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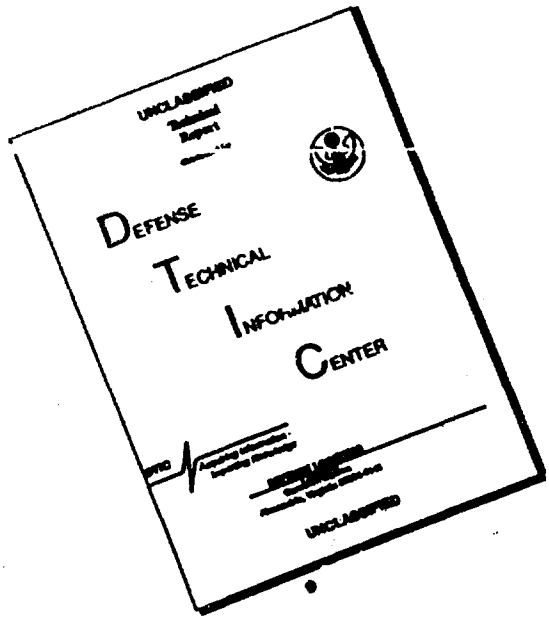

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On the Therapeutic Effects of Certain Protein Fractions of Anti-Plague Serum

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(From the Moscow Institute of Vaccines and Serums, imeni Mechinkov, The Central Asian Scientific Research-Institute and Government Control Inst.)

In connection with the fact that the protective actions of immune serums are associated with a determined portion of its albumen, at the present time, for therapeutic and prophilactic purposes, there are applications of cleansed and concentrated preparations, consisting mainly of globuline fractions. Thus, the successful use of injections of cleansed antitoxic and anti-infection serums is well known during diphtheria, measles, anaerobic and other infections.

In order to obtain more effective preparations there are studies to further define the specific components of immune serum and improve the method of cleansing and concentrating these preparations. In this respect the study of antiplague serum is necessary, because the required large injection of this preparation leads to a great albumen intoxication in many cases.

There has been little work on the problem of obtaining cleansed and concentrated antiplague serums, and also, their therapeutic and prophilactic properties.

The first works on the possibility of utilizing cleansed and concentrated antiplague serums, and also their effects, were conducted by Kaganov and Pokrovski. The authors proved that the therapeutic antiplague serum could be freed from the ballast albumen, even though the concentrated preparation was not obtainable. Positive results are reported on by Harvey, Pirie and Grasset, but the data are insufficient.

Thus, our problem was to establish whether or not certain protein fractions of antiplague serum possess therapeutic and prophilactic properties, and to what extent.

A liquid and dry gamma-globuline, liquid beta-globuline fraction and globuline of antiplague serum (so called dialyzed serum) were subjected to study.

The gamma- and beta-globuline fractions were prepared in the Mechifikov Institute by the spirit sedimentation method. The dialyzed serum was prepared in the Central Asian Scientific Research Institute. Experiments were conduted on white mice, weighing 15-20 grams, and guinea pigs, weighing 250-400 grams.

One particularity in the determination of the characteristics of the serums was the dose introduced, it was significantly larger than that in analogic experiments. Thus, we injected, into the animals' right inguinal region, subcutaneously, 2500 microbe bodies, which is 250 MLD. The cultures were injected in the increased doses to obtain better comparative data and to conclude whether or not the certain fractions of protein of antiplague serum, being cleansed and concentrated, would render a more effective action.

During the study of the prophilactic action, the fractions being studied were introduced once, simultaneous with the infection. During the determination of the therapeutic properties of the fractions, the injections were given 24-26 hours after infection and followed for 10 days, daily. The serum, as its fraction, was injected into the subcutaneous tissue of the peritoneal wall in volumes of 1 ml for guinea pigs, and 0.5 ml for white mice. Observations were conducted for 45 days, after which the animals were killed, dissected and their organs

bacteriologically studied. Animals dying during the studies were also studied. As a control we used a therapeutic antiplague serum, series No. 653 (pure) and normal equine serum.

The control group animals also received the virulent plague cultures, but not the preparations.

The criteria of the effectiveness of the serums was served by the results of survivorship of the animals, and also data on the average life of the dying animals (post infection life).

Four tests in all were conducted, one on white mice and 3 on guinea pigs.

The first orientating test on the study of the prophilactic properties of the liquid gamma-globuline fraction and liquid dialyzed serum was conducted on 25 guinea pigs (5 per group). Results were as follows: the best protective characteristics, in contrast with the pure antiplague serum, were rendered by the liquid gamma-globuline fraction. Of 5 pigs receiving the test fraction, 3 lived. The average life of the pigs after infection was greater in this group (18 days) than the life of the control (8 days). In the groups receiving the dialyzed and pure antiplague serums, 4 pigs died. Those animals receiving the preparation, as well as those receiving the normal equine serum, died om the 5-7th day.

Having obtained results in the preliminary experiments on guinea pigs which indicated the presence of protective properties in certain fractions of albumen of antiplague serum, we started our tests on mice. We studied the therapeutic action of various fractions. For this we divided 110 animals into 6 groups (5 groups of 20 and 1 of 10). The results are as follows: all those animals receiving the normal equine serum, or not any serum, died in periods from 4-8 days after infection (% of survivors equaled 0). It is necessary to note that those animals receiving the

normal equine serum died in somewhat earlier periods, in comparison with the control. Of those mice receiving the antiplague serum, series 653, 30% lived. Better results were obtained with the use of the liquid gamma-globuline fraction (40%), and liquid dialysed antiplague serum (45%). Thus, the preliminary tests on white mice also indicate the presence of therapeutic properties in the gamma-globuline fraction and dialysed serum.

After the preliminary tests on the mice, conforming experiments were conducted on guinea pigs (two tests).

Fifty guinea pigs were taken for the first test (5 groups). The test serums (liquid gamma-globuline fraction and dialysed serum) were introduced daily for ten days, 1 ml per day, for therapeutic results(the animals receiving the liquid gamma-globuline fraction received it for only 5 days because of insufficient preparation on hand.).

The results of this test can basically be considered analogical with those results obtained in the preliminary experiments on white mice and guinea pigs, with only one difference, the later test had a therapeutic effect expressed in the lenghtened post infection life of the animals (the culture was injected in doses of 250 MLD, and the test fraction-one ml daily for 10 days). As in the preliminary tests, so here, the gamma-globuline showed the best therapeutic properties.

Having obtained results indicating the introduction of 1 ml of the test serum into the guinea pigs does not insure life, but only extends the post infection life period, we decided to pin point the variations needed; introduce the preparation earlier, in smaller doses, etc. We also wanted to know what effect the test fraction had on the character of course of the experimental plague, what moments extended the pre-death period.

To clearify the above, we ran a third test on 395 pigs. In this

test we studied the therapeutic properties of the liquid and dry gamma-globuline and the beta-globuline fraction. We introduced the serums pure and in dilutions of 1:5, 1:10, 1:20, 1:30. Thus, we had 23 groups of pigs, with 20 in each group. Also, the dose of serum in the first three days of treatment was two times greater. Thus, the doses introduced the pigs (test fraction and control) was 2 ml at the start of treatment and later only 1 ml. Daily, for ten days, we killed one animal from each group, subjected it to dissection and bacteriological study. The group receiving the beta-globuline fraction had only 5 animals, due to the lack of preparation, and therefore daily dissections were not made.

From the table it is seen that all the animals in the three control groups, including those receiving the pure antiplague serum, died in 5-12 days. There were deaths in the other groups also; in the group being treated with liquid beta-globuline and dry gamma-globuline, all the pigs died, only in the group receiving liquid gamma-globuline did some of the pigs survive (pure gamma-globuline and dilutions of 1:5 and 1:10). The percentage surviving was 30-50%. All the pigs died in those sub-groups receiving the liquid gamma-globuline in the smaller doses (1:20-1:30).

The post infection period of life was greatest in the group receiving the liquid gamma-globuline; next was the dry gamma-globuline, then beta-globuline and last-pure antiplague serum.

Thus, the most effective treatment was rendered by the liquid gamma-globuline fraction. Thus, there was a 30-50% survivorship among those pigs receiving this fraction, during the injection of 250 MLD; the remaining groups, animals treated with pure serum as well as the control, died in 6-19 days.

Results of bacteriological study of the organs were as follows: in the control groups and in those animals receiving the normal equine serum,

there was a generalized process on the 2nd or 3rd day. Cultures of plague baccilli were isolated from the lymph nodes, lungs, kidneys, liver, blood, etc. Somewhat different results were obtained with the use of the pure antiplague serum. The generalization of the process in these animals was prevalent on the 5th or 6th day. In those groups treated with dry and liquid gamma-globuline fractions the process was localized, regional. It was possible to isolate microorganisms from the place of injection of the culture or from the inguinal regional lymph nodes only.

Thus, these gamma-globuline fractions slowed the process to the 10th day or later, and localized the process.

A. G. Stogov studied the specificity of the test fractions. The specificity was determined with the aid of the precipitation reaction. The question was whether or not the test fractions possessed the antibody characteristics and, consequently, the ability to react with specific antigens. A capsule-somatic antigen was prepared from the plague culture according to Zhukov-Vereshnikov and Lipatov. The results obtained were as follows: a ring of precipitation with all the test serums (pure antiplague, and also gamma- and beta-globuline fractions) was present if the antigen was added undiluted.

During the running of reactions with diluted antigens (1:2, 1:4, 1:8, and 1:16) the ring of precipitation was obtained only with the cleansed gamma- and beta-globuline fractions. Consequently, both of these fractions possess the characteristics of specific antigens.

CONCLUSIONS:

1. Certain fractions of antiplague serum albumen (gamma- and beta-globuline, and also globuline in the form of dialyzed serums), being introduced into the organism of experimental animals, renders a therapeutic action.

2. The therapeutic characteristics are expressed greatest by the liquid cleansed concentrated gamma-globuline fraction. This preparation surpasses the action of the original antiplague serum (series No. 653).

3. The introduction of the test fractions of antiplague serums aids in the localization of the plague infection in the regional lymph nodes.

4. The gamma- and beta-globuline antiplague serums possess characteristics of antibodies, which are expressed by the formation of a ring of precipitation during the addition of capsule-somatic antigens of cultures of plague microbes to them.

5. Because of the therapeutic characteristics, and also the antibody characteristics of the gamma- and beta- globuline fractions, they can be used at the present, even though their full evaluation demands more study.

Zhukov-Vereshnikov, N. N., Immunology of Plague, M. —L., M., 1940.—
Pirie, J. H. and Grasset E., Brit. Journ. Exp. Pathol., 1935, v. XVI, p. 126-128.

One(1) Table.

Restuls of survivorship of guinea pigs, receiving fractions of antiplague serum albumen in various dilutions

GROUP	GROUP NUMBER	NUMBER OF ANIMALS IN GROUP	INFECTION		IDENTIFICATION OF SERUM	INJECTION OF SERUM		PURE SERUM				DILUTED PHYSIO- +1:5+			
			METHOD	DOSE(QUANTITY OF MICROBE BODIES)		METHOD	DOSE (IN ml)	DIED	PERIOD OF LIFE AFTER INFECTION (DAYS)	SURVIVED	% OF SURVIVORSHIP	DIED	PERIOD OF POST-INFECTION LIFE (DAYS)	SURVIVED	
TESTED	1	10	Subcutaneous	2500	Liquid cleansed, concentrated gamma-globuline fraction of anti-plague serum	Subcutaneous	1-2	7	19.0	3	30	5	23.2	5	
	2	10		2500	Dry cleansed, concentrared gamma-globuline fraction etc.		1-2	—	—	—	—	10	19.7	0	
	3	5		2500	Liquid cleansed concentrated Beta-globuline fraction etc.		1-2	5	14.0	0	0	5	10.8	0	
CONTROL	4	10	Subcutaneous	2500	Pure antiplague serum (653)	Subcutaneous	1-2	10	12.8	0	0	10	12.8	0	
	5	10		2500	Normal equine serum		1-2	10	6.5	0	0	10	6.1	0	
	6.	10		2500	Control(without treatment)		—	10	5.4	0	0				

LOGICAL SOLUTIONS

1:5	1:10				1:20				1:30			
% surviving	DIED	POST INFECTION PERIOD	SURVIVED	% SURVIVING	DIED	POST INFECTION PERIOD	SURVIVED	% SURVIVING	DIED	POST INFECTION PERIOD	SURVIVED	% SURVIVING
50	6	16.3	4	40	10	14.6	0	0	10	10.7	0	0
0	10	13.8	0	0	10	12.6	0	0	10	9.6	0	0
0	5	12.4	0	0	5	10.8	0	0	5	10.9	0	0
0	10	11.3	0	0	10	10.5	0	0	10	9.5	0	0
0	10	6.2	0	0								