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PREPARATION OF STANDARD CLOSTRIDIAL ANTITOXINS IN SHEEP

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Horse and sheep have so far been used for preparation of Clostridial antitoxins by several workers (1,2,6). The present communication reports the results of an attempt which was made at this laboratory to prepare standard antitoxin against several types of Clostridium perfringens, Clostridium oedematiens and Clostridium septicum by hyperimmunisation of sheep.

MATERIALS AND METHODS

Preparation of toxins – Toxigenic strains of Clostridium perfringens, types A,C,D, Cl. septicum and Cl. oedematiens type B were used in this study.

The medium for production of Cl. perfringens types C and D toxins was consisted of 3% Difco proteose peptone, 0.9% Na_2HPO_4 and 0.25% sodium chloride. The pH was adjusted to 7.5 and sterillised by autoclaving at 110°C. for 30 minutes.

The VF (viande-foie) medium was used for preparation of Cl. perfringens type A, Cl. oedematiens type B and Cl. septicum toxins.

The toxin was obtained by filtration of cultures and kept at 4°C. until use. Two hours before injecting sheep, sterile 10% potash alum solution was added to the toxin to give a final concentration of 1.5%, and the pH was adjusted to 5.8 by using 5N caustic soda.

Selection of the animals – Thirty two native sheep were each given 2 injections of 2 ml. of Alum Precipitated Toxoid (A.P.T.) enterotoxemia polyvalent vaccine. Two weeks after the second injection the level of immunity of each animal was determined by assaying the serum of each individual. The animals with high level of potency against each type were selected for preparation of standard Clostridial antitoxins against that particular strains. Ten selected sheep were used for preparation of the antitoxins.

The animals were hyperimmunised in three cycles. In the first cycle, the animals were given a series of 8 injections of 1,2,3,5,10,10, 20 and 40 ml. of alum precipitated toxin at 4 days interval. In the second cycle of immunisation the animals were given 3 injections of 20,40, and 80 ml. with four days interval. The third cycle consisted of 3 injections of 20,40, and 80 ml. of antigen with intervals of four days. After each cycle of immunisation the animals were given four weeks rest. Seven days after the last injection of each cycle of immunisation 1000 to 1500 ml. of blood was obtained and the serum was separated from each sample. To the pooled sera of each cycle 0.3% tricresol was added and stored at 4°C, until use.

Purification and Concentration

The purification and concentration of each type of collected crude sera of Cl. perfringens types A,C,D, Cl. oedematiens type B and Cl. septicum was performed by the following method (4).

One litre of the crude serum was added to two litres of distilled water and the temperature brought up to 30°. The pH of the mixture was carefully adjusted to 3.2 with 1/4 dilution of concentrated HCl. Five grams of pepsin was then stirred in and an incubation of 30 minutes at 30°C, was allowed for digestion. 390 grams of ammonium sulphate and 1% toluen was then added and the pH was carefully adjusted to 4.3 using 10% sodium hydroxide. The temperature was immediately raised to 55°C, and the temperature kept the same for a period of 30 minutes, 108 grams of ammonium sulphate was added and stirred in. The mixture was then cooled to 48°C, and filtered, using a No. 3 filter paper and cetite 503 (Hyflo). To the filtrate 570 grams of ammonium sulphate was added and thoroughly stirred in until the precipitation was completed. The obtained precipitate was then dissolved in adequate amount of buffer pH 7.0.

The resulting solution was then dialysed against normal saline at pH 7.0 for two days at 4°C. To the final product 0.3% tricresol was added after being filtered and kept at 4°C, for further procedure.

Standardization

For standardization of the potencies of concentrated sera the following materials were used.

1- Dried alpha, beta and epsilon toxins (5).

2- Clostridium oedematiens type B and Clostridium septicum toxins concentrated by polyethylene glycol compound 20-M (Carbowax) (3).

3– Gas gangrene antitoxin (Cl. perfringens type A), Gas gangrene antitoxin (Cl. oedematiens) and Gas gangrene antitoxin (Cl. septicum) supplied by Statens Seruminstitut (*). Cl. perfringens type C antitoxin and Cl. perfringens type D antitoxin obtained from Central Veterinary Laboratory Weybridge (**).

The standard antisera which previously adjusted to 1 unit per ml. was mixed with the various dilutions of dried or concentrated toxins in a total volume of 2 ml. Toxin–antitoxin mixtures were shaken thoroughly, and left at room temperature for half an hour before injection. O.5 ml. of the contents of each tube was injected intravenously into each of 3 mice and the result of the test read up to 3 days. To assay the unknown antiserum, the test dose toxin was mixed with the serial dilutions of the serum. Toxin antitoxin mixtures were held at room temperature for 30 minutes, then 0.5 ml. of the contents was injected intravenously into each of 3 mice. The 50 per cent end point was then calculated and considered as the titre of the concentrated serum.

RESULTS

The titre of the sera collected from sheep following the 1st, 2nd and 3rd cycles of immunisation against Cl. perfringens types A,C,D,Cl. oedematiens type B and Cl. septicum could be seen in Table 1.

Concentration and purification of the immune sera however, resulted the serum samples with 1000, 15000, 3000, 250 and 200 International Units/ml· Cl. perfringens types A,C,D, Cl. oedematiens type B and Cl. septicum, respectively. The prepared sera were standardized and successfully used for identification and other studies of the Clostridial organisms. This study however, indicated that sheep could successfully be used for preparation of standard Clostridial antitoxins.

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TABLE 1

Microorganism	Number of sheep immunised	Titres of sheep serum in International Units/ml. after: Hyperimmunisation		Concentration of pooled serum
Cl. perfringens type A	2	first inoculation	300	
		second »	350	1000
		third »	500	
Cl. perfringens type C	2	first »	2500	
		second »	4500	15000
		third »	6000	
Cl. perfringens type D	2	first »	400	
		second »	600	3000
		third »	650	
Cl. oedematiens type B	2	first »	150	
		second »	200	250
		third »	250	
Cl. septicum	2	first »	180	
		second »	290	200
		third »	700	

SUMMARY

A procedure for preparation of the standard Clostridial antitoxin in sheep was described. The native sheep used for this investigation were highly resistant to hyperimmunisation with Clostridial toxins which high titre of the Clostridial antitoxins were obtained. The standard antitoxins were prepared by purification and concentration of hyperimmune sera.

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REFERENCES

(1) Bittner, J., et coll., (1966). Arch. Roum. Path. Exp. Microbiol., T. 25, No 1.

(2) Erdogan, I., et al. (1968). Pendik Vet. Kontrol Ar. Enst Derg. Vol. 1, No. 2.

(3) Phillips, A.W. and Batty, I. (1963). Nature, Vol. 198, No. 4886.

(4) Pope, C.G. (1938). Bri. J. exp. path. 19.

(5) Schuchardt, L.F., Munoz, M.S., and Vervey W.F., (1954). Am. J. Vet. Res., 15.

(6) Weinberg, M., et Guillaumie, C.R. (1935). Soc. Biol., 120.