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ANAPHYLACTOGENIC, ANTIGENIC AND AVID PROPERTIES OF ANTIBOTULIN --ETC(U)
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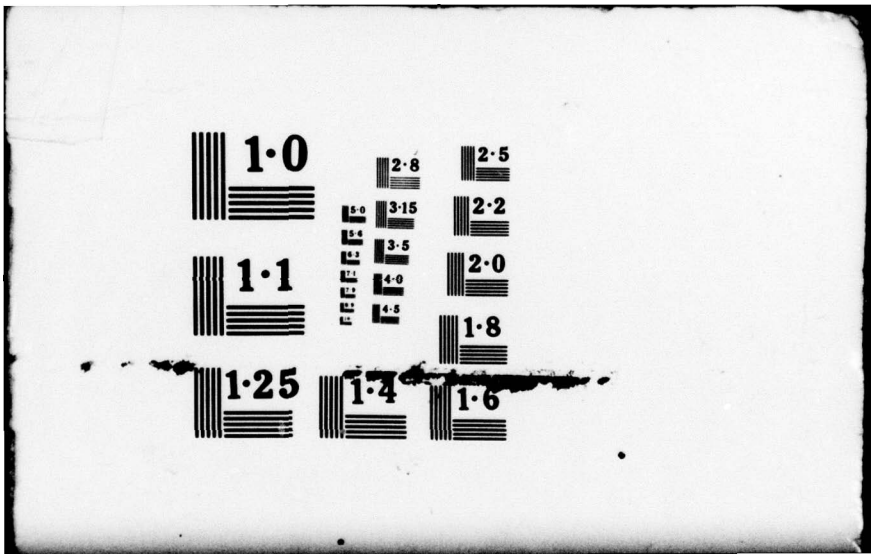
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ANAPHYLACTOGENIC, ANTIGENIC AND AVID PROPERTIES OF ANTIBOTULIN SERUM FROM THE BLOOD OF VARIOUS SPECIES OF ANIMALS

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Introduction. The authors demonstrated earlier [4] that purified concentrated antibotulin serum from the blood of cattle assures a less pronounced degree of humoral immunity than does serum from the blood of horses. At the same time, as regards prophylactic and therapeutic effects, cattle serum is in no way inferior to horse serum. First of all, the therapeutic effect of horse serum is reduced [2] whenever the organism is sensitized to horse protein. In this case, in order to increase the effectiveness of the preparation, it is possible to use serum from another species of animal (including horned varieties) in the treatment of botulism--a serum to which the organism is less sensitive than to horse serum [5].

In the present report we give the results of a comparative study of the anaphylactogenic, antigenic and avid properties of purified, concentrated antibotulin serum of Type B from the blood of both horned cattle and horses.

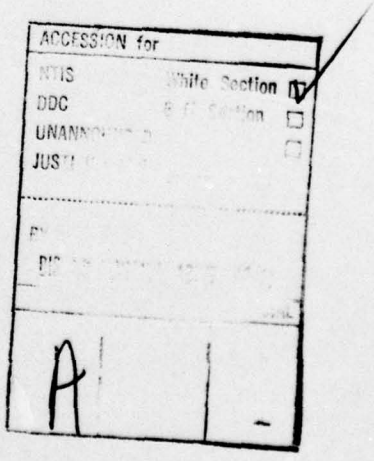
Materials and methods. Our study of anaphylactogenic properties of preparations was conducted on guinea pigs ranging in weight from 350 to 400 grams; two methods were used. With use of the first method [1] we employed 94 guinea pigs as test animals, these being sensitized with a single 0.1 mg subcutaneous injection of protein: animals of the first and third groups received horse blood serum which had been purified and

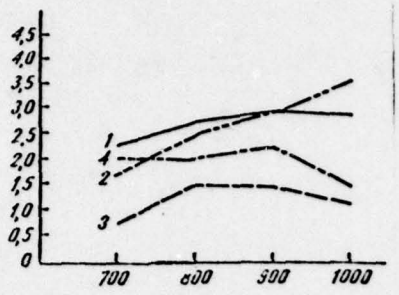
and concentrated by the "Diatherm" method; animals of the second group received cattle blood. After 2 weeks following sensitization, guinea pigs of the first and second groups received a definitive intracardiac dose of cattle blood serum, the dose varying from 1 to 400 mg in terms of protein; while animals of the third group received horse blood serum in the same quantities. The guinea pigs were observed for a period of two hours following the definitive dose, the result being recorded in terms of survival time. In following the second method [6] 58 guinea pigs were sensitized a single time, using 20 mg of protein administered subcutaneously, one group being sensitized with horse blood serum, the second with cattle blood serum, the definitive doses being 700-800-900-1,000 mg given on the 21st day following sensitization. Each group was split up into 2 subgroups: the animals of one of these were injected with horse blood serum, those of the second with cow blood. The anaphylactogenic properties of the preparations were estimated on the basis of the reaction which the animals showed following administration of the definitive dose. The test animals were then killed. Observations were conducted for a period of two hours. The results were evaluated in terms of the number of reactions per test animal (guinea pigs).

For a comparative value of the antigenic property and species specificity of the serums, we used the reaction of dual diffusive precipitation (RDDP) in agar gel. The precipitating sera were obtained from rabbits by means of hyper-immunizing the animals with antibotulin (Diatherm) serum from horse blood (the first group, 4 test animals) and also from cattle blood (second group, 3 test animals). Blood gamma-globulin was also used (third group, 3 animals). The preparations were administered

subcutaneously with a 7-day interval, in doses of 50, 100, 200, 400 and 600 mg of protein. Blood tests were made on the seventh day following immunization. The antisera obtained, whole and in solutions, were tested with homological and heterological antigens containing a standard amount of protein (100 mg).

The avid properties were studied, following Freyman's method [7] as adapted to antibotulin serum. Here we made use of unpedigreed white mice ranging in weight from 16 to 18 g. Observations were made over a period of 4 days. To determine the rate and strength of bonding of the toxin with the antitoxin, we first of all took titered series of the sera, which, in combination with the toxin following a 45-minute exposure with intravenous administration, produced only illness in the animal, but not its death. To study the rate of interaction of toxin and antitoxin, the animals were injected immediately following mixing of those substances, then again after 5, 10, 20, 30 and 45 minutes. To determine antitoxin-toxin bonding strength, we waited 45 minutes, following which various doses of antitoxin, from 0.01 to 0.05 ml, were added. Following a 1-hour exposure, the mixtures were administered to the white mice. In the comparative test we studied 5 series of the preparation from both cattle and horse blood.





Anaphylactogenic properties of blood sera from various animals.

1 - sensitizing and definitive injections of blood sera from cattle; 2 - from horse blood; 3 - sensitizing injection of serum from horse blood, definitive injection from cattle blood; 4 - sensitizing injection of serum from cattle blood, definitive injection from horse blood. On the x-axis is shown the amount of protein (in micrograms) in the definitive dose; on the y-axis, the number of reactions exhibited by the individual test animal (guinea pig).

Results and discussion. Analysis of the material demonstrated that with subcutaneous sensitization of guinea pigs with sera containing 0.1 mg of protein, and with intracardiac administration of a definitive dose of serum protein 2 weeks following a definitive dose of blood serum from the same species of animal whose serum was used to produce sensitization, or else from another species in an amount from 1 to 250 mg (in terms of protein), there was no difference whatever in anaphylactogenic properties. With increase in the definitive dose up to 300 and 400 mg, however, some differences appeared: with a single subcutaneous sensitization with 0.1 mg of protein from horse serum, and with intracardiac administration of a definitive dose of horse serum with amount of protein given to a single guinea pig of 300 mg (in one case) and 400 mg (in another), all of the animals remained alive; while with sensitization and definitive administration to a single pig (horse-horse or cattle-cattle)

of a dose of 300 mg and 400 mg (to two different test animals), a number of the tested animals died. With subcutaneous sensitization of guinea pigs 21 days following the definitive dose of serum in various amounts (of protein), the anaphylactic reactions appeared only 3-5 minutes following the injection, and maintained themselves for periods of 20-30 minutes. Calculation of the number of exhibited reactions (per pig) showed (See Illustration) that as regards anaphylactogenic reactions, antibotulin sera from horse and cattle blood differ only insignificantly; although, as Pushkarev [3] has pointed out, the native horse sera are rather more anaphylactogenic than the cattle sera. Sensitization and subsequent administration of single-type sera were found to produce a greater number of anaphylactic reaction than did the use of sera from various different species of animals.

The results of our study of antigenic properties of purified and concentrated sera from cattle and horse sera showed (Table 1) that the antigenic properties of sera from various animal species, when purified by the "Diatherm" method, differ quite little. However, gamma-globulin from cattle blood was found to be more active in the antigenic sense: the lines of precipitation appeared with greater dilutions of the antisera.

Comparison of the species specificity of heterogeneous serum preparations, conducted in RDDP in a cross test, shows conclusively that various nonspecific precipitins, quite apart from species-specific antibodies, are produced in response to cattle and horse sera. These are formed at a lower titer, upon the administration of either horse or cattle protein.

TABLE 1

Results of RDDP in Agar Gel

Antiserum	Antibody titer in RDDP (Means)			
	with homologous antigens		with heterogeneous antigens	
	antigen	serum titer	antigen	serum titer
Against horse sera	horse serum	1:7	cattle serum	1:0
Against cattle sera	cattle serum	1:8	horse serum	1:0
Against cattle gamma-globulin	cattle gamma-globulin	1:21	" "	1:1

TABLE 2

Rate of Union of Toxin with Antitoxin of Antibotulin Sera from the Blood of Various Species

Time of persistence of toxin with antitoxin min.	Cattle blood anti.				Horse blood antitoxin			
	Results of observations							
	healthy	slight intoxication	ill	death	healthy	slight intox.	ill	death
	number of mice							
0	4	0	2	4	0	0	0	10
5	4	2	4	0	2	0	8	0
10	6	4	0	0	4	2	4	0
20	2	6	2	0	8	0	2	0
30	4	2	4	0	6	0	4	0
45	0	4	6	0	0	6	4	0
No. of mice. abs.	20	18	18	4	20	8	22	10
%	33,4	30,0	30,0	6,6	33,3	13,3	36,7	16,7

Verification, in the comparative test, of the avidity of cattle and horse sera purified by the Diatherm method based on the rate of union of toxin with antitoxin (Table 2) showed that sera from cattle blood do not differ in this respect from horse sera. Analogous data were obtained in testing the strength of the antitoxin-toxin bond (Table 3).

Thus, the results of our comparison of the properties of purified, concentrated antibotulin sera from the blood of various animal species justify speculation on the possible use of horse- and cattle-derived sera in the prophylaxis and treatment of botulism. At the same time, apart from sensitization of the organism to horse-derived protein, a second administration of cattle serum would assure a lesser degree of reactogenicity of the preparation. All this argues the advisibility of the manufacture of such preparations from cattle blood.

TABLE 3

Strength of Union of Toxin with Antitoxin in the Antibotulin "Diatherm" Sera of Type B, from Various Animal Blood

Amount of Type B anatoxin, ml	Cattle blood ana.				Horse blood anatoxin			
	Results of observations							
	healthy	slight intoxication	illness	death	healthy	slight intoxication	illness	death
	number of mice							
0,01	0	4	6	0	0	2	8	0
0,02	0	2	4	4	0	0	8	2
0,03	0	0	4	6	0	0	4	6
0,04	0	0	2	8	0	0	4	6
0,05	0	0	2	8	0	0	4	6
No. of mice, abs.	0	6	18	26	0	2	28	20
%	0	12	36	52	0	4	56	40

Conclusions

1. Differences in anaphylactogenic properties of purified and concentrated sera from horse and cattle blood were not observed. At the same time, during sensitization of the organism to protein from one species, the use of sera from a second species was found to assure a lesser degree of reactogenicity of the preparation.

2. Antigenic activity of purified and concentrated sera from horse and cattle blood during tests on rabbits was found to be identical; but, upon the administration of cattle gamma-globulin, the animals responded with a more intensive production of precipitins.

3. Avidity of cattle- and horse-derived purified antibotulin sera was found to be identical, as judged on the basis of bond strength (with the corresponding toxin).

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ANAPHYLACTOGENIC, ANTIGENIC AND AVID PROPERTIES OF THE ANTIBOTULIN SERUM FROM THE BLOOD OF VARIOUS TYPES OF ANIMALS

G. E. Sinelnikov, S. M. Preger, N. Kh. Muzafarova

It was shown that purified and concentrated by the «Diaferm» method antibotulin sera from horse and cattle blood failed to differ by anaphylactogenic properties; at the same time in sensitization of the organism to protein of one animal species the use of the sera of another species provided a lesser reactogenicity of the preparation.

The antigenic activity of the purified and concentrated sera from the blood or horses and cows in testing on rabbits was identical, but in response to cow α-globulin the animals responded by a more intensive production of precipitins.

The activity of cow and horse antibotulin serum (determined by the rate and stability of their association with the corresponding toxin) proved to be identical.