample No d) quantity of EC with immunization e) Toxin Dim [minimum lethal dosage] f) results g) immunogenic properties of mono-toxoids.

Note: The numerator = number of survived animals, and the denominator = number of animals that died.

Table 2.

Comparative immunogenic properties of penta-toxoid and monotoxoids in experiments on rabbits

a) Тип антигена	b) Иммуногенность пентаанатоксина				г) Иммуногенность моноанатоксина							
	c) № образца	d) в прививочной дозе		e) № групп кроликов	ж) содержание антитоксина (в АЕ/мл) в разные дни		z) № образца	и) в прививочной дозе		k) № групп кроликов	л) содержание антитоксина (в АЕ/мл) в разные дни	
		EC	Al(OH) ₃ (в мг)		15-й	30-й		60-й	EC		Al(OH) ₃ (в мг)	15-й
A	21	100	2	1	0,05	<0,05	114	100	2	<0,3	>3<51	
B		50			<0,05	<1	35	50	2	>0,5<1	<3	>1<31
C		50			<0,1	<0,5	79	50	2	0,3	>5<10	>5<10
D		50			<0,05	>0,5<1	187	50	2	>1<3	3	>5<10
E		25			0,05	0,3	166	25	2	>1<3	3	>3<5
A	21	20		7	<0,05	<0,05	114	20	0,4	<0,05	<0,1	
B		10	0,4		<0,05	<0,1	35	10	0,4	<0,1	0,5	—
C		10			<0,05	<0,3	79	10	0,4	0,05	<0,1	—
D		10			<0,05	<0,3	187	10	0,4	<0,05	<0,1	—
E		5			0,05	0,5	166	5	0,4	<0,05	>1<3	—

[Legend:] a) Type of antigen b) immunogenic properties of penta-toxoid c) Sample No d) inoculation dosage e) (in milligrams) f) rabbit group number g) anti-toxin content (in AE/milliliter) at various intervals in days h) immunogenic properties of mono-toxoids.

Note: There are five rabbits in each group.

Table 3

Comparative immunogenic properties of penta-toxoid and mono-toxoids in experiments on guinea-pigs

Вид препарата	Тип антигена	В прививочной дозе			Содержание антитоксинов AE/мл (среднее)	Напряженность иммунитета				
		ЕС	Al(OH) ₃ (в мг)	число животных		доза токсина (в Дпт)	число животных	из них:		
								здоровы	больны	погибли
Пента-анатоксин	A	100	2-3,8	75	0,3 (0,1-0,4)	2000 4000 8000	1 4 4	1 4 4	— — —	— — —
	B	50			0,4 (0,3-0,5)	1000 2000 4000	4 5 3	2 3 2	1 1 1	1 1 —
	C	50			0,8 (0,5-1)	1000 2000 4000	4 4 2	4 2 2	— 2 —	— — —
	D	50			0,07 (0,05-0,1)	25 50 100	2 6 6	1 4 4	1 2 2	— — —
	E	25			0,08 (0,05-0,1)	250 500 1000	1 3 5	1 3 5	— — —	— — —
Моно-анатоксин	A	100	0,5	15	0,4 (0,3-0,5)	2000 4000 8000	5 3 2	5 3 2	— — —	— — —
	B	50	0,14-0,23	15	1,1 (0,5-2)	1000 2000 4000	4 3 1	— — —	2 1 1	2 2 —
	C	50	0,22-0,25	15	1,6 (1-3)	1000 2000 4000	5 3 2	2 — —	1 1 1	2 2 1
	D	50	0,22-0,32	15	0,06 (0,05-0,1)	25 50 100	3 4 2	2 2 —	1 2 2	— — —
	E	25	0,75-2,8	15	1,16 (0,5-2)	250 500 1000	3 4 2	— — —	1 2 —	2 2 2

[Legend:] a) form of preparation b) type of antigen c) inoculum dosage d) antitoxin content e) degree of immunity f) (in milligrams) g) number of animals h) AE/milliliter (average) i) toxin dose (in Dim) j) healthy k) sick l) died m) penta-toxoid n) mono-toxoids.

Note: The minimal and maximal antitoxin titers are given in parentheses.

Table 4

Comparative immunogenic properties of penta-toxoid and mono-toxoids with increased adsorbent content in the latter

Вид препарата (m)	Тип антигена (b)	В прививочной дозе (c)		Содержание антитоксина (d)		Напряженность иммунитета (e)						
		ЕС	Al(OH) ₃ (в мг) (f)	число свинок (g)	АЕ/мл (h)	Доза токсина (в Длм) (i)	число свинок (j)	из них:				
								здоровы (k)	больны (l)	погибли (l)		
Пентаанатоксин (m)	A	100	2	25	<0,1	1 000	2	2				
						2 000	2	2				
	B	50						0,3	1 000	2	2	
						2 000	2	2				
	C	50						0,5	500	1	1	
					1 000	2	2					
					2 000	2	2					
	D	50			<0,05	25	1		1			
						50	2		2			
						100	2		2			
	E	25			0,05	250	1	1				
						500	1	1				
						1 000	1	1				
Моноанатоксины (n)	A	100	10	5	0,5	1 000	2	2				
						2 000	2	2				
	B	50	10	5	<3	1 000	2		2			
						2 000	2		2			
						4 000	2		2			
	C	50	10	5	<3	1 000	1		1			
						2 000	2		2			
						4 000	2		2			
	D	50	10	5	<1	10	1	1				
						25	2	2				
						50	2		2			
	E	25	10	5	1	250	1		1			
						500	2		2			
						1 000	1		1			

[Legend:] a) form of preparation b) type of antigen c) inoculum dosage d) antitoxin content e) degree of immunity f) (in milligrams) g) number of guinea-pigs h) АЕ/milliliter i) toxin dose (in Dlm) j) healthy k) sick l) died m) pentatoxoid n) mono-toxoids.

THE ROLE OF REGIONAL LYMPH NODES IN THE MECHANISM
OF POST-INOCULATION PRODUCTION OF IMMUNITY

[Following is a translation of an article by B. A. Godovanny, from the Moscow Institute of Vaccines and Sera imeni Mechnikov, in the Russian-language periodical Zhurnal mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, 1963, pages 51-55.]

The question of the locus of production of antibodies in an immune organism is, at the present level of development of immunology, an extremely important problem related with the study of the mechanism of production of post-inoculation immunity. As we know at the present time, through a large number of observations it has been established that one of the primary sites of production of antibodies in the organism is lymphoid tissue (McMaster, 1953; Harris and Harris, 1945-1960; Fagraeus, 1948; Zdrodovskiy, 1956-1962; Fontalin, 1959, and others).

Both the earlier investigations (Huebschmann, 1913; Bing and Plum, 1937) and the most recent research (Coons et al, 1955-1960; Igonin, 1962, and others) have established the most likely producers of antibodies -- plasma cells whose origin and purpose remain a subject of discussion. The question of the nature of the reactions to introduction of antigen of individual portions of the lymphatic system is far from being fully resolved. It is known that of all of the forms of lymphoid tissue accumulation in the organism the production of antibodies in response to immunization is first of all and most illustratively demonstrated in the lymph nodes that are regional in relation to the site of introduction of the antigen (McMaster and Hudack, 1935; Sagalovich, 1954; Azetskaya-Matsiyevskaya, 1948; Meshalova and Amirov, 1962, and others). However, the reaction of regional nodes to the introduction of various antigens depends to a considerable extent on the physical condition of the latter. It has been shown (Stavtskiy, 1954; Gurvich and Shumakova, 1960) that with immunization with soluble antigens (diphtheria or tetanus toxoid) the reaction is primarily observed in the regional lymph node, while the reaction to administration of corpuscular antigen is generalized, extending over the

entire system of lymphoid organs, the role of the draining node being secondary in this case. Lymphoid tissue reaction to administration of soluble antigens has been investigated thoroughly enough. At the same time, the question of the reaction to introduction of corpuscular antigen of the various parts of the lymphatic system upon primary immunization is covered only by isolated works, while research on this reaction under conditions of re-inoculation is almost non-existent. Yet the existence in practice of a large quantity of corpuscular vaccines demands that the mechanism of production of post-inoculation immunity following immunization with corpuscular antigens be investigated.

The purpose of the present work was to determine the extent and duration of regional lymph node participation in the production of humoral post-inoculation immunity after primary immunization with corpuscular bacterial antigen.

For the study of such questions the methods of extirpation of the regional node or comparative study of antibody titer in the lymph flowing to and from the node are used the most often. Some authors (Shumakova, 1960; Stepin, 1960) used in their investigations the method of determination of antibody titer in the blood serum in relation to the quantity of regional lymph nodes involved in immunogenesis. This method, which we have also used, has the advantage that it obviates the necessity of surgical intervention and of the concurrent "surgery control," and particularly that it eliminates the post-operative changes in the animal's organism.

Governed by the fact that every pattern can be demonstrated with particular clarity under certain conditions, we determined first of all the vaccine dosage producing the most marked "regional effect," i.e. accumulation of antibodies primarily in the regional node with minimal response by the remote portions of the lymphatic system. Such a dosage (one billion bacterial cells) was found by a purely trial-and-error method in preliminary experiments.

The first series of experiments was conducted on 28 chinchilla rabbits weighing 2.4 to 3 kilograms. The animals were divided into two equal groups, selected in such a manner that the average normal antibody titers, determined prior to inoculation, were the same in both groups. We immunized the animals with single-dose subcutaneous injections of heated typhoid vaccine, prepared from a Ty₂ No 4446 strain. We used vaccine from a homologous strain, killed by heat, as a diagnosticum. The rabbits in the first group (in this instance the control group) were inoculated with one billion bacterial cells in the external surface of the lower third of the right hind leg, the animals in the second group (experimental) were given one-quarter the full dose (i.e. 250 million bacteria) in the external surface of both hind and forelegs. Blood for testing was taken on the fifth, eighth, twelfth, fifteenth, twenty-fifth, forty-fifth and sixtieth day from the time of the first inoculation.

It was established (figure 1) that during the first twelve days following immunization there was a very distinct difference in the dynamics of accumulation of agglutinins in the serum of the experimental and control rabbits: at the height of antibody production (eight day after immunization) the average titer was two and a half times higher in the former group than in the latter. Starting with the fifteenth post-inoculation day and up to the sixtieth day, the agglutinin titers became equalized, however they remained higher in the blood serum of the experimental animals for the duration of the follow-up period. The same pattern was demonstrated upon analysis of the agglutinin titer dynamics in the serum of rabbits taken separately. The accuracy of the results of this experiment was confirmed by variation statistical processing (probability of null hypothesis $P < 0.05$).

The presented data can be explained from the standpoint of simultaneous participation of four groups of regional nodes in the production of antibodies. However, other explanations are also possible, first of all from the position of nonspecific stimulation of several areas of the skin surface, as well as reflex from interoceptors in the stroma of the lymph nodes. It is known that these factors, while they do not actually cause the production of antibodies in the organism, regulate antibody production and can be instrumental in elevating the antibody titer of blood serum.

In order to show that in the described experiments these factors did not play a decisive role, a second series of experiments was conducted on 26 rabbits of the same breed and weight. The animals were divided into four groups: one experimental and three controls. The ten rabbits in the first group were given heated typhoid vaccine, in the amount of one billion bacterial cells, in the right posterior extremity; ten rabbits in the second (actually the experimental group) were immunized in all four extremities with doses of 250 million bacterial cells; the three rabbits in the third group were given the full dose of vaccine divided into four equal portions of 250 million bacterial cells, introduced simultaneously into four adjacent points on the external surface of the right hind leg, and the three rabbits of the fourth group were given one billion bacterial cells in the right hind extremity and simultaneously 0.25 milliliters of saline was injected into the other three extremities. Blood was taken for testing on the fifth post-inoculation day. The average antibody titers in the serum of experimental and control animals were the same: for the first group -- 1:230, for the second (experimental) -- 1:980, for the third -- 1:300, for the fourth group -- 1:370 (figure 2). It may be considered that the data obtained in this series of experiments permit exclusion of nonspecific stimulation of skin and lymph node receptors as the primary cause of the higher antibody titer in the serum of the rabbits inoculated in all four extremities.

Thus, the results of the two described series of experiments demonstrate that there is a relationship between the quantity of regional lymph

nodes involved in the immunological process and the antibody titers in the blood serum. However it was interesting to investigate the relationship between the extent of lymph tissue involvement in immunogenesis and the degree of humoral immunity as reflected by other indicators of immunity, and first of all by the preventive properties of blood serum. For this purpose a third series of experiments was conducted: eight rabbits were inoculated with a single subcutaneous injection of heated typhoid vaccine, prepared from Ty₂ No 4446 strain; four rabbits (the control group) were vaccinated on the external surface of the lower third of the right hind leg with one billion bacterial cells; four other animals -- in the external surface of both hind and forelegs with 250 million bacterial cells each. Blood was taken for testing prior to immunization as well as on the fifth, tenth, fifteenth and fiftieth days post-inoculation. After settling and freezing, the serum was lyophilized in a Dolinov vacuum desiccating apparatus for 68 hours. All subsequent work was done on desiccated serum diluted with saline on the day of the experiment. The serum tests for both groups were done simultaneously. Albino mice, weight 14--16 grams were used in the experiments. Serum dilutions of 1:3, 1:9, 1:27 and 1:81 were prepared and injected intraperitoneally, in doses of 0.4 milliliter, to four mice. The mice were also inoculated intraperitoneally 24 hours after injection of serum with a culture of Ty₂ 1203 in 0.4% agar. The dosage which we used, one million bacterial cells (0.2 milliliter of bacterial mass suspension in saline and 0.8 milliliter of agar) equalled on an average 6 LD₅₀ of culture. Every stage of the investigation was accompanied by a test of the virulence of the culture and determination of the preventive properties of the standard serum administered in the experiment. A record was kept of the death rate for a period of three days. The preventive activity of the blood serum was expressed in PD₅₀, i.e. minimal dose of serum (in milliliters) that protected 50% of the mice from the given culture dose. The results were processed statistically ($P < 0.01$).

The follow-up observations showed that in the rabbits immunized in one extremity the preventive activity of the serum remained essentially the same as the pre-immunization level (only on the tenth day did it demonstrate an 0.3% rise) while the preventive activity of the serum of rabbits inoculated in all four extremities grew to a considerably higher degree. This difference in changes in preventive properties of the serum of rabbits in different groups is illustrated on figure 3, where the degree of change in preventive activity of the serum is shown as compared to the initial level. The preventive properties grew in a larger measure in the early postinoculation stages then gradually began to drop to the initial level. It is interesting to note that on the fiftieth day only the serum of the experimental group of rabbits still demonstrated preventive activity.

The presented results of the three series of experiments warrant the conclusion as to the considerable role of regional lymphoid formations in the production of post-inoculation immunity with corpuscular

antigen inoculation. Apparently, we must also broaden the concept of the possibility of directed regulation of humoral immunity which formerly (Zdrovskiy, 1960) was recognized only for dissolved antigens. This view can also be confirmed by our research on antibody production and cell reactions in the lymph nodes proper, which will be shown in subsequent reports.

Conclusions

1. After primary subcutaneous immunization with corpuscular typhoid vaccine (one billion bacterial cells) agglutinin titers and preventive activity of blood serum was directly proportional to the quantity of lymph nodes stimulated by the antigen.

2. The data obtained indicate the predominant role of the regional lymph nodes in the production of humoral immunity following immunization with corpuscular antigen.

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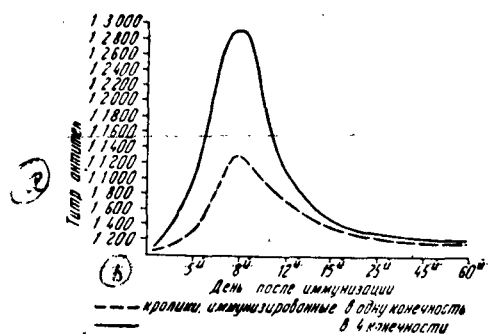
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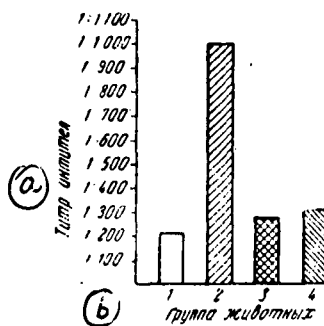
Figure 1.



Dynamics of agglutinin accumulation
in the blood serum of rabbits in
relation to the inoculation method

[Legend:] a) antibody titer b) day, post-inoculation
--- rabbits inoculated in one extremity — rabbits inocu-
lated in four extremities.

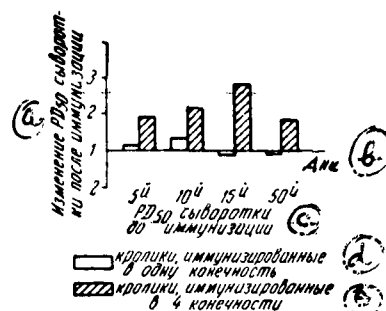
Figure 2.



Average antibody titers of blood serum in
experimental and control rabbits in rela-
tion to method of primary immunization

[Legend:] a) antibody titer b) group of animals.

Figure 3.



Comparative data on the change in average serum PD₅₀ following immunization of rabbits in one or four extremities (dispensed dose -- 6 LD₅₀ of culture).

[Legend:] a) change in serum PD₅₀ following immunization
 b) days c) serum PD₅₀ prior to immunization d) rabbits immunized in one extremity e) rabbits immunized in four extremities.

CHANGES IN FRACTIONAL SERUM GLOBULIN AND LYMPH NODE
EXTRACT CONTENT UNDER THE EFFECT OF
IMMUNIZATION AND CORTISONE

Communication I

[Following is a translation of an article by A. N. Meshalova, A. V. Beylinson, K. L. Shakhanina and I. B. Fryazinova, from the Moscow Institute of Vaccines and Sera imeni Mechnikova and the Institute of Epidemiology and Microbiology imeni Gamalei of the Academy of Medical Sciences USSR, in the Russian-language periodical Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow Vol 40, No 7, 1963, pages 55-60.]

The cellulo-humoral factors of immunogenesis and the effect of steroid hormones on these processes have been studied by a number of investigators (Gaydamovich and Solov'yev, 1946; Gurvich and Shumakova, 1957; Fontalin, 1957 and 1960; Stavitsky, 1954 and 1957; Thompson, 1957; White et al, 1955).

There are data on the]changes in serum globulin and globulin fractions under the effect of immunization and cortisone (Mikhnev et al, 1958; Yegorova and Savina, 1958; Lebedeva, 1956; Blecher and White, 1958; Weimann and Hammerl, 1953; Aschkenasy, 1957, and others).

Biochemical changes in the lymphatic nodes and spleen have been established as an index of increased protein synthesis under the effect of antigen (S. Harris and T. Harris, 1950).

Bosşak et al (1955) observed an increase in alpha₂-globulin under the effect of cortisone in the serum of normal dogs. Aschkenasy (1957) noted an increase in albumins in normal rats due to decrease in gamma-globulin, reduced weight of the spleen and enlargement of the liver. He concludes that cortisone causes a decrease in gamma-globulins and elicits destruction of lymph nodes.

Halpern et al (1957) also observed increase in alpha-globulin and decrease in gamma-globulin content with destruction of lymph nodes following administration of cortisone to young rats.

Weinmann and Hammerl (1953) described a reduction in alpha- and gamma globulin and increase in beta-globulin after injecting cortisone to immunized rabbits, as compared to immunized rabbits that were not given cortisone.

The purpose the present investigation was to study the biochemical changes of blood serum proteins, lymph node extracts and spleen of immunized animals following administration of cortisone.

Two series of experiments were conducted.

In the first series 19 rabbits, weighing two to three kilograms, were used. Nine of these rabbits were given intramuscular injections of cortisone, 10 milligrams per kilogram of body weight, for three consecutive days, and the blood serum and organ extracts were examined on the third, seventh, tenth, fifteenth and twentieth day after injection of cortisone. The remaining ten rabbits were not given any cortisone; they served as controls.

In all of the normal rabbits the fractional protein content was determined by the electrophoretic motility in the blood serum, lymph nodes and spleen, and concurrently the plasmocytic reaction was determined on impression preparations.

The obtained data served as the reference criteria to be compared with the results of analagous tests in the experimental animals.

The rabbits were anesthetized with urethane for removal of the organs. The sterilely removed lymph nodes -- subpatallar, inguinal, axillary, mesentery -- and spleen were weighed on a torsion balance, shredded in a manual homogenizer, diluted to 1:10 by weight, and centrifuged.

The Kjeldahl method was used to determine the total protein in the obtained centrifugate; then it was desiccated by lyophilization and used for biochemical and seralegical analysis. The plasmocytic reaction was tested on the impression preparations made from the lymph nodes and spleen.

The fractional protein content was determined by means of electrophoresis on paper with an LKV instrument in a veronal buffer (pH= 8.6, ion intensity -- 0.05). Prior to applying the serum to paper it was diluted in equal parts with saline, and the organ extracts -- with one-fifth the initial volume of saline. The electrophoregrams were stained with amid-black, cleared with vaseline oil and interpreted with a densitometer.

The cited tests demonstrated a significant difference between the electrophoregrams of serum and organ extracts.

While in the blood serum there was a distinct division of globulin into gamma-, beta- and alpha-fractions, in the organ extracts this division was not distinct, yet a considerable fraction is observed with a low electrophoretic motility. On the electrophoregram of spleen extracts the maximal protein content was observed in the form of a distinct band between beta- and gamma-globulin.

Thus the electrophoretic motility of serum proteins and organ extracts of normal animals was not the same.

Weinmann and Hammerl (1953) indicate that in rabbits immunized with the use of cortizone there is a decrease in gamma globulin and increase in beta-globulin content in the blood serum (table 1) as compared with the control animals.

Cortisone was administered daily, intramuscularly, in doses of 12 milligrams per kilogram of weight.

The data we obtained demonstrate the reduction in the gamma-fraction of globulin and increase in beta-fractions and blood serum albumin of normal rabbits under the effect of cortisone. In the organ extracts there is increase in albumin and an inappreciable change in globulin fraction (table 2).

The plasmocytic reactions of normal and cortisone-treated animals reveal that there is total disappearance of plasma cells from the lymph nodes and spleen under the effect of cortisone. The quantity of plasmocytic cells (lymphocytes and lymphoblasts) in normal rabbits ranges from 10 to 16--17 in 50 fields of vision; after administration of cortisone, on the third and seventh day, no plasma cells are found in the lymph nodes and spleen; on the tenth and fifteenth days isolated cells are observed.

Thus, our experiments permit us to conclude that the effects of cortisone on normal rabbits are a reduction of the gamma-globulin fraction, increase of the beta-fraction and albumins, inhibition of plasmocytic reaction.

In the next series of experiments we used 34 rabbits, weighing 1.5 to 2 kilograms, which were divided into two groups, 17 rabbits in each. The first group was immunized twice intravenously with typhoid vaccine, 400-800 million and one billion bacterial cells, seven days apart. The other group was immunized in the same manner, but five days prior to immunization the rabbits were given intramuscular injections of cortisone, 20 milligrams per kilogram of weight. Tests were run on the third, seventh, twelfth, fifteenth, twentieth and twenty-fifth days after the last inoculation.

We present the data on electrophoretic motility of serum protein fractions and organ extracts under the effect of immunization and cortisone (tables 3 and 4). The organ extract globulin was not broken down into fractions.

The data demonstrate the change in serum globulin of immunized animals due to increase in gamma-globulin fraction. In the lymph node extracts there is an increase from 0.42 to 0.57 percent in protein by the end of the observation period; and the globulin content is increased by 22% (from 0.36 to 0.44%). No particular changes in fractional composition of protein in the spleen extracts were noted.

The pre-immunization introduction of cortisone also elicited an increase in protein content, but this was the result of a large amount of albumin and not increase in gamma-globulin content. During the first post-immunization days a lower gamma-globulin content was observed with gradual increase towards the fifteenth--twentyfifth day from the time of the last inoculation.

Under the effect of cortisone the lymph node extracts demonstrated increase in total protein due to increase in albumins, and the quantity of globulin was almost unchanged (table 5).

No changes in the fractional composition of protein were observed in the spleen extracts.

On the basis of the foregoing it may be considered that cortisone administered to immunized animals elicits globulin synthesis disturbances in the lymph nodes which, apparently may produce changes in antibody synthesis.

The comparative data on gamma-globulin content in the serum of immunized and cortisone-treated animals demonstrate that there is a considerable difference in gamma-globulin content in the various groups of rabbits. The antibody level dropped under the influence of cortisone, both in the blood serum and in the lymph node extracts (figure 1).

Thus, the investigations we conducted indicate that there is a considerable change in protein composition under the effect of specific antigen and cortisone.

Conclusions

1. The electrophoretic motility of blood serum proteins and organ extracts of normal rabbits varies. In contrast to serum, there is indistinct globulin fraction division in the organ extracts. At the same time we observe the presence of a fraction with low electrophoretic motility.

2. Administration of cortisone to normal rabbits causes reduction in the gamma-fraction of globulin and increases the beta-fraction and albumins in blood serum. The albumin content increases in the organ extracts.

3. Following immunization with typhoid vaccine there is a rise in globulin content in the blood serum in the form of increased gamma-fraction content. In the lymph node extracts there is an increase in protein content owing to an increase in globulin.

4. Pre-immunization administration of cortisone to rabbits resulted in changes in protein content in the lymph node extracts due to an increase in albumin content and impaired globulin synthesis.

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Table 1.

Исследованный материал	Фракционный состав белка (в %)			
	γ	β	α	альбу-мин
Сыворотка нормальных кроликов	15,9	14,4	10,2	59,3
Сыворотка иммунизированных кроликов	22	13,1	10,5	53,1
Сыворотка кроликов, иммунизированных с применением кортизона	13,3	19,9	8,8	58,3

Change in fractional serum protein content in immunized rabbits as compared to the serum of normal rabbits (according to Weinmann and Hammerl)

[Legend:] a) Material examined b) Fractional protein content (in %) c) albumin d) Normal rabbit serum e) Immunized rabbit serum f) Immunized, cortisone-treated rabbit serum.

Table 2.

Условия опыта	Исследуемый материал	Общий белок (в %)	Фракционный состав белка (в %)				
			глобулины (в %)	γ	β	α альбу-мин	
Нормальная	Сыворотка	6,29	49,6	20,2	13,9	15,6	50,4
	Экстракт селезенки	0,71	94,8	—	—	—	5,2
	Экстракт лимфатических узлов	0,56	88,8	—	—	—	11,2
После воздействия кортизоном	Сыворотка	5,71	45,1	17,7	15,2	12,2	54,9
	Экстракт селезенки	0,69	93,1	—	—	—	6,9
	Экстракт лимфатических узлов	0,51	87	—	—	—	13

Change in fractional composition of blood serum and organ extracts under the effect of cortisone in normal rabbits

[Legend:] a) Experimental conditions b) material examined c) total protein (g) d) Fractional protein content (g) e) globulin (g) f) albumin g) normal h) serum i) spleen extract j) lymph node extract k) after administration of cortisone.

Table 3.

3 Изученный материал	4 Наименование фракций	5 Обработка кроликов	6 Белок (в %)	7 Содержание белка (в % к общему белку) по дням иммунизации					
				3-й	7-й	12-й	15-й	20-й	25-й
8 Сыворотка	Общий белок	Иммунизация	6,29	6,26		5,8	6,14	5,94	6,11
		Кортизон		5,61	6,50	7,26	5,48	4,93	
		Иммунизация + кортизон		5,93		6,64	7,18	6,63	5,65
	γ	Иммунизация	20,2	19,7		22,8	25,3	25,6	25,7
		Кортизон		16,3	14,5	14,7	14,3	20,7	
		Иммунизация + кортизон		18,8		19,5	20,3	22,2	22
	β	Иммунизация	13,9	17		12,5	15,3	13,7	11,7
		Кортизон		15,5	15,8	19,1	17,8	15,5	
		Иммунизация + кортизон		17,2		14,4	11,9	11,3	11,5
	α	Иммунизация	15,6	16,7		10,9	14,4	13,3	13,9
		Кортизон		19,1	19,6	12,1	19,4	17,3	
		Иммунизация + кортизон		18,1		13,4	13,2	12,9	14,6
9 Альбумин	Иммунизация	50,4	46,7		53,9	45,4	47,4	42,6	
	Кортизон		48,9	50,2	54,1	48,7	46,9		
	Иммунизация + кортизон		45,7		52,3	54,7	53,8	51,9	

Change in fractional protein content of blood serum in rabbits under the effect of cortisone and immunization

[Legend:] a) Material examined b) fraction name c) rabbits treated with: d) protein (%) e) protein content (in % of total protein) on the following post-immunization days g) total protein h) immunization i) cortisone j) immunization + cortisone k) albumin.

Table 4.

a) Изученный материал	b) Наименование фракции	c) Обработка кроликов	d) Белок (в %)	e) Содержание белков (в % от общего белка) по дням иммунизации					
				3-й	7-й	12-й	15-й	20-й	25-й
f) Экстракт лимфатических узлов	g) Общий белок	Иммунизация	0,42	0,48	0,4	0,51	0,52	0,51	0,52
		Кортизон		0,19		0,44	0,23	0,12	
		Иммунизация + кортизон		0,56		0,46	0,48	0,46	0,51
	h) Глобулин	Иммунизация	85,9	87,1	70,7	89	84	77	83,6
		Кортизон		81,9		77	83,8	82,4	
		Иммунизация + кортизон		85		81	77,9	85	86,2
	i) Альбумин	Иммунизация	14,1	12,9	29,3	11	16	22,5	16,4
		Кортизон		18,2		23	16,3	17,6	
		Иммунизация + кортизон		15		19	22,1	15	13,8
m) Экстракт брыжечных лимфатических узлов	g) Общий белок	Иммунизация	0,55	0,52	0,66	0,67	0,66	0,58	0,68
		Кортизон		0,58		0,75	0,63	0,55	
		Иммунизация + кортизон		0,56		0,64	0,57	0,62	0,55
	h) Глобулин	Иммунизация	88,8	87,4	80	89,6	88,7	83,7	89,2
		Кортизон		86,6		79,5	84,9	86,3	
		Иммунизация + кортизон		87,2		85,7	84,8	84,2	88,8
	i) Альбумин	Иммунизация	11,2	12,6	20	10,4	11,3	16,3	10,8
		Кортизон		13,4		20,6	15,1	13,7	
		Иммунизация + кортизон		12,8		14,3	15,2	15,8	11,2
n) Экстракт селезенки	g) Общий белок	Иммунизация	0,71	0,65	0,87	0,67	0,68	0,73	0,75
		Кортизон		0,7		0,81	0,69	0,74	
		Иммунизация + кортизон		0,67		0,66	0,67	0,79	0,65
	h) Глобулин	Иммунизация	94,8	95,2	93,8	93,4	94,3	93,1	94,4
		Кортизон		93,8		90,1	92,6	94,6	
		Иммунизация + кортизон		94,1		93,4	93,6	92	92,1
	i) Альбумин	Иммунизация	5,2	4,8	6,2	6,6	5,7	6,9	5,6
		Кортизон		6,3		9,9	7,6	5,4	
		Иммунизация + кортизон		5,9		6,6	6,4	8	7,9

Change in fractional protein content in organ extracts from rabbits following cortisone injection and immunization

[Legend:] a) material examined b) fraction name c) rabbits treated with d) protein (%) e) protein content (in % of total protein) on the following post-immunization days f) lymph node extract g) total protein h) immunization i) cortisone j) immunization + cortisone k) globulin l) albumin m) mesenteric lymph node extract n) spleen extract.

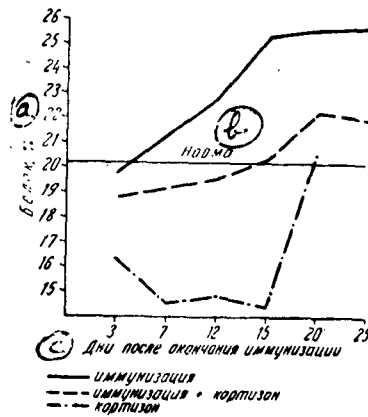
Table 5

Обработка кролика	Содержание белка по дням иммунизации						
	норма	3-я	7-я	12-я	15-я	20-я	25-я
Иммунизация	0,36	0,42		0,44	0,44	0,44	0,43
Кортизон		0,16	0,28	0,34	0,19	0,1	
Кортизон + иммунизация		0,48		0,37	0,37	0,39	0,44

Change in globulin content of lymph node extracts following cortisone injection and immunization

[Legend:] a) rabbits treated with b) protein content on the following post-immunization days c) normal d) immunization e) cortisone f) cortisone + immunization.

Figure 1.



Change in gamma-globulin content of blood serum under the effect of cortisone and immunization

[Legend:] a) protein (б) b) normal c) post-immunization days — immunization - - - immunization + cortisone cortisone.

THE DISTINCTIONS OF IMMUNOGENESIS IN GUINEA-PIGS INOCULATED
WITH ADSORBED TETANUS TOXOID UNDER CONDITIONS
OF IONIZING RADIATION INJURY

[Following is the translation of an article by A. Yu. Ilyutovich, B. N. Raykis and I. I. Labetskiy, from the Stavropol Institute of Vaccines and Sera, in the Russian-language periodical, Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, pages 61-64.]

In recent years the problem of the effect of ionizing radiation on the production of immunity is being given serious attention. It is known, in particular, that roentgen rays have an inhibitory effect on immunity.

However, as has been correctly indicated by Troitskiy and Tumanyan (1958) the detection of these effects, although they are very useful in the sense of being able to contribute a number of practical conclusions and theories, does not mean that the mechanisms of radiation effect on immunity are known.

In order to shed some light on the intimate processes occurring in an immunized organism on a background of ionizing radiation effects, it is important to study the cytomorphological changes that reflect to a certain degree the immunological shifts occurring in such animals.

As is known, the morphological expression of immunological changes in the tissues of an immunized (but not irradiated) animal consists of hyperplasia arising in individual organs, cellular transformation and phagocytosis (Rapoport, 1957). It has also been established that the rate of mitotic division may be one of the indicators of degree of immunity (Zalkind, 1959), while transformation of reticular cells into plasma cells is a necessary link in the process of antibody synthesis (Fagraus, 1948).

In view of the fact that antibodies have been discovered in plasmocytes (Coons, 1955), these cells are considered (Zdrodovskiy, 1961) to be the principles locus of immune globulin synthesis. However, there are

not many data in the literature describing the cytomorphological changes occurring in lymph nodes of animals immunized following irradiation. A study of this question is of general biological significance and of particular interest in determining the process of production of immunity under conditions of preliminary ionizing radiation.

The present paper submits the results of a study of the immunological shifts in the irradiated animal organism through a determination of antitoxin titer increment in the blood as well as cytomorphological changes in the regional lymph nodes.

A total of 60 guinea-pigs was used.

The experimental animals were divided into three equal groups: the animals in the first group were immunized with two subcutaneous injections of adsorbed toxoid, 0.5 milliliters per injection spaced 30 days apart; the animals in the second group were immunized in the same manner beginning on the fourth day after single total roentgen irradiation, in a dosage of 300 roentgen units (with an RUM-3 machine, 190 volts, 10 milliamperes, filters: 1 Cu - 1 Al, half-strength layer -- 1.43 centimeters, target to focus distance -- 40 centimeters, rate of delivery: 26.75 roentgen units per minute to the surface, time -- eleven minutes and 39 seconds); the animals in the third group received only roentgen treatment under the same conditions as those in the second group. As a control we used healthy guinea-pigs that were not irradiated or immunized.

The follow-up observation period extended for 30 days from the time of the second antigen injection.

The blood antitoxin titer was determined (by a biological test) on the second, fifth, twelfth, twentieth and thirtieth post-inoculation days.

The cytological changes in the lymph nodes were determined on moist impression preparations (Revutskaya method), stained with methyl green and pyronine. In the preparations we calculated the total number of plasmocytes in relation to all of the lymph node cells, the quantitative ratio of the various forms; we also analyzed other cytomorphological changes.

As a result of our observations it was established (figure 1) that in guinea pigs immunized but not irradiated the antitoxin titer (21 AE) in the blood serum attained a maximum level on the twelfth day following the second antigen injection. Subsequently it dropped somewhat, but still remained at a relatively high level (15-17 AE). In the regional lymph nodes of the guinea-pigs in this group a large amount (up to 3%) of plasma cells appeared. The increase in plasmocyte content (figure 2) was accompanied by development of plasmocytes up to mature forms with destruction of the latter. Increased mitotic division was observed primarily in the lymphoblastic cells.

In addition to increased plasmocytic activity and cellular division, we were also able to detect a large accumulation of cytoplasmic sphere-like formations, strongly stained by pyronine and consequently rich in ribonucleic acid (figure 2, k).

Immunization of the animals in the second group, begun on the fourth day following irradiation, elicited less marked immunological changes in the blood and lymph nodes. For almost the entire follow-up period the antitoxin titer was lower than in the non-irradiated animals. Immunization did elicit an increase in plasmocyte content, mitosis and cytoplasmic spheres rich in ribonucleic acid, however, the number of plasma cells began to increase much later than in the guinea-pigs that were only immunized (first group). Thus, while in the non-irradiated animals the plasmocyte content was considerably higher than normal on the second day (1.7%, with a normal of 0.2 to 0.3%) and attained 3% on the fifth day, in the group of irradiated animals the rise in plasmocyte content (to 2.7%) occurred only on the fifth day following the second toxoid injection. Thus, in the group of guinea-pigs treated with roentgen radiation, the plasmocytic reaction occurred later and was less pronounced than that of the non-irradiated animals at the same period (after the second toxoid injection).

In addition to an increase in total number of plasma cells, the quantitative relationship between the different forms in the lymph nodes also changed.

The morphogenetic pattern in the lymph nodes of animals that received only roentgen radiation (the third group) was characterized by a somewhat higher than normal plasma cell content (primarily owing to transitional and a few immature forms).

Thus the differentiated morphological analysis confirmed the importance of plasma cells for antibody synthesis and showed that irradiation inhibits the processes leading towards increase in number of plasmocytes.

Apparently the accumulation of cytoplasmic spheres containing ribonucleic acid also has a certain significance in immunogenesis, inasmuch as a high ribonucleic acid content is in turn an indicator of increased synthesis of proteins in general and consequently of immune protein as well.

Conclusions

1. Accumulation of plasma cells in the regional lymph nodes of animals immunized with adsorbed tetanus toxoid preceded the appearance of antitoxins in the blood.

2. Animals immunized following roentgen irradiation demonstrated a lower plasma cell content in the lymph nodes and lower antitoxin content in the blood than nonirradiated animals.

3. The less pronounced plasmocyte reaction in animals immunized following ionizing radiation is explained by inhibition of the processes leading towards multiplication of this type of cells.

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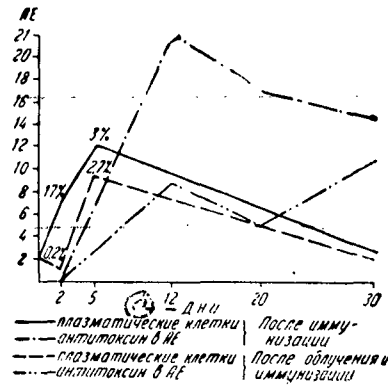
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Figure 1.

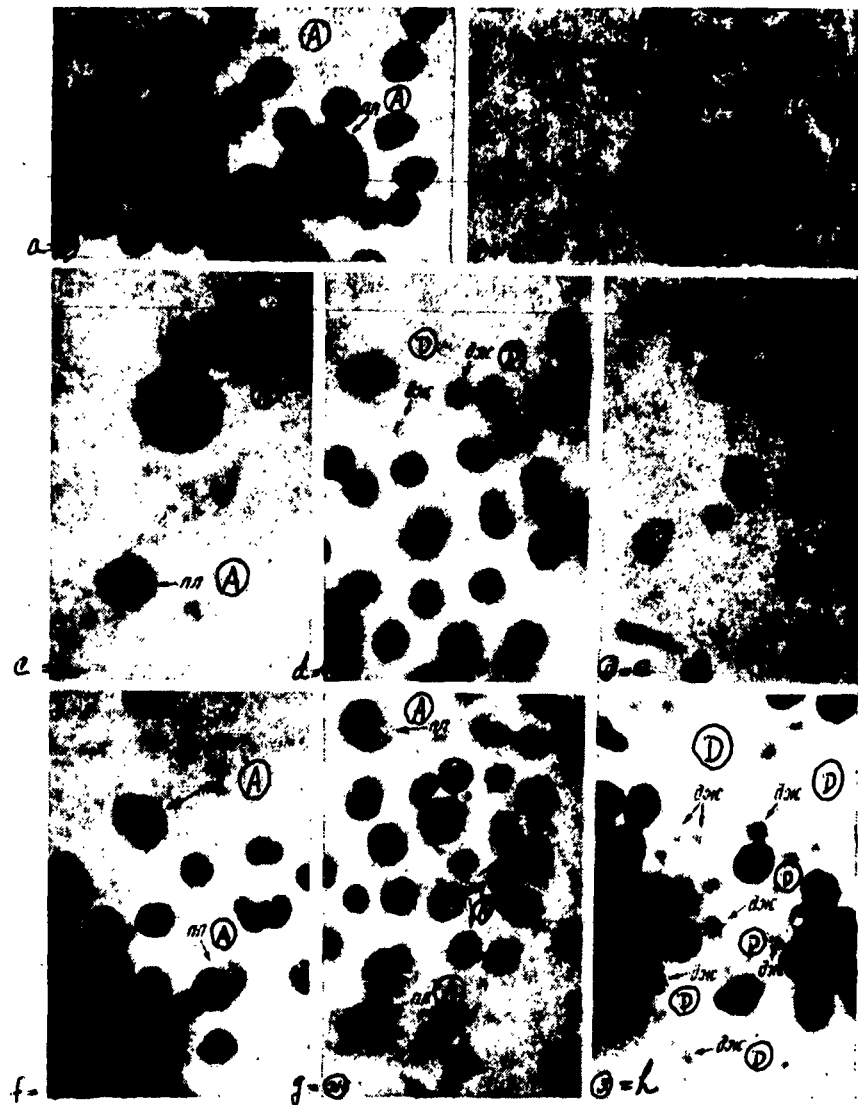


Quantitative fluctuation of antitoxins in the blood and plasma cells in the regional lymph nodes at various intervals following the second injection of adsorbed tetanus toxoid

[Legend:] a) days

—○—○—○—	plasma cells	after immunization
- - - - -	antitoxin in RE	
—□—□—□—	plasma cells	after irradiation and immunization
- - - - -	antitoxin in RE	

Figure 2.



Plasma cells (A) at various stages of maturation, basophilic spheres (B) and gemination of the latter (C) in controls (a and b), twice immunized animals (c, d and e) and animals irradiated after immunization (f, g and h). moist impressions, stained by the Brashe method. Emersion -- 90, ocular -- 10x.

[Legend:] A=pl (plasma cells) B=bsh (basophilic spheres)
 C=otp (gemination of the latter)
 D=dzh [Comment: expansion unknown].

IMMUNIZATION OF IRRADIATED GROUND-SQUIRRELS (*CITELLUS SUSLICA*
GUELLENSTAEDT) WITH PENICILLIN AND PENICILLIN-ALUM
LEPTOSPIRA VACCINE

[Following is a translation of an article by V. P. Morozova, from the Voronezh Medical Institute, in the Russian-language periodical Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, pages 65-67.]

A study of the pattern of artificial immunization in irradiated animals has established that when vaccines that are easily resorbed from bacilli coli are injected in a single dose shortly before and after irradiation they did not protect the organism from death following infection (Troitskiy and Tumanyan, 1958; Klemparskaya et al, 1958). Consequently, for immunization on a background of ionizing radiation other vaccines are required; those that are capable of eliciting an immunological shift in the organism at short intervals between irradiation and with subsequent inoculation.

The purpose of this investigation was to study the anti-Leptospira immunity in ground-squirrels (*Citellus suslica* Gueldenstaedt) against an infectious *Leptospira* culture.

We conducted experiments on 150 ground-squirrels, weighing from 150 to 250 grams. The animals received single doses of total irradiation from an RUM-11 machine. Conditions of irradiation: 195 kilovolts, 15 milliamperes, filters: 0.5 millimeters Cu + 1 millimeter Al, rate -- 19 roentgens per minute, total dosage -- 200 roentgen units.

The ground-squirrels of one group were inoculated with an easily resorbable penicillin preparation of *Leptospira*, the second group -- with the same vaccine but adsorbed with alum. The vaccines were introduced once, subcutaneously, in a dose of 2 milliliters. After injection of penicillin-alum vaccine a firm infiltrate the size of a bean was formed under the skin which did not resolve for the duration of the experiment. There was no local reaction to the penicillin vaccine.

The animals were infected 20--25 days after immunization with adsorbed vaccine and 10-12 days after inoculation with the nonadsorbed vaccine. These intervals coincided with the maximum production of antibodies (Zemskov and Khomik, 1951; Khomik, 1951). The animals were infected with a ten-day old culture of *Leptospira grippotyphose* (Ovtsa strain 964) containing 100-150 *Leptospira* in a field of vision.

Five series of experiments were conducted with each vaccine, and the animals were immunized two and ten days prior to irradiation, on the day of irradiation and two and ten days after irradiation. In each series of experiments there were control animals that were irradiated and infected at the same times as the experimental animals, but the controls were not vaccinated. There were also two other control groups of ground-squirrels that were not irradiated but were inoculated with adsorbed (first group) and nonadsorbed (second group) vaccine. Infection of the controls was done simultaneously with the experimental animals. A third control group consisted of animals receiving only irradiation.

Changes in weight and quantity of leukocytes in the blood were determined in the irradiated animals. The ground-squirrels that died were submitted to pathological examination. For the purpose of demonstrating *Leptospira* infection a bacteriological examination was also made.

Table 1 shows that in the first series of experiments, of the 13 irradiated animals immunized with adsorbed vaccine six survived the subsequent infection, while none of the five irradiated animals that were inoculated with nonadsorbed vaccine survived. In the second series, of the eleven irradiated animals inoculated with adsorbed vaccine and infected, five survived: all ten of the ground-squirrels, immunized two days after irradiation with nonadsorbed vaccine and infected, died of leptospirosis. Of the eight animals inoculated with adsorbed vaccine on the day of irradiation, five became immune, while the six animals that received nonadsorbed vaccine at the same times died as a result of the infection. Of the 14 ground-squirrels in the fourth group, immunized with adsorbed vaccine, nine survived following infection; of the six inoculated with nonadsorbed vaccine -- three survived. In the fifth series, seven animals survived infection of the fifteen immunized with adsorbed vaccine, and one survived of the six inoculated with nonadsorbed vaccine.

All of the irradiated and infected, but not inoculated, animals in all of the series of experiments died of leptospirosis.

Table 2 shows that of the eight animals receiving radiation only, six survived. Two ground-squirrels died of radiation sickness. All of the non-irradiated animals that were immunized with adsorbed vaccine and infected acquired immunity to leptospirosis. Of the eight non-irradiated ground-squirrels inoculated with nonadsorbed vaccine and infected, two died of leptospirosis.

As can be seen from the foregoing, immunization of ground-squirrels shortly before and after irradiation with nonadsorbed, easily resorbed vaccine did not produce immunity: the animals inoculated on the day of irradiation and two and ten days after irradiation did not survive experimental infection and died of leptospirosis.

When immunized at the same periods under the same conditions with nonadsorbed *Leptospira* vaccine a considerable portion of the animals was immune to the infectious *Leptospira* culture. The nonadsorbed vaccine elicited some immunological reaction in the animals only when injected two and ten days prior to irradiation, but even under these conditions there were considerably fewer immune animals than in the analagous series of animals inoculated with adsorbed vaccine.

It must also be stated that in the first series of experiments inoculation with either adsorbed or nonadsorbed vaccine was performed at the height of radiation sickness which, according to a number of authors, not only does not lead to the production of immunity but even aggravates the course of radiation sickness. Nevertheless, even under these difficult experimental conditions, of the thirteen animals inoculated with adsorbed vaccine, seven acquired immunity while all of the animals inoculated at the same times with nonadsorbed vaccine died following control infection.

In the fifth series, infection of the animals, inoculated with nonadsorbed vaccine, coincided with the second day of radiation sickness and in those inoculated with adsorbed vaccine -- with the height of radiation sickness. As is known, when animals, that have been immunized with killed vaccines easily resorbable from intestinal bacilli, are infected at the height of radiation sickness they are susceptible, but immunization with adsorbed vaccine resulted in considerable resistance of the animals to leptospirosis even when they were infected during this same period. Apparently this may be explained by the fact that adsorbed vaccine provides gradual and prolonged emergence of antigens in the blood and, consequently, prolonged stimulation of the lymphoid elements that produce antibodies. Thus, Hawkins and Wormall (1961), for instance, proved that when alum-adsorbed ox albumin was injected to rabbits, it remained at a relatively constant level for a long time. As a result, adsorbed antigen is capable of eliciting immunizing stimulation in irradiated animals even when the acute phase of radiation sickness has passed (Morozova, 1961). This property of the vaccine probably provides the production of a sufficiently stable immunity in animals receiving single inoculations even shortly before and after irradiation.

Conclusions

1. In ground-squirrels, receiving single inoculations with *Leptospira* adsorbed vaccine two and ten days before and after irradiation, as

well as on the day of irradiation, a sufficiently high specific immunity was observed.

2. In ground-squirrels, receiving single inoculations with nonadsorbed *Leptospira* vaccine two and ten days prior to irradiation, the specific immunity was considerably lower than that of the animals inoculated with adsorbed vaccine. Immunization of animals with nonadsorbed vaccine on the day of irradiation and two and ten days after irradiation did not elicit development of immunity.

3. Adsorbed *Leptospira* vaccine was found to be a more effective means of immunizing irradiated animals than nonadsorbed vaccine.

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Table 1.

№ серии	Срок облучения	Депонированная вакцина				Недепонированная вакцина				Контроль			
		обл. чело	заражено	выжило	погибло	обл. чело	заражено	выжило	погибло	обл. чело	заражено	выжило	погибло
1	За 10 дней до иммунизации	14	13	7	6	6	5	—	5	5	5	—	5
2	За 2 дня до иммунизации	14	11	5	6	10	10	—	10	5	5	—	5
3	В день иммунизации	13	8	5	3	6	6	—	6	5	4	—	4
4	Через 2 дня после иммунизации	14	14	9	5	6	6	3	3	3	3	—	3
5	Через 10 дней после иммунизации	15	15	7	8	6	6	1	5	5	5	—	5

Effectivity of single immunization of ground-squirrels with penicillin and penicillin-alum Leptospira vaccine

[Legend:] a) Series No. b) Time of irradiation c) Adsorbed vaccine d) nonadsorbed vaccine e) control f) irradiated g) infected h) survived i) died j) 10 days prior to immunization k) 2 days prior to immunization l) on the day of immunization m) 2 days after immunization n) 10 days after immunization.

Table 2.

Группа животных	Привитые	Число животных	Срок вакцинации	Заражено	Облучено	Погибло	Выжило
Облученные	—	8	—	—	8	2	6
Необлученные	Депонированной вакциной	9	За 20 дней до заражения	9	—	—	9
	Недепонированной вакциной	8	За 10-12 дней до заражения	8	—	2	6

Survival rate of control animals

[Legend:] a) animal group b) inoculated c) number of animals d) time of inoculation e) infected f) irradiated g) died h) survived i) irradiated j) nonirradiated k) adsorbed vaccine l) nonadsorbed vaccine m) 20 days prior to infection n) 10-12 days prior to infection.

ACTIVE IMMUNITY AGAINST GAS GANGRENE IN MONKEYS
UNDER CONDITIONS OF ACUTE IRRADIATION

[Following is a translation of an article by B. K. Dzhi-
kidze, A. S. Aksenova and S. K. Stasilevich, from the In-
stitute of Experimental Pathology and Therapy of the Acad-
emy of Medical Sciences of the USSR, in the Russian-lang-
uage periodical Zhurnal mikrobiologii, epidemiologii i
immunobiologii (Journal of Microbiology, Epidemiology
and Immunobiology, Moscow, Vol 40, No 7, pages 68-72.)]

The study of the effect of ionizing irradiation on the production of immunity has been broadly covered in the literature (Klomparskaya, Troitskiy et al, Troitskiy and Tumanyan, Goncharenko, Taliaferro, Kohn, Dixon et al, Jacobson et al, Craddock, and others).

From the work of Soviet and foreign investigators it is known that acute irradiation has an inhibitory effect on active and passive immunity. It has also been established that the degree of disturbance of immunity is in close relationship with the irradiation dosage and the length of time between immunization and irradiation. The general conclusion drawn from these investigations is arrived at in the following manner: with inoculation prior to irradiation immunogenesis is not essentially impaired, while irradiation prior to administration of antigen elicits inhibition of immunogenesis. Yet in both instances, upon testing the active immunity of inoculated animals it has been found that there is a marked reduction in general resistance and a mild increase in specific insusceptibility (Tumanyan and Izvekova).

Not much work has been done on the question of effectivity of delayed revaccination under conditions of acute irradiation, and the results of investigations are contradictory. Some authors indicate the high protective role of re-inoculation in acute experimental radiation sickness (Klomparskaya, Krivenkov), others hold the exact opposite view. (Shabarov).

In our preceding investigation ([Note]: Zh. mikrobiol. ((Journal

of Microbiology), No 1, 1963, pages 132-137) it was demonstrated that revaccination six months after immunization with adsorbed tri-toxoid was effective and protected monkeys from fatal illness after they had received fractional irradiation (1.17 roentgen units per day) for a cumulative dosage of 500 and 600 r [roentgen units]. The monkeys were resistant to infection with three to six D₁₀ [minimal lethal dose] of *Cl. perfringens*

In the present investigation we attempted to study on monkeys the effectivity of revaccination in gas gangrene under conditions of radiation sickness produced by delivery of sublethal dosage of acute irradiation. The investigation was conducted on 30 monkeys.

Nineteen monkeys (*Macacus Rhesus*), two to three and a half years old, were used in the first series of experiments. All of the monkeys were inoculated twice with adsorbed tri-toxoid (Institute of Epidemiology and Microbiology imeni Gamaled) containing 40 EC of *Cl perfringens* antigen. The preparation was injected twice subcutaneously at a 20-day interval, 1 milliliter each time, in the left thigh. Five months after immunization the monkeys were divided into three groups: six monkeys in each of two experimental groups and seven in a control group. The monkeys of the first group were reimmunized 24 hours prior to irradiation and those of the second group -- 24 hours after irradiation. The control group did not receive any radiation, but was revaccinated at the same times. For re-immunization 1 milliliter of tritoxoid was used. The serum was titrated in the lecitho-vitellin reaction by the usual method.

Radioactive cobalt was used as a source of radiation energy. The irradiation dosage was 388 r, delivered at a rate of 111 r per minute, exposure time -- 3½ minutes. Three monkeys from each of the two experimental groups were irradiated with multiple fractional doses -- 10 and 20 r per day up to a cumulative dosage of 490 r -- sixteen months prior to the acute irradiation. Four of the seven control animals were irradiated in the same manner.

The irradiation caused development of acute radiation sickness of moderate severity on the tenth--fourteenth day, with pronounced leukopenia, very negligible erythropenia and reduced hemoglobin content, and appearance of pinpoint hemorrhages. Outwardly the monkeys' general condition changed little.

As can be seen from the table, which presents the data on determination of quantity of antitoxin against *Cl perfringens* antigen before and after revaccination (on the fourteenth day), revaccination stimulated the accumulation of specific antitoxin in the monkeys of the three groups. It must also be noted that the antitoxin titer in the animals revaccinated prior to irradiation was ten to thirty times higher than the previous indices; in the group irradiated prior to revaccination it was five to 30 times higher in five instances and in one instance the initial titer did not change. In the control animals the antitoxin titer was

two and a half to ten times higher. The mean antitoxin titer in the re-vaccinated group was higher than in the controls. Thus, the mean titer in the animals reimmunized prior to irradiation was 1.9 AE, in those re-immunized after irradiation -- 0.85 AE and in the control group -- it did not exceed 0.53 AE.

Thus it has been shown that sublethal doses of irradiation not only did not inhibit the organism's secondary immunological reaction to revaccination, but on the contrary, they stimulated accumulation of antitoxins at rather high titers. These data are in agreement with those of Kovtunovich who established, under conditions of spaced re-immunization, that there was a higher production of antitoxin in irradiated guinea pigs as compared with the controls, regardless of the irradiation dosage and of the periods at which the antigens were inoculated.

Further, we tested the resistance of irradiated animals to infection by the gas gangrene pathogen. This permitted us to evaluate the effectiveness of reimmunization as well as the relationship between antitoxin level in the blood of irradiated animals and their resistance to infection. For this purpose all of the immunized animals were infected with 2.5 milliliters of *C. perfringens* on the sixteenth day after irradiation; this dosage, as was shown by preliminary titration on healthy monkeys, corresponded to 3 DLM. For infection we used an 18-hour culture grown on Kitt-Tarotstsi medium. The culture, which was activated by the addition of a 50% calcium chloride solution (0.1 per milliliter), was injected into the left thigh muscle.

On the day after infection, all of the monkeys became listless and could not step on the affected extremity. Examination revealed local phenomena typical of gas gangrene: edema and blue discoloration of the skin at the site of inoculation, crepitus. Two to three days after the inoculation, we were able to observe a difference between the course of illness in the irradiated and control animals. In the former, it was very severe with sharp disturbance of the general condition and extensive gangrene of the thigh muscles. The control monkeys demonstrated a light course, the process was localized, fistulae were formed on the fifth day after infection, the wounds demonstrating healing by first intention within a month. Infection of control animals elicited a rise in antitoxin titer of the blood in contrast to the two irradiated monkeys that survived, in whom a reduction of antitoxin titer was observed.

The table also presents the results of testing the immunity. They show that all of the animals irradiated after revaccination died from three to eleven days after experimental infection, four of the six animals irradiated prior to revaccination died on the second to fourth days after infection, while all of the control animals survived. Thus no correlation was observed between the antitoxin level of blood serum of irradiated monkeys and their resistance to infection.

Consequently, reimmunization performed 24 hours before or 24 hours after irradiation was found to be little effective.

In the second series of experiments we attempted to determine the significance of intervals between revaccination and irradiation in the development of immunity to gas gangrene. For this purpose six monkeys, revaccinated six months after immunization and one month prior to irradiation with a dose of 400 r, were infected with *Cl perfringens*. In this instance, three of the six monkeys died, the others experienced a very severe illness from which they recovered within a month.

Thus, revaccination performed one month prior to irradiation was found to be effective enough and resulted in the survival of half of the experimental animals. The practice of two-phase revaccination, the first one month prior to irradiation and the second seven days after irradiation did not enhance the effectivity of inoculation immunity. Thus, of the five monkeys irradiated with a dose of 400 r and infected after two inoculations, three died.

On the basis of the presented data it becomes apparent that revaccination, performed 24 hours before or 24 hours after acute irradiation, is inadequate for the prevention of development of fatal illness after infection with 3 Dlm of gas gangrene pathogen. At the same time, we see that immunity to gas gangrene is not governed solely by the level of specific humoral protective factors. As can be seen by the foregoing data, with relatively high antitoxin titers in the blood, in monkeys irradiated 24 hours after reimmunization the immunity test showed a total lack of immunity. Analogous findings were obtained by Nechayevskaya et al who proved the absence of resistance to minimal doses of anaerobic infection pathogen in guinea-pigs with high antitoxin titers in the blood.

The survival of monkeys revaccinated 24 hours after irradiation can apparently be explained on the basis of individual peculiarity of these animals. We are not at all inclined to evaluate this as the result of increased radioresistance due to previous administration of small doses of radiation energy, although there are such reports in the literature. On this basis it may be assumed that in the mechanism of immunity against gas gangrene a leading role is played by a change in cellular reactivity which is impaired to a great extent by the effect of radiation. The results obtained confirm the opinion that irradiation causes a reduction in animals' resistance to infection, although production of antibodies during this period may not be altered (Troitskiy et al, Tumanyan and Izvekova).

The survival of three out of the six monkeys revaccinated one month prior to acute radiation can apparently be explained by the fact that during this period there is time for the development of adequate specific immunity which is only partially impaired by irradiation.

Conclusions

1. Revaccination 24 hours prior to or 24 hours after acute irradiation stimulated the accumulation of antitoxin in the blood against *C1 perfringens* antigen. However gas gangrene immunity tests show the absence of correlation between the antitoxin titer and degree of immunity.
2. Revaccination 24 hours prior to or 24 hours after irradiation of animals in sublethal doses was little effective.
3. Revaccination one month prior to acute irradiation caused increased survival rate of monkeys when they were infected with lethal doses of gas gangrene pathogen.

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№ обезьяны	Условия опыта	Количество антитоксина (в АЕ)							Исход заболевания					
		после первой прививки		после второй прививки		перед ревакцинацией	после ревакцинации через 14 дней	средний титр		после заражения в разные дни				
		через 20 дней	через 14 дней	7-й	14-й					21-й	28-й			
3193	Ревакцинация за 24 часа до облучения	<0,1	0,1	0,1	1									Гибель на 4-е сутки
3209		<0,1	0,5	0,1	3									То же на 3-и сутки
3216		<0,1	0,1	0,1	2									>>> 4 >>>
3315		<0,1	0,5	0,1	2	1,9								>>> 3 >>>
3317		<0,1	0,25	0,1	2,5									>>> 1 >>>
3730		<0,1	0,25	0,1	1									>>> 11 >>>
3173	Ревакцинация спустя 24 часа после облучения	<0,1	0,1	0,05	1,5		1	1	0,25	0,1				Здоров
3175		<0,1	0,1	0,1	0,5									Гибель на 4-е сутки
3185		<0,1	0,5	0,1	1,5	0,85	0,5	0,5	0,5	0,5				Здоров
3728		<0,1	<0,1	0,1	0,1									Гибель на 2-е сутки
3729		<0,1	0,1	0,1	0,5									То же на 3-и >>>
3731		<0,1	0,25	0,1	1									>>> 3 >>>
3212	Контроль	<0,1	0,1	0,1	0,25		2	2	0,5	0,5				Здоров
3215		<0,1	0,1	0,05	0,5		2	2	1					>>>
3188		<0,1	0,1	0,1	0,5		5	4	2					>>>
3192		<0,1	0,25	0,1	0,5	0,53	2	2	1	0,5				>>>
3283		<0,1	0,1	0,1	1		1	1	0,5					>>>
3287		<0,1	0,25	0,1	0,5		1	1	0,5					>>>
3735		<0,1	<0,1	0,1	0,5		1	2	1					>>>

Immunity test and determination of antitoxin titer in the blood of immunized monkeys

[Legend:] a) monkey No b) experimental conditions c) quantity of antitoxin (in AE) d) after first inoculation e) after 20 days f) after second inoculation g) after 14 days h) prior to revaccination i) 14 days after revaccination j) mean titer k) days after infection l) outcome of illness m) revaccination 24 hours before irradiation n) died on the 4th day o) died on the 3rd day p) revaccination 24 hours after irradiation q) in good health r) died on the 2nd day s) control.

THE USE OF HAMSTERS (*CRICETULUS TRITON*) FOR THE STUDY
OF IMMUNOGENIC PROPERTIES OF WOUND INFECTION
AND BOTULISM TOXOIDS

[Following is a translation of an article by L. M. Samorodov in the Russian-language periodical Zhurnal Mikrobiologii, epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Moscow, Vol 40, No 7, page 72-76.]

At the present time the harmlessness and immunogenicity of wound infection and botulism toxoids are being tested on guinea-pigs. However these animals multiply slowly (eight to ten offspring per female per annum), they are relatively expensive, and their maintenance in a laboratory is involved with certain difficulties. Therefore it seemed desirable to investigate the possibility of using hamsters (*Cricetulus triton* Winton species, 1899) instead of guinea-pigs for testing toxoids; the hamsters multiply rapidly under laboratory conditions (40-60 offspring per annum per female) and they are available in large quantities (Vinogradov and Gromov).

The data in the literature concerning the use of such hamsters for laboratory investigations are extremely sparse.

Only a few papers have been published on the question of inoculating plague (Suknev), tularemia (Dunayev), Prowazeki rickettsiosis (Kulagin) and recurrent tick fever (Mishchenko) to hamsters. All of the authors established the susceptibility of these small hamsters to the cited infections. There are also indications in the literature, by Kovalevskiy, on the possibility of using hamsters in work with *Leptospira*, tularemia and rickettsiosis.

All of the above-mentioned investigators reached the same conclusion as to the possibility of using the *Cricetulus triton* as a suitable biological indicator in the study of infections.

We did not find any data in the literature concerning the susceptibility of these hamsters to the toxins of anaerobic infection and

botulism pathogens; nor did we find indications as to the possibility of using these animals for the study and testing of immunogenic properties of the corresponding toxoids.

We studied the possibility of using *Cricetulus triton* for the determination of immunogenic properties and harmlessness of native and purified concentrated as well as aluminium hydroxide adsorbed toxoids of type A, B, C and E Cl, botulinum, Cl tetani, perfringens and oedematiens. We conducted our research on animals weighing 150 to 180 grams. Guinea-pigs weighing 300 to 400 grams were used as controls.

The animals were immunized subcutaneously with a single dose (of varying amounts) of mono- and polyvalent toxoids containing a constant amount of aluminium hydroxide with total adsorption of antigen. At least five to seven hamsters and five control guinea-pigs were given the same immunizing dose. The immunization results were evaluated by the specific antitoxin titer in the blood serum of the immunized animals and by their resistance to administration of the corresponding toxins.

Prior to immunization the animals' blood serum was examined for the presence of natural specific antibodies. We did not detect any anti-botulin (type A, B, C and E), antigangrene or antitetanus toxoids in any of the 14 readings within the range of sensitivity of the method used (Sbornik instruktsiy po kontrolyu bakteriynykh preparatov [Collection of Instructions for the Testing of Bacterial Preparations], Moscow, 1946).

By testing the sensitivity of the hamsters to botulin toxins and to toxins of wound infection pathogens as compared to the sensitivity of guinea-pigs to these toxins it was established that the D_{1m} [minimal lethal dosage] quantities of toxin as related to the animals' weight (we used dry standard toxins) were the same as for guinea-pigs. However, in view of the fact that these little hamsters weigh 50% less than guinea-pigs, it follows that their sensitivity (on the basis of one gram of body weight) is half that of the guinea-pigs.

The clinical picture of intoxication in hamsters following injection of one D_{1m} of toxin was the same in guinea-pigs. Thus, with injection of botulin toxins the hamsters died at the same times as the control guinea-pigs with phenomena of severe muscular weakness, sharp decrease in dermal turgor, collapse of abdominal walls ("wasp waist"). Tetanus toxin elicited the death of the hamsters with phenomena of sharply marked tetanus and deviation of the spine. Injection of gangrene toxins resulted in a clinical picture of gangrene and death of the hamsters which in no way differed from the clinical picture in the control guinea-pigs.

Thus, these hamsters cannot be distinguished from guinea-pigs, with respect to susceptibility and sensitivity to toxins of wound infection and botulism pathogens.

Further, we determined the immunogenic properties of concentrated adsorbed monotoxoids of all of the wound infection pathogens and those of botulism, types A, B, C and E, after being injected subcutaneously in a single dose. We used the method of single dose immunization inasmuch as the quality of the antigen or immunological reactivity to it of either species can be evaluated objectively only in experiments with single dose immunization, which has been indicated by Khalyapina and Roskovskaya, Mel'nik and Starobinets, Zubova, Chertokova et al, Vorob'yev in their investigations.

The antibotulin and antitetanus antitoxin titers in the blood serum of the hamsters were dozens of times higher than in the guinea-pigs (table 1). Gangrene toxoids elicited about the same immunological response. In testing the degree of immunity it was established that in parallel with the high antibotulin and antitetanus antitoxin titers the hamsters were better protected from injection of larger doses of the corresponding toxins than guinea-pigs. The immunity to gangrene toxins was about the same in the hamsters and guinea-pigs. Consequently, the hamsters had a rather high immunological reactivity with respect to wound infection and botulism toxoids, which justifies their being considered suitable for the determination of immunogenic properties of adsorbed monotoxoids of anaerobic infection.

In the next series of experiments we studied the immunological reactivity of hamsters as compared to that of guinea-pigs following immunization with compound preparations including purified adsorbed types A, B, C and E botulin, tetanus and gangrene toxoids. The results showed that in response to single dose immunization with adsorbed polyvalent toxoids the hamsters demonstrated higher antitoxin production and were more resistant to administration of higher doses of toxins than the control guinea-pigs (table 2). Thus, with immunization of hamsters with polytoxoids we obtained the same results as with immunization with adsorbed monotoxoids.

In view of the fact that the hamsters responded to immunization with a full dose of adsorbed polytoxoid with higher antitoxin production than the control guinea-pigs, it seemed of interest to determine the optimal-minimal immunizing dosages of polytoxoids for the hamsters which would permit obtaining antitoxin titers comparable to those of the control guinea-pigs. This was also necessary in order to be able to compare the results of testing the immunological effectivity of compound preparations on hamsters with the results obtained previously on guinea-pigs. For this purpose the hamsters were immunized with one-tenth and one-fifteenth of the doses of polytoxoid administered to the guinea-pigs. We found that the antitoxin content in the blood serum of the hamsters that had been immunized with one-fifteenth of the guinea-pig dose was about the same as in the serum of guinea-pigs immunized with a full dose of polytoxoid.

For the purpose of determining the harmlessness for hamsters of botulin (type E), tetanus toxoids and native wound infection toxoids these preparations were injected subcutaneously in a total dose of 10 milliliters (5 milliliters in each hip). Infiltrates appeared at the site of injection three to five days later. By the end of the follow-up period (15-20 days) areas of thickening and intantion remained at the site of the infiltrates. No general phenomena of intoxication were present for the duration of the follow-up period and the animals gained weight normally. In the control guinea-pigs the formation of infiltrates, intantion and thickening were observed in lesser quantity and dimensions.

Apparently the local phenomena are caused by a large amount of free formalin in the native toxoids (0.3 to 0.4%) and by the specific distinctions of the skin and subcutaneous cellular structure of the hamsters. Injection of deformedalized (by dialysis through saline and addition of sodium sulfate salt) toxoid in a subsequent experiment did not elicit local phenomena in the hamsters or guinea-pigs.

It must be noted that the dosage of toxoid used for the determination of harmlessness, 10 milliliters, is large for the small hamster and in subsequent investigations of the harmlessness of native and concentrated toxoids this circumstance must be taken into consideration.

Conclusions

1. The little hamster (*Cricetulus triton*) is susceptible to botulin (types A, B, C and E), tetanus and gangrene (*perfringens*, oedematiens) toxins. The clinical picture of intoxication in the hamsters could not be distinguished from the intoxication of guinea-pigs.
2. In the normal blood serum of hamsters there were no natural antitoxin, antigangrene or antitetanus antibodies.
3. The immunological reactivity of hamsters with respect to tetanus and botulin toxoids is higher than that of guinea-pigs. In response to single-dose immunization with mono- and polytoxoids the corresponding antibodies were produced in the blood serum of hamsters in higher titers than in guinea-pigs.
4. These hamsters may be used instead of guinea-pigs for the qualitative determination of toxins of wound infection and botulism pathogens as well as for the determination of immunogenic properties of adsorbed monotoxoids and compound preparations.

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Table 1.

Сорбированный анатоксин	№ серии	Иммунизирующая до-за (в мл)	Антитоксический титр (в АЕ) через 25-27 суток после прививки		Напряженность иммунитета через 30 суток после прививки				
			крысовид-ные хомяч-ки	морские свинки	крысовидные хо-мячки		морские свинки		
					число вве-денных Dlm	результ-тат	число вве-денных Dlm	результ-тат	
i) Ботулиновый типа А	10	0,1	>10	<0,1	2000	2/0	2000	0/2	
					4000	3/0	4000	0/2	
	» » В	14	0,1	>50	>1<3	5000	2/0	5000	0/3
	» » С	18	0,1	3	<0,025	20000	3/0	20000	0/2
					500--	3/0	500	0/2	
k) Столбнячный . . .	13-20	0,1	≈ 1	≈ 0,01	-1000	3/0	1000	0/2	
					3000	3/0	3000	0/2	
					10000-	3/0	3000	0/2	
					-15000				
					20000	2/0	5000	0/3	
Perfringens	7	0,5	>0,5<1	>0,5<1	15000--	3/0	2000	2/0	
					-20000				
					25000	0/3	3000-	0/3	
Oedematiens	53	0,3	>80<100	>50<60	-30000	2	2	2/0	
						3/0	4	2/0	
						3/0	400	1/1	
					800	0/2	600	0/2	

Comparative immunogenicity of adsorbed monotoxoids of wound infection and botulism pathogens with single-dose immunization of hamsters and guinea-pigs

[Legend:] a) Adsorbed toxoid b) series No c) immunizing dosage (in milliliters) d) antitoxin titer (in AE) 25-27 days after inoculation e) cricetus triton f) guinea-pigs g) degree of immunity 30 days after inoculation h) number of Dlm injected i) result j) type A botulin k) tetanus.

Note: the numerator = number of animals that survived
the denominator = number of animals that died

Table 1.

№ серии полимиктоксина	Компоненты полимиктоксина	№ серии	Иммунизирующая доза (в мл)	Антитоксический титр (в АЕ) через 25 суток после прививки		Напряженность иммунитета через 30 суток после прививки			
				крысидные хомьячки	морские свинки	крысидные хомьячки		морские свинки	
						число введенных DIm	результат	число введенных DIm	результат
1	Ботулиновый типа А	22a	1,5	>1<3	>0,1<0,5	2000	2/0	500	1/0
	» » В	15		>20<25	>1<3	4000	2/0	1000	1/1
	» » С	19		>0,5<1,0	>0,05<0,1	5000	2/0	1000	1/0
	» » Е	31		>0,1<0,5	0,05	10000	2/0	2000	2/0
	Столбнячный	6		0,01	<0,05	500	1/1	300	0/1
	Perfringens	20		>0,1<0,5	<0,1	1000	2/1	500	1/1
	Oedematiens	2		>3<5	=1	3000	1/2	3000	2/0
2	Ботулиновый типа А	22a	0,5	>1<3	<0,01	2000	2/0	500	1/0
	» » В	15		>3<5	0,1	5000	2/0	800	1/1
	» » С	19		>0,5<1	0,05	3000	2/0	500	1/0
	» » Е	31		1	0,05	5000	2/0	1000	1/1
	Столбнячный	6		>0,5<1	0,1	1000	2/0	250	2/0
3	Столбнячный	6	0,5	>0,5<1	0,1	3000	2/0	500	1/1
	Столбнячный	6		>0,5<1	0,1	500	2/0	500	1/0

analysis of immunogenic properties of adsorbed polytoxoids in hamsters (Cricetus triton)

[Legend:] a) Polytoxoid series No 1) polytoxoid components
 c) series No d) immunizing dose (in milliliters) e) anti-toxin f) cricetus triton g) guinea-pigs h) degree of immunity 30 days after inoculation i) number of DIm injected
 j) result k) botulin type l) tetanus.

Note: the numerator = number of animals that survived
 the denominator = number of animals that died

THE DETECTION OF BOTULIN TOXINS AND TYPE B AND C BACILLI
IN THE ORGANISM OF PATIENTS, ANIMALS AND CADAVERS

[Following is a translation of an article by T. I. Serge-
yeva, from the Institute of Epidemiology and Microbiology
imeni Gamaled of the Academy of Medical Sciences of the
USSR, in the Russian-language periodical, Zhurnal mikro-
biologii, epidemiologii i immunobiologii (Journal of Mic-
robiology, Epidemiology and Immunobiology), Moscow, Vol
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In recent years a number of reports have appeared in the foreign
literature concerning cases of botulism in humans caused by type C Cl.
botulinum (Meyer and Eddie, 1953; Prevot and Terras, 1955).

Up to 1956 no type C Cl botulinum had been seen on the territory
of the Soviet Union. In 1956, in a study of a mass outbreak of botulism
in minks near Kazan' for the first time in the Soviet Union we (Matveyev,
Bulatova, Sergeeva, 1957) isolated a highly toxigenic strain of type
C Cl botulinum. Later on a report was published on the isolation of this
type of bacillus from the soil of the USSR (Kravchenko, Shishulina, 1960).
In this connection the question of diagnosing botulism caused by the
type C Clostridium gained important significance.

Our goal in the present investigation was to study the possibility
of detecting botulin toxins and type B and C bacilli in the organism of
sick animals and in the organs of cadavers in relation to the adminis-
tered dose and method of administering the toxin. For the purpose of pro-
ducing experimental botulism various doses of type B and C botulin toxin,
or five-day broth cultures of the same types, were introduced into the
stomach or respiratory tract of guinea-pigs and rabbits. The toxin was
introduced into the stomach with a cannula made of a long, thick needle
with an olive-shaped tip soldered to the end; it was introduced into the
respiratory tract through the nose, with a dropper, in various dilutions
of the toxin in a dosage of 0.2 to 0.5 milliliters.

The minimal lethal doses of toxin for the guinea-pigs and rabbits

were first determined for the enteral and intranasal methods of administration. Proof that the toxin reached the respiratory tract (with intranasal administration) was that the minimal lethal doses were four to five times smaller than when it was administered by mouth.

The clinical picture of the disease in animals experimentally infected with types B and C botulin toxins was similar, and was manifested by listlessness, refusal to eat, paresis of the hind extremities and death due to paralysis of the respiratory center. When the toxin was administered intranasally the illness developed more rapidly with pronounced symptoms of impaired breathing.

On the basis of our previous investigations conducted with type A Cl botulinum toxin, in the present work, for the detection of toxin and bacilli with the enteral method of infection, we used only the liver, stomach and small intestine contents, and with the intranasal method of infection -- the lungs, liver and blood from the cadavers; only blood samples were taken from sick animals.

To detect toxin in the blood and organs we used the neutralization reaction with types B and C antitoxin serum on albino mice, and to isolate the bacillus we made cultures from the organs on the following media: casein-fungus, pepsin-peptone broth with pieces of meat, and Hottinger's broth with pieces of meat in vaseline oil. Prior to inoculation of the media 0.5% sterile glucose solution was added to them. The material was inoculated in two flasks, one of which was heated in a water bath at 80° for 20 minutes. The cultures were incubated at 37° for 48 hours then tested for neutralization of the corresponding serum on albino mice.

Examination of the organs of 14 guinea-pigs for the presence of toxin after it had been introduced by mouth in doses of one and two Dlm [minimal lethal dosage] did not yield positive results. Upon infection of four animals per os with five Dlm of type B Cl botulinum culture, we were able to detect the toxin only in the contents of the small intestine of three of the guinea-pigs, while with administration of 10 Dlm the toxin was found in the small intestine of all four and in the stomach contents and liver of two out of the four experimental guinea-pigs.

Of the 18 animals that had been given dry type B botulin toxin by mouth, in various doses, it was found in the small intestine only in the animals who received at least 20 Dlm. Thus, in order to detect the toxin it must be administered in a dosage four times greater than when producing the infection with a culture.

The bacillus was consistently isolated from the small intestine and liver of the guinea-pigs when they were infected (per os) with 1, 2, 5 Dlm of culture, while it was present with less consistency in the other organs. The greatest amount of positive results were obtained on casein-fungus medium without preliminary heating after inoculation of the material.

Experiments performed on 22 guinea-pigs showed that intranasal infection led to localized accumulation of toxin in the lungs and as a result, after administration of 2 Dlm of dry toxin or culture, the toxin was found in the lungs of three out of four guinea pigs. When the dosage was increased, the toxin was also found in the liver and blood. The type B botulism pathogen was readily isolated from the lungs after introduction of 1 Dlm of culture.

In the organs of 15 guinea-pigs, infected per os with 1, 2, 5 and 10 Dlm of type C culture, we were unable to detect the toxin. Only after administration of 15 Dlm did we find the toxin in the small intestine of three out of the four experimental animals, and in two out of four guinea-pigs we found the toxin in the liver and gastric contents.

With administration of dry toxin to 18 guinea-pigs per os, we were unable to isolate it from the organs only of those animals that had been given at least 30 Dlm.

Intranasal administration of 2 Dlm of dry toxin or culture of type C bacillus was accompanied by detection of toxin in the lungs of the animals that died in all of the examined cases; we were only able to demonstrate the toxin in the liver and blood after administration of doses that were five times greater.

Upon bacteriological examination of the organs of the guinea-pigs type C Cl botulinum was isolated from the small intestine of every guinea-pig that had been infected per os with one or two Dlm and from the lungs of the animals that had been infected intranasally with one Dlm. The best results were obtained on Hottinger broth with pieces of meat in vaseline oil. Less positive results were obtained upon examination of heated cultures than unheated ones.

In comparing the results of detection of types B and C botulin toxins, we can note that the former are easier to demonstrate in the organs of the dead guinea-pigs, i.e. with smaller infecting doses than the latter. This applies primarily to the enteral method, inasmuch as when introduced intranasally the toxins of both types were detected in the lungs with equal infecting doses. This difference in the frequency of detection of toxins in the gastrointestinal tract is probably related to the lower resistance of type C toxin in the digestive canal.

In experiments on rabbits we attempted to settle the question of the possibility of detecting types B and C botulin toxins in the blood of infected animals. For this purpose the rabbits were given 0.75, 1, 2, 5 Dlm of dry types B and C toxins or broth cultures of these bacteria, per os and intranasally.

Four, 24, 48 and 72 hours later blood samples were taken from the rabbits' vena auditiva [vein of the ear] and examined for toxin by

the neutralization reaction method; after the rabbits died the blood and organs were examined.

When the rabbits were infected per os with 0.75 Dlm of type B broth culture, no toxin was found in the blood of three rabbits, whereas when they were given one Dlm, the toxin was found four hours after ingestion and remained in the blood for one to two days; on the third day we were unable to isolate any toxin (table 1). In the blood of rabbits infected per os with two and five Dlm of culture we found toxin over the entire period of illness, however, after these animals died we did not find any toxin in the organs and blood whereas the bacillus was isolated from the intestine and liver of all the animals examined.

Per os administration of the same doses of dry toxin was accompanied by a briefer circulation of the toxin in the blood of the sick animals, since it was demonstrated through the neutralization reaction only four hours after ingestion of 1--5 Dlm.

With intranasal administration of 1 and 2 Dlm of culture or dry Type B toxin we found toxin in the blood of the sick rabbits for the first twenty-four hours after infection, and in the case of dry toxin there were less positive results. Upon administration of 5 Dlm the rabbits died 24 to 36 hours later, therefore we were able to find toxin in the blood in four and 24 hours; in the agonal stage and after the rabbits died no toxin was found in the blood, whereas it was found in the lungs in every case.

In the blood of rabbits infected enterally with 1, 2 and 5 Dlm of broth type C culture, we were able to find toxin for the first two days from the time of infection, whereas with administration of dry toxin we could find it in the blood of the rabbits during the first hours after administration (table 2). After the animals died, we were unable to detect toxin in the blood and organs.

When the rabbits were given dry toxin or type C broth culture through the nose, comparable results were obtained, i.e. toxin was found in the blood after administration of 1--2 Dlm of culture for the first 24 hours, and after administration of dry toxin -- primarily during the first hours from the time of infection. Toxin was found in the blood of rabbits that had died after intranasal administration of 2 and 5 Dlm.

From our experiments it can be seen that upon reaching the organism through the digestive or respiratory tract the type B and C botulin toxin penetrated into the blood and circulated in it for a certain length of time, part of it was adsorbed by the tissues and as a result the toxin could be found in the blood and organs of the animals that died only when administered in large doses. The fact that the toxin remained in the blood for a longer time when infection was produced with broth cultures may be explained by additional production of toxin in the organism as

well as better penetration through the mucosa of the gastro-intestinal tract of native toxin than of dry toxin.

Concurrently with the animal experiments we made an examination of clinical material from patients and patho-anatomical material from human cadavers.

In examining the blood and excretions of 31 patients with symptoms resembling botulism in only one instance, patient I, age 33, did we find type B botulin toxin in the blood on the twenty-third day of illness (at earlier periods no botulism tests were run). Repeated examination of the blood of this female patient made after administration of antibotulin type B serum did not yield positive results. After treatment with specific serum the patient was released from the hospital in good condition.

Analysis of the case histories of the other patients revealed that in three instances the patients probably were suffering from botulism, since treatment of these patients with bivalent types A and B antibotulin serum had a positive therapeutic effect. However, neither in the blood nor in the excretions of the patients were we able to detect bacilli or toxin. This may be explained by the fact that the blood was examined after repeated administration of types A and B antibotulin serum to the patients.

In other cases, during the patients' stay at the clinic, the diagnosis was changed to "gastroenteritis," "paralysis of the facial nerve," "sequelae of periaxial encephalitis," "luminal poisoning," "poliomyelitis," etc.

Examination of the organs of 82 human cadavers suspected of toxic infection revealed five cases where the botulism pathogen was present.

Case No 1. Patient C, 73 years old, died on the nineteenth day following ingestion of boiled, reheated sturgeon. In the small intestine of the cadaver, using the reaction of neutralization with types A, B, C and E antibotulin serum, type A botulin toxin was found (up to 1,000 Dlm per millimeter). We failed to detect the toxin in the brain, liver, spleen and blood. Cultures from the small intestine and liver permitted isolation of type A botulin bacillus which developed a toxin with a potency of up to 50,000 Dlm (for the mouse) per milliliter on casein-fungus No 3 medium.

In two other instances the patients had been victims of a botulism outburst observed in Moscow in May, 1959, which hit five people who had eaten home-smoked salmon.

Cases No 2 and 3. Patient O, 52 years old and patient K, 33 years old, became ill four to five hours after

consuming some fish, and died 24 to 36 hours later with phenomena of acute intoxication. The small intestine from the cadaver of patient O and the blood and large intestine from that of patient K were sent down for examination. We were unable to detect any toxin in the blood or intestine of cadaver K. Cultures of intestine from both cadavers permitted isolation of a highly toxic strain of type B *Ci botulinum*.

Case No 4. Patient B, age 34, became ill after eating reheated boiled duck and died within 24 hours. Type B botulin bacillus was isolated from the liver of the cadaver, and it produced a toxin with a potency of over 50,000 Dlm per milliliter on artificial media. We were unable to find any toxin or bacilli in the other organs.

Case No 5. Patient C, 55 years old, became ill six to eight hours after eating reheated fried cod fish and died on the third-fourth day. Examination of the organs (liver, spleen, small and large intestine) revealed toxin in the small intestine and type B bacillus was isolated (with a toxicity of 1,000 Dlm, mouse doses, per milliliter). No toxin or bacilli were found in the other organs. In all of the described cases the botulism pathogen was isolated in cultures (unheated) on casein-fungus medium.

Conclusions

1. The possibility of detecting types B and C botulin toxins in the organisms of experimental animals depends on the dosage of administered toxin and method of administration.
2. With enteral inoculation, the toxin was found in the contents of the small intestine after administration of at least 5 Dlm of native toxin with type B bacilli and at least 15 Dlm of type C toxin, whereas for the detection of dry toxin two to four times higher doses had to be administered.
3. With intranasal administration types B and C toxins were found in the lungs of the animals that had died after receiving 2-3 Dlm.
4. In the blood of sick rabbits infected per os through the nose types B and C toxins were found 4, 24 and 48 hours after administration of 1-2 Dlm (for the rabbit) of native toxin with bacilli. With inoculation of dry toxin we were able to detect toxin in the animals' blood only during the first hours after infection.
5. Types B and C botulin bacilli were always isolated from the

small intestine and lungs of animals infected per os or intranasally with 1-2 Dlm, and we were most successful when working with unheated cultures on casein-fungus media (for type B) and Hottinger broth (for type C).

6. Examination of section material from human cadavers also showed that only with massive infection, leading to rapid death in the absence of specific treatment, was it possible to detect the toxin in the small intestine. The bacillus was usually isolated even in the instances where no toxin was found.

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Table 1.

a) Метод введения	b) Дозы токсина для кроликов (в D1m)	c) Вид токсина	d) Число опытных животных	e) Исследование крови через				
				4 часа	24 часа	18 часов	72 часа	96 часов
g) Через рот	1	Сухой k	3	3/2	3/0	3/0	3/0	3/0 пал
	2	Культура k	3	3/3	3/3	3/1	3/0	Пали
		Сухой k	3	3/3	3/3	3/0	Пали	
	5	Культура k	3	3/3	3/3	3/1	Пали	
		Сухой k	2	2/2	2/0	Пали		
Культура k	3	3/3	3/3	Пали				
j) Через нос	1	Сухой k	3	3/3	3/1	3/0	3/0	Пали
	2	Культура k	3	3/3	3/3	3/2	3/0	Пали
		Сухой k	3	3/3	3/3	3/1	Пали	
	5	Культура k	3	3/3	3/3	3/1	Пали	
		Сухой k	2	2/2	2/1	Пали через 36 часов		
	Культура k	3	3/3	3/2	Пали через 36 часов			

Detection of type I botulin toxin in the blood of rabbits

[Legend:] a) method of administration b) dosage for rabbits (D1m) c) form of toxin d) number of animals in the experiment e) blood examined after f) ...hours g) orally h) dry i) culture j) intranasally k) died l) died within 36 hours.

Note: the numerator = number of tests
the denominator = number of positive results

Table 2.

a) (g)	b) Дозы ток- сина в Dlm для кро- ликов	c) Вид токсина	d) Количество животных в опыте	e) Исследование крови через				
				f) 4 часа	f) 24 часа	f) 48 часов	f) 72 часа	f) 96 часов
g) Через рот	1 Dlm	Сухой k)	3	3/2	3/0	3/0	3/0	3/0
		Культура i)	5	5/5	5/5	5/5	5/1	Пали
	2 Dlm	Сухой k)	3	3/3	3/0	3/0	Пали	Пали
		Культура i)	4	4/4	4/4	4/4	Пали	—
	5 Dlm	Сухой k)	3	3/3	3/1	Пали	—	—
		Культура i)	4	4/4	4/2	Пали	—	—
g) Через нос	1 Dlm	Сухой k)	3	3/2	3/0	3/0	3/0	Пали
		Культура i)	3	3/3	3/3	3/1	3/0	Пали
	2 Dlm	Сухой k)	3	3/3	3/1	Пали	—	—
		Культура i)	3	3/3	3/3	Пали	—	—
	5 Dlm	Сухой k)	3	3/3	3/1	Пали	—	—
		Культура i)	3	3/3	Пали	—	—	—

Detection of type C botulin toxin in the blood of rabbits

[Legend:] a) method of administration b) dosage for rabbits
(Dlm) c) form of toxin d) number of animals in the experi-
ment e) blood examined after f) ...hours g) orally
h) dry i) culture j) intranasally k) died

Note: the numerator = number of tests
the denominator = number of positive results

- END -