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THE NATURE OF ANTISTREPTOLYSIN S IN THE SERA OF MAN AND OF OTHER SPECIES. THE LIPOPROTEIN PROPERTIES OF ANTISTREPTOLYSIN S.

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EVIDENCE has been presented elswhere (Humphrey, 1949) to suggest that antistreptolysin S does not behave as a true antibody, and that inhibition of streptolysin S is a non-specific property of the serum of numerous species. Because of the interesting properties of streptolysin S, which in serum broth cultures appears to be a lipoprotein and in nucleic acid broth cultures to be a nucleoprotein (Herbert and Todd, 1944 ; Bernheimer and Rodbart, 1948), and because such non-specific inhibitors may play a part in natural resistance to disease, it seemed desirable to study further the nature of antistreptolysin S activity.

A possible clue appeared to lie in the observation of Hewitt and Todd (1939) that streptolysin S is powerfully inhibited by lecithin in the absence of protein, particularly since the levels of serum lipoids have been found to fall during acute disease and to rise to normal or supra-normal values during convalescence (Peters and Van Slyke, 1946), in a rather similar manner to the antistreptolysin S titres. An investigation of the effect of gentle extraction of the sera with lipoid solvents showed that such treatment did in fact remove much of the antistreptolysin S activity, but that the question was complicated by marked differences in behaviour between species and even between the sera of individuals within the same species. Further work on the behaviour of antistreptolysin S after proteolytic digestion of the serum, and of its distribution among serum fractions, has thrown some light upon its nature, although this is far from being completely revealed.

MATERIALS AND METHODS.

Streptolysin S was obtained from a strain of streptococcus (Group A, Type 11) which was known to produce streptolysin S, but not streptolysin O. Two preparations were used, as previously described, one being a horse serum extract of streptococci grown in serum broth, and the other a filtrate of a yeast nucleic acid broth culture. Sera were stored frozen at − 20° C. Antistreptolysin estimations were performed as described by Todd (1938) with slight modifications. In the case of streptolysin S the haemolysin was standardized against a human serum to which had been assigned an arbitrary value of 10 units per ml.

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Lipoid extractions.

Lipoid extractions were made with ether below − 25° C. as described by McFarlane (1948) or with alcohol-ether at − 10° C. as decribed by Hartley (1925). Three or four extractions were made with ether unless stated otherwise. All solvents were of Analytical Reagent grade and were free from peroxides and all but traces of aldehyde.

Lipoid phosphorus.

Lipoid phosphorus estimations were made by a modification of the method of Youngburg (Hawk, Oser and Summerson, 1947).

Cholesterol estimations.

Cholesterol estimations were performed by the digitonide precipitation method of Schoenheimer and Sperry as modified by Hawk, *et al.* (1947).

Total carbohydrates.

Total carbohydrate estimations were made by the orcinol method of Sørensen and Haugaard (1933) in terms of glucose.

EXPERIMENTAL.

Inhibition of streptolysin S by lecithin, cholesterol and cholesterol esters.

Preliminary experiments were performed to test quantitatively the inhibition of streptolysin S by lecithin and other lipoids. Antistreptolysin S estimations were made in the usual way, and it was found that, in the absence of serum, ovo-lecithin was powerfully inhibitory to both preparations of streptolysin S. A dispersion of highly purified lecithin at a concentration of 0·018 per cent in 0·9 per cent NaCl solution corresponded to 1 unit per ml. antistreptolysin S. When the streptolysin was diluted with normal rabbit serum, however, instead of buffer, the inhibitory effect of lecithin was reduced by more than 9/10ths, and it seems unlikely that lecithin, in the concentrations found in normal serum, could account for more than 0·5–1 unit antistreptolysin S per ml.

One sample of cholesterol, of which a very fine suspension was obtained, inhibited streptolysin S comparably with ovo-lecithin. This result was not in agreement with the findings of Hewitt and Todd (1939) and the experiments were repeated with recrystallized cholesterol. It was not found possible to obtain so fine a dispersion of the recrystallized cholesterol as of the first preparation, but with it no significant inhibition was obtained. Cholesterol esters (from wool fat) likewise caused negligible inhibition.

The effect of neutral fat was tested by measuring the antistreptolysin S of the serum of an individual taken while fasting and after a fat meal. Both the clear and lipaemic sera had the same antistreptolysin S titres.

The effects of ether and alcohol-ether extraction.

A number of sera, both from normal and sick people and from other animals, were extracted with ether below − 25° C. or with alcohol-ether at − 10° C. Treatment with ether removed nearly all free cholesterol, most of the ester cholesterol, and a variable fraction of the phospholipids. Alcohol-ether treatment removed nearly all detectable lipoids. The antistreptolysin S content of the sera were measured before and after extraction, and the results are given in

Table I. In order to ensure that the treatment was not so drastic as to destroy other known antibodies, these were also estimated in a number of cases by conventional methods. The results are summarized in Table II.

TABLE I.—*The Effect of Ether Extraction Upon the Antistreptolysin S Titres of Various Sera.*

(Ranges are given in parenthesis.)

Species.	Number of sera.	Mean antistreptolysin S units/ml. Before extraction.	After extraction.	Removed.
Human	10	8·9 (6–12·5)	3·6 (1·5–5·6)	60%
Horse	6	16·5 (10–27)	3·5 (2·4–6·4)	79%
*Horse (anti-*welchii*)	6	5·5 (4·8–7·2)	2·5 (2·2–2·8)	55%
Rabbit	2	5	1·3	74%
Rat	2	55	6	89%
Ox	1	23	2·9	87%
Sheep	1	12	3·1	74%
Plaice	1 pool	48	23	52%
Guinea-pig	3 pools	46	43	6%
Effect of Alcohol-ether Extraction.				
Human	4	8·7 (6·5–11)	3·7 (3·1–5·4)	58%
Horse	2	18·5	4·3	77%
Guinea-pig	1 pool	36	17	53%

* Sera preserved with 0·3 per cent tricresol.

TABLE II.—*The Effect of Ether Extraction Upon the Titre of Various Antibodies in Sera.*

(Ranges are given in parenthesis.)

Species.	Number of sera.	Nature of antibody.	Mean value. Before.	After.	Removed.
Human	9	Antistreptolysin O units/ml.	303 (28–1200)	260 (21–1000)	14%
Horse	1	Ditto	100	100	0%
Horse*	1	Diphtheria antitoxin. Units/ml.	420	390	7%
Horse*	5	*Cl. welchii* β-antitoxin. Units/ml.	1300 (900–1800)	1250 (950–1800)	4%
Human	4	Iso-agglutinin titre	1 : 46	1 : 39	15%
The Effect of Alcohol Ether Extraction.					
Human	5	Antistreptolysin O Units/ml.	872 (31–1200)	523 (20–810)	39%
Horse*	1	Diphtheria antitoxin. Units/ml.	420	340	19%

* Estimations by Dr. C. L. Oakley, Wellcome Physiological Research Laboratories, Beckenham.

From Tables I and II it is clear that whereas lipoid extraction has a relatively minor effect upon a variety of known antibodies, it causes a major reduction in the antistreptolysin S titres of all the sera examined (human, horse, rabbit, rat, ox) except for guinea-pig, and possibly plaice sera, in which considerable antistreptolysin S activity remained even after alcohol-ether treatment.

It was of interest to examine the ether extracts for antistreptolysin S activity. These were accordingly either recombined with the extracted sera or added to equivalent volumes of 0·9 per cent NaCl, and the ether was removed *in vacuo* over H_2SO_4. Both the saline and the serum preparations were very much more opalescent than the original sera, and a layer of fat formed on the surface after standing for some hours. Thus it was evident that the original lipoid complexes were not wholly restored.

Representative results are given in Table III in which the behaviours of antistreptolysin S and antistreptolysin O are contrasted. It will be observed that in each case the antistreptolysin S activity of the extracted lipoid only partly accounted for the difference between original and extracted sera, and that the reconstituted serum behaved with respect to antistreptolysin S as a simple mixture of the extracted serum and the lipoids. In the case of antistreptolysin O, however, the lipoid extracts had activities far greater than those of the original sera, yet the reconstituted sera were equivalent to the original sera. This rather puzzling observation is explained by the observation (Hewitt and Todd, 1939)) that *free* cholesterol is a very powerful inhibitor of streptolysin O. Thus lipoid extraction, while leaving the true antibody unchanged, frees cholesterol from the inactive protein complexes in which it occurs in serum. It is clear, however, that such complexes must be formed anew when the extracts are recombined, although this is not the case with the complexes concerned with antistreptolysin S activity.

TABLE III.—*Antistreptolysin S and O Contents of Sera Before and After Extraction with Ether, Compared with Titres of the Extracted Lipids Added to Saline and to the Ether Extracted Serum.*

		Original serum.	Ether extracted serum.	Extracted lipoids.	Reconstituted serum.
Human A	A.S.S.	10	4	1·5	5·8
	A.S.O.	28	28	450	53
Human B	A.S.S.	6·2	4	$<<1$	4·7
	A.S.O.	1200	1000	2000	1200
Horse (anti-scarlatina)	A.S.S.	13	2·5	<1	2·6
	A.S.O.	100	100	900	100

The effects of proteolytic digestion.

In order to disrupt the protein moiety of the lipoid complexes in serum use was made of proteolytic digestion. Of the available enzymes pepsin seemed suitable, since it would be inactive at pH 8·0, even in the presence of reducing agents, and since serum contains no natural inhibitor for it. Sera were treated with pepsin (B.D.H.) at pH 3 and at 37° C. until approximately 20 per cent of the protein nitrogen was no longer precipitable by tungstic acid. The sera were adjusted to pH 7, and the antistreptolysin S and antistreptolysin O contents were

measured before and after extraction with ether. Control samples were incubated under similar conditions without pepsin.

Through the kindness of Dr. C. L. Oakley there were available sera from horses immunized against *Cl. welchii* which contained considerable amounts of β-antitoxin, and which had been subjected to pepsin treatment so brief as to leave the antitoxin titres substantially unchanged (Harms, 1948), The antistreptolysin S and β-antitoxin of these sera were estimated before and after extraction with ether.

The results are summarized in Table IV. From this it will be seen that prolonged incubation with pepsin, actually tended to increase the antistreptolysin S values of the sera, although antistreptolysin O was largely destroyed. Furthermore, after such prolonged treatment almost all the antistreptolysin activity could be extracted by ether. From the control experiments without pepsin it appears that most of these effects were obtained by incubation at pH 3 alone.

TABLE IV.—*Effect of Pepsin Treatment upon Antistreptolysin S, Antistreptolysin O, and Anti-β-toxin Contents of Sera.*

(Mean values are given as percentages of those of the original sera.)

Treatment.	Type of serum	Number.	Antistreptolysin S. Before ether.	Antistreptolysin S. After ether.	Antistreptolysin O. Before ether.	Antistreptolysin O. After ether.
Prolonged with pepsin	Human	3	190%	9%	7%	Not done
	Horse	2	100%	6%	Not done	,,
Prolonged without pepsin	Human	2	100%	6%	1%	,,
	Horse	2	78%	6%	Not done	,,
					Anti-β-toxin	
Brief with pepsin	Horse	5	135%	62%	77%	71%

Brief incubation with pepsin also caused a rise in antistreptolysin S, but in this case there was no increase in extractability with ether. Presumably under such mild conditions (in which the β-antitoxin was largely unchanged) the lipoprotein complexes were only slightly disrupted.

The correlation between serum lipoids and antistreptolysin S titres.

The experiments described above suggested that lipoid-protein complexes were responsible for the greater part of the antistreptolysin S activity of sera, with the possible exceptions of guinea-pig and plaice sera. In order to test whether any direct correlation could be found between the presence of particular lipoid constituents and antistreptolysin S activity, the lipoid phosphorus and free and total cholesterol content of a number of sera from different species were estimated, both before and after extraction of lipoids. From Table V it is apparent that there was no correlation between antistreptolysin S titres and any of the serum constituents measured. It is particularly notable that the alcohol-ether extracted sera contained only very small amounts of phospholipid or cholesterol, and yet had considerable residual antistreptolysin S.

TABLE V.—*Correlation Between Antistreptolysin S and Serum Lipoid Content.*

Species.	No.	Antistreptolysin S. (units/ml.) Range.	Antistreptolysin S. (units/ml.) Mean.	Mean lipoid P. (mg./100 ml.)	Mean free cholesterol. (mg./100 ml.)	Mean total cholesterol. (mg./100 ml.)
(1) *Untreated sera.*						
Human	6	9–13	10	8·4	65	161
,,	8	7–9	7·8	6·3	37	138
,,	12	2–7	5·5	6·8	42	140
Horse	2	—	18·5	4·3	20	89
Rat	2 pools	—	55	1·4	0	31
Guinea-pig	1 pool	—	35	1·72	Not done	37
Sheep	1	—	12	3·2	,,	53
Plaice	1 pool	—	50	9·5	,,	109
Dog fish	,,	—	5	3·1	,,	104
Spider crab (whole blood)	,,	—	1·2	0·24	,,	<10
(2) *Ether extracted sera.*						
Human	4	3·9–5·6	4·5	7·2	2	18
Horse	2	—	6	2·5	11	21
Rat	2 pools	—	6	1·3	0	5
Sheep	1	—	3·1	1·9	Not done	<10
(3) *Alcohol-ether extracted sera.*						
Human	3	—	3·7	0·5	0	7
Horse	2	—	4·4	0·25	0	0
Guinea-pig	1 pool	—	1·7	0	0	0

The antistreptolysin S content of serum fractions.

Effect of dialysis.—The distribution of antistreptolysin S activity in serum between the euglobulin fraction (precipitated by prolonged dialysis in the cold against distilled water saturated with CO_2) and the remainder was studied in several sera (Table VI). Somewhat surprisingly it was found that the two

TABLE VI.—*Distribution of Antistreptolysin S Between Euglobulin Fraction and Residue in Various Sera.*

Serum.	Original titre (units/ml.).	Per cent original activity in Euglobulin.	Per cent original activity in Remainder.	Recovery.
Human	12	7	81	98
Ox	23	130	108	238
Guinea-pig	32	146	148	264
Horse 7629	27	80	30	110
,, ,, (after ether extraction)	4	58	19	77
Horse 7637	10	45	100	145
,, ,, (after ether extraction)	3·3	70	30	100

fractions of ox, guinea-pig and horse sera taken together had more antistreptolysin S activity than the original serum. Furthermore, the two horse sera differed sharply from one another—that with a high antistreptolysin S having relatively a much larger activity in the euglobulin than the other, while the activity of the remaining fractions was about equal. After ether extraction the two horse

sera behaved similarly, and their euglobulin fractions contained almost 3/4 of the residual antistreptolysin S.

These experiments demonstrated that the separate antistreptolysin S activities of serum components were not necessarily equal to the activity of the original serum, and that not only were there differences between species but also between individuals within a species. It was nevertheless decided to attempt to follow the behavious of antistreptolysin S in human serum fractionated by ammonium sulphate and by electrophoresis.

Ammonium sulphate fractionation.—Two normal human sera, before and after extraction with ether, were fractionated with ammonium sulphate solution (buffered at pH 6·7) in the cold. The fractions were freed from ammonium sulphate, by dialysis in the cold first against distilled water and then against 0·9 per cent NaCl. The antistreptolysin S and O, and the nitrogen, total cholesterol, lipoid phosphorus, and total carbohydrate content of each fraction were then estimated. Table VII shows the distribution of antistreptolysin S and antistreptolysin O between the fractions. The percentages are given in terms of the original serum activity, and the differences between the two sera are largely due to poorer recovery of activity in serum B than serum A.

TABLE VII.—*Percentages of Original Antistreptolysin S and O Activity Recovered in Different Fractions of Human Serum.*

	Per cent saturation with ammonium sulphate.							
	0–33		33–40.		40–50.		50–80	
Serum.	S.	O.	S.	O.	S.	O.	S.	O.
Human A	7	77	9	14	16	9	59	0
Human A (ether extd.)	7	64	7	9	7	2	7	0
Human B	5	53	6	31	8	16	58	0
Human B (ether extd.)	3	30	4	15	4	8	4	0

Electrophoretic Fractions.

	γ-glob.		β- + γ-glob.		α-glob. + alb.		Albumen.	
Antistreptolysin	S.	O.	S.	O.	S.	O.	S.	O.
Human C	(?5)	58	(?12)	42	16	0	67	0

It is clear, however, that (in contrast to antistreptolysin O) the antistreptolysin S activity is mainly in the albumen containing fractions, and that it is from these that the activity is removed by treatment with ether.

No quantitative correlation was found (Table VIII) between the antistreptolysin S and the total cholesterol, the lipoid phosphorus, the protein nitrogen or the total carbohydrate in the fractions of the unextracted sera, and although after ether extraction there was apparently a fair correlation between carbohydrate content and antistreptolysin S in serum A, there was more variation in serum B.

In contrast to the results obtained with the human sera, similar fractionation of pooled guinea-pig serum, before and after extraction with alcohol-ether, showed that the antistreptolysin S activity was initially high both in the albumen and globulin fractions, and that after extraction the activity of the globulin fraction was diminished more than that of the albumen.

TABLE VIII.—*Correlation of Antistreptolysin S and Serum Constituents in Fractions of Serum A.*

	Antistreptolysin S in units per mg. of			
Before extraction.	Nitrogen.	Cholesterol.	Lipoid P	Total carbohydrate
0–33% Am. Sulph.	0·33	8·1	136	5·4
33–40% ,, ,,	0·78	6·8	238	8·9
40–50% ,, ,,	1·59	9·1	200	16·5
50–80% ,, ,,	1·06	5·2	107	22·7
After ether extraction.				
0–33% Am. Sulph.	0·29	17	84	5·0
33–40% ,, ,,	0·37	9	70	6·5
40–50% ,, ,,	0·79	38	64	7·0
50–80% ,, ,,	0·14	10	37	6·2

Electrophoretic fractionation.—Through the kindness of Dr. C. L. Oakley an electrophoretic separation was performed at the Wellcome Research Laboratories on a sample of normal human serum. The separation was carried out at pH 8·0 in phosphate buffer, $\mu = 0{\cdot}2$. Albumen, albumen + α-globulin, β–+γ-globulin and γ-globulin fractions were examined for antistreptolysin S and O.

The results were in agreement with those found with the ammonium sulphate fractions, except in one respect. It was found impossible to measure the antistreptolysin S activity of the fractions containing γ-globulin when yeast nucleic acid broth streptolysin was used, irregular results with apparently very high titres being obtained despite several repetitions of the assay. When serum streptolysin, which in other estimations behaves in the same way as nucleic broth streptolysin, was used for the assay end-points were obtained, but the apparent antistreptolysin S activities both of the γ-globulin and of the β–+γ-globulin fractions were still greater than the total activity of the original serum. Similar phenomenona, though less well marked, were observed when estimating antistreptolysin S on separated ox and guinea-pig euglobulin fractions, and it appears that the combination between γ-globulin and streptolysin S is very much firmer in the absence of other serum components.

The effect of acid, alkali and heat.

It has already been mentioned (Table IV) that incubation of serum at pH 3 for 48 hr. caused only a small decrease, if any, in the antistreptolysin S content. Other samples of normal human serum—untreated, ether extracted and reconstituted after ether extraction—were kept at pH 9–10 for 3 days in the cold without significant change in antistreptolysin S. The same samples were heated at 65° C. for 1 hour, also without effect upon the antistreptolysin S.

Heating of normal and ether extracted horse serum at 100° C. for 5 minutes caused coagulation. The clear fluid which separated from the coagulum in both cases had considerable antistreptolysin S activity, approximately equal to that of the unheated ether-extracted serum. This activity was shown to be associated with uncoagulated protein, being reversibly precipitated by trichloracetic acid, and was not affected by further extraction with ether.

The effect of varying treatment with ether.

It had been observed that successive extractions of serum with ether below − 25° C., up to four in number, all removed antistreptolysin S activity, though in diminishing amounts. It was of interest to note the effect of saturation of serum with ether, with gentle shaking, followed by removal of ether by evaporation on a pump so as to leave behind all the original serum components. When a horse and a human serum were thus treated at room temperature their antistreptolysin activities fell to 55 per cent and 71 per cent, and when treated below − 25° C. to 26 per cent and 40 per cent respectively. Thus it is evident that the lipoprotein complexes concerned in antistreptolysin S activity are extremely labile to ether and are irreversibly broken simply by saturating at room temperature.

DISCUSSION.

The evidence presented above supports the suggestion made on serological grounds that antistreptolysin S is not an antibody in the accepted sense, and furthermore that its nature varies between species, and even between individuals. In human serum most of the antistreptolysin S activity is carried by a labile lipoprotein complex associated with the albumen fraction, but the globulin fractions also have some activity, most of which is retained after gentle treatment with fat solvents.

Mild denaturation or peptic digestion may increase the antistreptolysin S activity by loosening the lipoprotein complexes without destroying that part of the proteins which carries the residual antistreptolysin S activity after ether extraction. More extensive digestion of the proteins, however, destroys this residual antistreptolysin S. Schematically this may be represented as follows :

Antistreptolysin S
- *Lipoprotein complexes*
  - *Loose* ; activity removed simply by ether extraction
  - *Firm* ; activity removed by ether only after digestion or denaturation.

  } Activity may be increased by digestion or denaturation of protein.
- *Non-specific proteins* — Activity remains even after alcohol-ether extraction, but destroyed by prolonged peptic digestion. No evidence that these are true antibodies.

The same scheme would also cover horse sera, with the proviso that in some sera a considerable amount of active lipoprotein complex may be found in the euglobulin fraction. Insufficient evidence was obtained for generalization about other species, but in guinea-pig serum, which behaved differently from other sera, lipoprotein complexes are either unimportant in this connection or else are so stable that they are unaffected by ether treatment.

Concerning the detailed nature of the inhibitors mainly negative evidence has emerged, for there was no apparent correlation between antistreptolysin S and any of the serum constituents examined. The methods of fractionation used were too crude to permit identification of the protein, or of the lipoprotein factors, which were included in the globulin fractions. Some information was gained by examination of the streptolysin S inhibition by human serum fractions prepared for another purpose by the cold ethanol fractionation method of Cohn and his colleagues (Edsall, 1947). Considerable inhibition was caused by the fractions containing the β_1 lipoprotein (Fraction III_0), the α_1 lipoprotein (Fraction IV_1),

and by the γ-globulin containing Fraction (II + III_w). The albumen fraction, however, presumably because of the alcohol treatment involved in fractionation, caused negligible inhibition. Fraction II and III (which contains the inhibitory III_0 and II + III_w) was notably less active than either of its constituent fractions demonstrating once again that inhibition of streptolysin S is a complex phenomenon, and that the affinity of a particular serum component may depend upon the presence or absence of others. Greater knowledge of streptolysin S itself is required before the exact mechanism of its inhibition can usefully be considered.

It may be of interest to compare antistreptolysin S with some other inhibitors of enzymes or toxins known to occur in normal sera. Duthie and Lorenz (1949) have recently studied trypsin inhibitor, and an inhibitor of the proteases of certain non-pathogenic bacteria, and have reviewed earlier work on these substances. Trypsin inhibitor is found mainly in the albumen fraction, although some activity was present in all fractions of serum examined. Unlike antistreptolysin S it is relatively unaffected by ether treatment, although much diminished by chloroform. The bacterial protease inhibitor was found to be a labile component of the globulin fraction, being present in varying amounts in different species. Plasmin inhibitor, studied by MacFarlane and Pilling (1946) and others, appears to resemble trypsin inhibitor in its behaviour. Among inhibitors of haemolysins may be mentioned an inhibitor of θ-toxin of *Cl. welchii* (private communication from Dr. C. L. Oakley), and a non-specific inhibitor of streptolysin O found by Packalén (1948) in sera from cases of hepatitis. The latter appears to be a lipoprotein present in α- and β-globulin fractions.

Normal sera also contain an inhibitor of hyaluronidase (McClean, 1942) and an inhibitor of the haemagglutinating and certain other properties of influenza virus which is found mainly in the β_1 globulins (McCrea, 1946; Hirst, 1949). In connection with this so-called "Francis inhibitor" it has been suggested by Smith and Westwood (1949) that such inhibitors may be biologically important in determining natural resistance to particular infectious agents. It is possible that the widespread occurrence of antistreptolysin S may account for the apparent unimportance of streptolysin S production in determining the virulence of streptococci.

SUMMARY.

(1) Treatment of sera with ether or alcohol-ether, in such a manner as to cause only minor changes in the titres of known antibodies, causes a major reduction in antistreptolysin S titre. This phenomenon was observed in the sera of man, horse, rabbit, rat, ox, sheep, but was less marked in guinea-pig and plaice sera.

(2) From a study of the effects on antistreptolysin S of peptic digestion of sera and of their fractionation by various methods, it is concluded that in man and the horse antistreptolysin S activity is a complex phenomenon, mainly dependent upon labile lipoprotein components associated with the albumen fraction.

(3) The antistreptolysin S activity of separated serum components may be greater than that of the original serum, presumably because some of the groups capable of linkage with streptolysin S are normally linked with one another.

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EXPERIMENTAL BERYLLIUM POISONING.

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THE work to be described in this paper was begun in January, 1947, to provide information on the toxicity of beryllium and its compounds. Hyslop, Palmes, Alford, Monaco and Fairhall (1943), in a long report, stated that no particular toxicity had been established for beryllium, and that whatever toxicity had been found with the salts of beryllium was due to the toxicity of the acid radicle and the hydrolysis of the salts. These writers had made an extensive review of the literature, but failed to emphasize the importance of earlier work by Siem (1886) and Comar (1935) which demonstrated the toxic properties of beryllium. When our preliminary experiments demonstrated beyond doubt that beryllium was a very toxic element, it became possible to assess the results of previous workers, and to explain many of the discrepancies and contradictions found in the literature. Hyslop *et al.* (1943) include in their report a very complete bibliography, and no useful purpose will be served by providing a similar list in this paper. It should,