THE NATURE OF ANTISTREPTOLYSIN "S" IN THE SERA OF MAN AND OF OTHER SPECIES: ANTISTREPTOLYSIN TITRES IN NORMAL AND DISEASED STATES.

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THE study of haemolysins produced by streptococci and of antibodies against them was greatly clarified by Todd (1938), who first clearly differentiated streptolysin "S," a serum-soluble, oxygen stable haemolysin, from streptolysin "O," which is readily formed in serum-free media and is inactivated by oxygen. It has been shown by him (Todd, 1932), and by many workers since, that streptolysin "O" is a powerful antigen, and that infection with strains of streptococcus which produce this haemolysin is generally followed by a sharp rise in the antistreptolysin "O" content of the serum. Streptolysin "S," on the other hand, does not readily give rise to antibodies, and although sera from various animals have long been known to contain inhibitors of "streptococcal haemolysin" (Lyall, 1914; McLeod and McNee, 1913), no increase in inhibitory action was observed after injection of haemolytic streptococci. Todd, however, claimed that by prolonged courses of intravenous injections of living Group A streptococci it was possible to obtain antisera in rabbits to streptolysin "S," that such antisera were specific for streptococci of this group, and that antibodies against streptolysin "S" were distinct from those against streptolysin "O" (Todd, 1938, 1939; Herbert and Todd, 1944). In 1939 Todd, Coburn and Hill published a study of the antistreptolysin "S" and "O" titres in normal adults, and in children with haemolytic streptococcal infections with and without rheumatic fever. The variations in antistreptolysin "S" titre were not large, but from a statistical analysis of their data these authors concluded that in response to infection with haemolytic streptococci there is a rise in the antistreptolysin "S" titre considerably above the normal level in the sera of all groups studied, with the exception of those children who developed clinical signs of rheumatic activity. Furthermore, during rheumatic attacks the antistreptolysin "S" titres tended to be lowest when the clinical symptoms were most pronounced.

Since antibodies to other streptococcal products (e.g. streptolysin "O," streptokinase, hyaluronidase, proteinase) have been shown by other workers to be increased in the sera of patients with rheumatic fever in much the same way as in the sera of persons with streptococcal infections, but without clinical rheumatic activity, it seemed that the antistreptolysin "S" response might be a clue to some significant difference between the rheumatic and the non-rheumatic groups. A limited study of such sera, which is reported below, showed that nearly all sera taken from sick persons had lower anti-streptolysin "S" titres

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than those from normal people, and failed to reveal the difference found by Todd, Coburn and Hill (1939) between those streptococcal infections which were accompanied by clinical rheumatic fever and those which were not. The investigation was extended therefore to attempts to produce immune sera to streptolysin " S " in rabbits and a horse, both by the injection of living streptococci and by potent filtrates of yeast nucleic acid broth cultures (Okamoto, 1939). These attempts were not successful, and since an examination of sera from several species of animal revealed considerable amounts of antistreptolysin " S " in cases where any streptococcal infection could be excluded, the status of antistreptolysin " S " as an antibody in the accepted sense of the word is called in question. In this paper are presented the results of antistreptolysin " S " investigations in untreated sera.

MATERIALS AND METHODS.

Sera.

Sera were obtained fresh and were stored without preservative at — 20° C. No alteration in antistreptolysin titres were observed during several months under these conditions.

Streptolysin " S."

Two strains of Group A streptococcus were used for production of streptolysin " S." During the earlier stages the strain used (NY5) was a Type 10 streptococcus, which produced powerful streptolysin " S " and some streptolysin " O." It was grown at 37° C. overnight in a beef heart infusion broth containing 10 or 20 per cent horse serum. The cocci were centrifuged down and were extracted with horse serum, thereby giving a powerful haemotoxin, which was stored in ampoules at — 76° C.

During the later stages there became available a Type 11 strain (Blackmore) which had previously been shown by Todd to produce streptolysin " S " but not " O." This strain was grown overnight at 30° C. in 20 per cent serum broth, and the euglobulin fraction of the supernatant fluid, which contained the bulk of the streptolysin activity, was precipitated according to the method described by Herbert and Todd (1944). The precipitate was dissolved in borate buffer saline pH 8·0, and stored in ampoules at — 76° C. At this temperature both the above preparations were stable for at least 6 months, although at — 20° C. or higher they rapidly declined in potency.

Towards the end of this investigation it became desirable to have a preparation of streptolysin " S " free from serum, in order to eliminate possible effects of the serum moiety. Use was made of the observation of Okamoto (1939), recently amplified by Bernheimer and Rodbart (1948), that in the presence of yeast nucleic acid large amounts of oxygen stable streptolysin are found even in the absence of serum. Strain Blackmore grown for 24 hours in infusion broth containing 1 per cent yeast nucleic acid yielded a potent haemolytic filtrate, which was reasonably stable at — 20° C. or at 4° C. As will be shown below, this filtrate was serologically equivalent to haemolysin produced in serum broth.

Finally, for comparison, a strain of group C streptococcus (Loewenthal M) was grown in 20 per cent horse serum broth in the same manner as the Group A Type 11 strain, and from it the haemolytic euglobulin fraction was also prepared.

Titration of antistreptolysin " S."

The method used was that given by Todd (1938), in which the unknown serum is titrated against a sample of streptolysin which has been standardized against a standard serum at the time of test. The estimations were carried out in borate buffer saline, at pH 8·0, and rabbit erythrocytes were used as indicators. It was found that a suitable amount of streptolysin was that which caused 50 per cent lysis of the erythrocytes in 2 hours under standard conditions. A normal human serum was set aside as a standard ; it was kept stored at — 20° C. To it was assigned an arbitrary value of 10 units antistreptolysin " S " per ml. Although this standard was not the same as Todd's standard, it was probably fairly close, since the range of antistreptolysin titres found did not differ greatly from his previous records.

Titrations of antistreptolysin " O."

These were done by Todd's (1938) method, modified by use of an isotonic phosphate buffer pH 6·5 for dilutions and of M/100 thiolacetic acid for reduction as recommended by Herbert and Todd (1941).

Immunization of experimental animals.

Rabbits.—Six rabbits of mixed breeds were given intravenous injections of Streptococcus Blackmore grown in 20 per cent rabbit serum broth, and suspended in the supernatant fluid. The first two injections consisted of killed organisms, but all later injections contained living streptococci. Injections were given 2 or 3 times weekly, with rest periods of a fortnight or more between courses. The total volumes of packed living organisms injected varied from 0·6 to 1·0 ml., and of culture fluid from 12 to 20 ml. Serum samples were taken at the beginning, in the middle, and one week after the end of each course.

Horse.—The course of immunization was kindly performed by Dr. C. L. Oakley, at the Wellcome Research Laboratories, Beckenham. Five intramuscular doses of formolized culture filtrate from streptococcus Strain Blackmore grown in yeast nucleic acid broth were followed by a total of nearly 3 l. of untreated filtrate, in 26 doses over a period of 15 weeks. Test bleedings were made weekly.

RESULTS.

Antistreptolysin " S " in human sera.

The summarized results of estimations on pathological and normal human sera are given in Table I, from which it appears that the highest antistreptolysin " S " titres were found in normal adults and in late convalescent cases of rheumatic fever. During the acute phases of streptococcal and other infections, and during the early stages of convalescence, the antistreptolysin " S " titres were comparatively low. It is also apparent that antibodies against streptolysins " S " and " O " behaved independently.

The lowest antistreptolysin titre encountered (not included in the table) was 2·7 units/ml. in the serum of a patient with steatorrhoea who was on a fat-free diet, and the highest value was 15 units/ml. in the serum of a patient with Type II nephritis and raised serum lipoids.

TABLE I.—*Antistreptolysin "S" Titres (units/ml.) in Human Sera.*

The mean antistreptolysin "O" titres are given for comparison.

Disease.	No. of sera.	Anti-streptolysin "S." Range.	Anti-streptolysin "S." Mean.	Anti-streptolysin "O." Mean.
Scarlet fever—acute	8	3·2–8	6·3	35
Convalescent (14–16 days)	8	4–8	6·2	240
Streptococcal pharyngitis—acute	4	4–6·7	5·2	410
Subacute and convalescent	4	2–9·5	6·1	240
Rheumatic fever—acute	8	5–6·7	6·3	428
Subacute	16	4–10	6·7	261
Convalescent	15	5·3–12·5	8·4	275
Rheumatoid arthritis—acute	4	5·3–9·5	6·5	92
Subacute	4	4–7·8	6·3	130
Normals	14	8–12·5	9·7	48

Antistreptolysin "S" in the sera of other animals.

In Table II are given antistreptolysin "S" titres of various mammals, of representative teleost and elasmobranch fishes and of a marine invertebrate. The marine animals were included because they are not known to be subject to streptococcal infections, and it was of interest to learn whether their sera would inhibit streptolysin "S." The high titres found in normal rat and guinea-pig sera are noteworthy.

TABLE II.—*Antistreptolysin "S" in the Sera of Various Animal Species.*

Species.	No. of sera.	Antistreptolysin "S" (units/ml.). Range.	Mean.
Normal human	14	8–12·5	9·7
Horse	7	10–27	16
*Horse (*Cl. welchii* antitoxin)	6	5–7	5·5
Rabbit	12	3·2–8·1	6·6
Rat	2 pools (36 sera)	50–60	55
Guinea-pig	4 pools (12 sera)	32–56	43
Ox	1	..	23
Sheep	1	..	12
Plaice (*Pleuronectes*)	2 pools	48–50	49
Dog fish (*Scyllium*)	1 pool	..	5
Spider crab (*Carcina Maia*)	1 pool (whole blood)		1·2

* These sera were preserved with 0·3 per cent tricresol. It was found that this preservative caused a fall of 25 per cent in the antistreptolysin "S" titre in one week at + 4° C.

Correlation between antistreptolysin " S " titres using different sources of streptolysin " S."

Duplicate estimations were made on a number of sera, both of human and of other species, covering a wide range of antistreptolysin titres, in order to determine whether significant differences in titre would be obtained when streptolysin " S " from different sources was used. The results (Table III) showed that no significant differences were found between streptolysins prepared in different ways from Group A streptococci, or between streptolysins from a strain of Group A and a strain of Group C streptococci.

The apparent serological identity between yeast nucleic acid broth streptolysin and serum broth streptolysin is interesting, since in chemical properties the partially purified streptolysins, although superficially different, both appear to have properties of lipo-nucleoproteins.

Immunization experiments.

The results of the rabbit experiments are given in Table IV. No significant increase in antistreptolysin " S " titres was observed. During periods when the rabbits were seedy owing to the injections, and were losing weight, the titres tended to be lower than during rest periods. That the animals were capable of producing antibodies was shown by measurement of the streptococcal agglutinins, which reached titres of more than 1:100,000. It is of interest to record, in confirmation of Herbert and Todd (1944), that the Strain Blackmore did not cause a rise in antistreptolysin " O," and that presumably not even traces of streptolysin " O " are elaborated by this organism.

In the horse the antistreptolysin " S " titre rose from an initial value of 9 units/ml. to 20 units/ml., but at the end of the course it was only 5 units/ml. These titres lie within the range found for normal horses, though the last is unusually low.

DISCUSSION.

An antibody in the commonly accepted sense of the term is a serum component which combines with the antigen and is produced in response to the introduction of the antigen into the tissues of the organism. It is a matter of observation that antibodies are proteins, and that they are found in the β- or γ-globulin fraction of the serum. In the investigation reported antistreptolysin S failed to conform to this definition of antibodies. It was found to occur in high titre in the sera of normal animals, in the complete absence of any evidence of either present or past streptococcal infection. It could not be produced either in rabbits or in a horse by intensive courses of " immunization " with streptolysin S. Furthermore, as will be shown in a subsequent publication, the antistreptolysin S activity of serum is not predominantly associated with the β- or γ-globulin fractions. For these reasons it is considered that antistreptolysin S is not a true antibody. It may well be associated with a normal constituent of serum which fluctuates in amount irrespective of streptococcal infection. An analogous situation is presented by the non-specific substances in many sera which inhibit the influenza virus haemagglutination reaction, and which fluctuate from time to time without known cause (Smith and Westwood, 1949). If individual fluctuations depend in some degree upon metabolic activity, illnesses of different types, including streptococcal infections, might be expected to produce similar

TABLE III.—*Correlation Between Antistreptolysin Titres of Sera when Assayed Against Streptolysin "S" from Different Sources.*

	Source of streptolysin.	No. of sera.	Nature of sera.	Antistreptolysin (units/ml.). Range.	Antistreptolysin (units/ml.). Mean.	S.D. of difference.
(1)	Euglobulin ppt. from Group A, Type 11	25	Human	4–12·5	7·34	0·31
	Serum extract Group A, Type 10	25	,,	4–11·5	7·33	
(2)	Euglobulin ppt. from Group A, Type 11	14	Human and other animals	2·5–50	12·8	1·8
	Yeast nucleic acid broth filtrate from Group A, Type 11	14	..	2–56	13·9	
(3)	Euglobulin ppt. from Group A, Type 11	11	Human and rabbit	4–25	11·2	1·4
	Euglobulin ppt. from Group C	11	..	3–25	12·6	

TABLE IV.—*Antistreptolysin " S " Titres in Sera of 6 Rabbits Given Repeated Intravenous Injections of Living Hæmolytic Streptococci.*

	Antistreptolysin " S " (units/ml.).		Mean weight (kg.).
	Range.	Mean.	
Preliminary	5–8·1	6·1	3·0
Middle of first course	2·7–3·2	3·1	2·7
End of first course	2·9–4·3	3·6	2·8
,, second course	3·1–6·0	4·9	2·7
,, third course	2·5–6·7	5·0	2·9

effects on antistreptolysin S levels, but the degree might depend upon the stage, activity and duration of the illness. This could account for the observation of Todd, Coburn and Hill (1939) on sera from cases of rheumatic fever, and our own failure to confirm any correlation between antistreptolysin S level and type of disease.

The present work failed to provide any explanation of Todd's results (1938), in which he obtained evidence of an immune response to streptolysin S in rabbits by the inoculation of living streptococci.

SUMMARY.

Antistreptolysin " S " titres have been measured in sera from human beings with streptococcal infections with and without rheumatic fever, from various mammals and from representative marine animals.

The level of antistreptolysin " S " was not specifically correlated with streptococcal infection.

No evidence was obtained of increase in antistreptolysin titre after prolonged immunization of rabbits and a horse with living haemolytic streptococci or haemolytic culture filtrates.

Streptolysin " S " produced in serum broth is serologically indistinguishable from streptolysin produced in yeast nucleic acid broth without serum.

It is suggested that antistreptolysin " S " is not normally an antibody in the accepted sense of the word.

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