NONSPECIFIC ANTISTREPTOLYSIN REACTIONS AND SERUM (OR PLEURAL-EXUDATE) CHOLESTEROL[1, 2]

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Hewitt and Todd (1939) occasionally noticed an obviously nonspecific increase in the antistreptolysin titer (AST) in sera that had been contaminated with certain bacteria. Independently, Löfgren (1944) has reported on the same phenomenon.

In comprehensive AST studies on clinical material, Westergren several years ago paid attention to the common occurrence of unexpected rises in AST, often to exceedingly high levels, in sera from hepatitis patients. Kalbak (1947) has had similar experiences. Recently, Sievers (1947), as well as Westergren (1948), has compared the occurrence of these high titers with the results of various liver function tests.

Systematic AST determinations in pleural exudates from patients treated at the Tuberculosis Department of St. Göran's Hospital have shown a high rate of elevated titers, often above 1,000, and not infrequently above 10,000, units in patients whose serum titers were normal or only moderately increased. In most of these cases no definite connection with streptococcal infections could be demonstrated.

When the conditions responsible for these apparently nonspecific AST reactions were studied, it was found that sera and exudates when treated with acid or alkali (and subsequently neutralized) showed striking increases in AST (tables 1 and 6).

At the first glance it seemed very unlikely that, in these four instances of nonspecific antistreptolysin reactions—viz., (1) sera (exudates) contaminated with certain bacteria, (2) a great number of hepatitis sera, (3) pleural exudates, and (4) acid- or alkali-treated sera (exudates)—there could possibly be a common causative factor. Yet from a biological point of view it would be far more logical if those findings were attributable to one and the same basic mechanism.

In the present paper a report is given on a series of experiments conducted in order to arrive at a uniform explanation of the foregoing nonspecific increases in the antistreptolysin titer.

For a better understanding of the problems involved an outline of the mechanism of the streptolysin O hemolysis will have to be drawn according to our

[1] An abbreviated version of this paper was read before the Eighth Scandinavian Pathological Congress, July 7 to 9, 1947, at Uppsala, Sweden.

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present knowledge. Since the pneumococcal hemolysin in all essentials appears to behave like the streptolysin O, the experiences gained with the former may, *mutatis mutandis*, be utilized in filling up the picture. The red cells are considered to have a mosaiclike membrane of thread-shaped protein and interspersed lipid micelles (Erickson *et al.*, 1938). Cohen *et al.* (1937, 1940) have found that the lytic activity of pneumolysin is associated with at least two functional groupings, one reversibly oxidizable thiol grouping, and the other more or less specific for a certain sterol grouping and configuration. The observations so far made seem to indicate that prior to hemolysis the red cell sterol combines with the lysin (Cohen and Shwachman, 1937; Cohen *et al.*, 1942). The state of oxidation or reduction of the thiol grouping of the lysin conditions its reactivity with the sterol, and thus its adsorption to the red cells (Cohen *et al.*, 1937). Though no direct proof is as yet available, it seems possible that the same thiol grouping provides also the site for attachment of the specific antistreptolysin. If this

TABLE 1

The effect of acid or alkali treatment upon AST

	AST IN SERUM B 246 TREATED 18 HOURS AT 56 C WITH	
Normality	HCl	NaOH
1/125	8,000	8,000
1/5	4,000	5,000
1/20	16	1,800
1/80	≦16	320
1/320	×	80
1/1,280	×	40
Normal saline	28	

assumption holds true, the antibody blocking of this grouping will, in a way similar to oxidation, prevent the attachment of the lysin molecule to the red cell sterol.

MATERIALS AND METHODS

Materials. Sera were heated for 30 minutes at 56 C. When not immediately examined they were stored at −16 to −20 C. Exudates obtained by thoracocentesis were centrifuged and the clear supernatants treated as the sera.

Antistreptolysin titration. Principally, the AST determinations were made according to Ipsen's (1944) technique. Sheep blood, however, was used instead of rabbit's blood. Todd's concentrated horse immune serum, or patients' sera, the titers of which had been determined by repeated titrations against Todd's serum, were used as standard sera. The calculation of the titers differed somewhat from that applied by Ipsen. A 50 per cent hemolysis end point was fixed for each serum (by direct observation or by interpolation) and compared with the 50 per cent hemolysis end point of the standard serum. Such a manner of proceeding simplifies the calculations, and is at the same time the more correct one

from a theoretical point of view, as shown by, among others, Herbert (1941). The method is described more in detail in a paper on antistaphylolysin determinations (Packalén and Bergqvist, 1947).

Specific absorption. Serum (or exudate) was diluted 1:50 with undiluted, active streptolysin broth. The mixture was incubated in a water bath for 15 minutes at 37 C, and subsequently heated for 30 minutes at 56 C. The effect of the absorption was determined by a routine AST titration in the inactivated mixture. Control absorption with heat-inactivated streptolysin broth or sterile broth showed no reduction of the serum titer.

Precursory adsorption of streptolysin to red cells. Precursory adsorption of streptolysin to the red cells was carried out by mixing both reagents, cooled down to 0 C, in the same proportions as used in ordinary AST determinations and keeping the mixture for 45 minutes in the ice bath. To cooled tubes containing serial dilutions of serum (or exudate) red-cell-streptolysin mixture was then added in amounts to make up the usual proportions of reagents. All transfers were made with cooled pipettes and in cooled tubes and bottles. The specific combination between serum (exudate) antibody and streptolysin, adsorbed in advance to the erythrocyte membrane, was allowed to go on for 45 minutes at 0 C; after which time all tubes were transferred for 15 minutes to a water bath of 37 C. Readings were made in the ordinary way.

Electrophoretic analysis. Electrophoretic fractionation of exudates and sera has been performed by Dr. B. Olhagen[3] according to Tiselius' method (1937). In order to obtain the purest possible albumin or γ-globulin, a compensatory hydrostatic pressure was applied in such a way that the boundary of either of these fractions was kept stationary for about 8 hours (Olhagen, 1945). After the dilution of the electrophoretic fractions was adjusted so that their protein content (determined by the micro-Kjeldahl method) became equal to that of the original dialyzed serum or exudate, their AST was determined in the ordinary way.

Cholesterol determinations. The content of total and esterified cholesterol in sera and exudates was determined photocolorimetrically according to a method described by Swedin (1946). For a part of the determinations a slight modification of this was used. Lipid-extracted sera and exudates were prepared according to Blix's (1941) acetone-ether method in the cold or McFarlane's (1942) ether extraction by freezing below -25 C.

RESULTS

The effect upon AST of specific absorption of sera and exudates, or of precursory adsorption of streptolysin to the erythrocytes at 0 C. The key function of the erythrocyte-membrane-sterol in the mechanism of hemolysis suggests that a nonspecific antistreptolysin reaction might be due to the presence of an excess of reactive cholesterol in serum or exudate. The active groupings of this cholesterol intercept the streptolysin molecules, thus preventing the combination of the

[3] The author is greatly indebted to Dr. Olhagen for his valuable co-operation.

streptolysin with the reacting sterol groupings in the erythrocyte membrane, which necessarily precedes hemolysis. If this reasoning holds true, prevention of the nonspecific reaction ought to be possible by allowing the streptolysin to combine with the red cells prior to the addition of the serum or exudate rich in cholesterol. Under ordinary experimental conditions, the red cells, when brought into contact with streptolysin, are all too quickly hemolyzed. Following adsorption to the erythrocytes at 0 C, however, there is no immediate hemolysis (Herbert and Todd, 1941). Under these circumstances serum antibody, even though added after the streptolysin, might be afforded an opportunity of specifically inhibiting the lysis that is otherwise certain to take place as soon as the temperature of the mixture is allowed to rise.

TABLE 2

The effect of absorption with streptolysin, and precursory adsorption to erythrocytes, at 0 C, respectively, upon AST in sera and pleural exudates

SERUM	AST			PLEURAL EXUDATE	AST		
	Before	After	After adsorption to r.b.c. at 0 C		Before	After	After adsorption to r.b.c. at 0 C
	Absorption with streptolysin				Absorption with streptolysin		
A 9931	100	≦18	25	A 9935	16,000	14,000	≦18
A 5667	160	25	36	A 5679	10,000	9,000	25
A 5833	140	≦16	32	A 5866	4,500	4,000	32
A 9865	700	90	180	A 9867	2,800	2,800	125
A 9822	180	≦16	56	A 9748	1,600	1,000	≦18
A 5024	360	22	90	A 4839	1,400	1,400	64
A 9874	250	≦18	50	A 9871	560	560	36
A 6262	320	≦16	64	A 6266	180	22	32
A 9844	100	≦16	32	A 9835	100	32	32
A 9944	100	25	25	A 9950	80	≦18	25

A series of absorption and adsorption experiments have been conducted in order to demonstrate the correctness of the foregoing conception. Typical records are presented in tables 2 to 4. As will be seen from table 2, absorption in serum with streptolysin always results in a more or less complete neutralization of the antibody inhibition. This occurred even in sera with considerably elevated AST values: A 9865, A 5024, and A 6262. The same was observed in exudates the (low) AST of which were in keeping with the corresponding serum titers.

After precursory adsorption of streptolysin to the red cells, the serum antistreptolysin as a rule showed titers that were only $\frac{1}{3}$ to $\frac{1}{4}$ of their initial values. It is thus obvious that the serum antibodies were not capable of completely preventing the streptolysin already attached to the erythrocytes from exerting its lytic action. Nevertheless, the remaining inhibitory effect usually exceeded that remaining after specific absorption.

Quite different conditions were encountered in pleural exudates, the AST of which are considerably above the serum titer level. Specific absorption did not

bring about any noticeable reduction of their titers. On the other hand, precursory adsorption of streptolysin to the red cells, as a rule, resulted in a practically complete disappearance of the strong inhibitory power of these exudates. Partial exceptions were exudates 9867 and 4839. However, in the corresponding sera, A 9865 and A 5024 (both showing specifically elevated titers), a remaining inhibition was also observed of approximately the same magnitude. Consequently, there is ample reason to assume that a part of the titer elevation in these exudates was due to the presence of antibody. This will explain why the inhibition in these cases could not be entirely blocked by precursory adsorption.

Corresponding observations were made with hepatitis sera (table 3). In the majority of these, specific absorption did not bring about any titer reduction,

TABLE 3

The effect of absorption with streptolysin, and precursory adsorption to erythrocytes, at 0 C, respectively, upon AST in hepatitis sera

PATIENT	HEPATITIS SERUM	AST			
		Before	After	After adsorption to r.b.c. at 0 C	Before or after the icteric stage
		Absorption with streptolysin			
G.M.E.	B 863	3,600	5,000	80	110–125
	B 1626	5,000	3,600	40	
E.A.S.G.	A 9444	9,000	8,000	≦16	32–70
	A 9461	4,000	4,000	32	
	A 9467	400	250	16	
S.A.E.Å.	A 9463	1,600	250	45	250–280
A.M.E.M.	A 9014	1,000	500	360	280–360
E.G.M.J.	A 8894	25,000	500	250	360–900

whereas precursory adsorption of the streptolysin to the red cells resulted in a more or less complete blocking of the inhibition. Some hepatitis sera (A 9463, A 9014, and A 8894), however, behaved differently, which was probably due to the presence of an increased amount of specific antibody, as indicated by the elevated AST values observed before or after the icteric stage.

Sera and exudates, the AST of which had increased owing to bacterial contamination or treatment with acid or alkali, behaved on the whole according to the general rule established above, as did the cholesterol suspension tested (table 4).

Electrophoretic fractionation of serum or exudate with high AST. Blix, Tiselius, and Svensson (1941) have shown that the bulk of the serum cholesterol migrates in an electric field with the β- and α-globulins, the γ-globulin and the albumin fractions carrying only small portions of the lipid. On the other hand, the electrophoretic mobility of most antibodies is that of γ-globulin.

In order to elucidate further the nature of the serum (or exudate) factor giving

TABLE 4

The effect of absorption with streptolysin, and precursory adsorption to erythrocytes, respectively, upon the elevated AST in contaminated or HCl-treated sera (exudate)

SPECIMEN	TREATMENT	AST		
		Before	After	After adsorption to r.b.c. at 0 C
		Absorption with streptolysin		
Serum				
T.P.	Sterile	70	≦16	32
T.P.	7ds *P. fluor.*	4,500	4,000	64
T.P.	7ds *P. aerug.*	1,800	2,800	45
Serum				
B 246	Sterile	90	25	≦18
B 246	7ds *P. fluor.*	18,000	18,000	≦18
B 246	7ds *P. aerug.*	4,000	4,500	≦18
Serum				
B 1043	Sterile	80	20	40
B 1043	HCl	3,600	2,500	160
Exudate				
B 59	Sterile	80	20	40
B 59	HCl	1,000	900	80
Cholesterol susp. 1%		14,000	18,000	≦18

TABLE 5

AST in electrophoretically separated fractions of exudate or serum

SPECIMEN	EXP.	AST							
		Whole dialyz.	Electrophoretically separated fractions						
			+ Top	+ Upper	+ Lower	Bottom	− Lower	− Upper	− Top
			−	*alb*	*alb*+α(+β)	*alb* + α + β	(*alb*+)α + β + γ	α + β + γ	β + γ
Exudate A 9747	I	2,000		360	2,000	2,000	2,200	2,000	1,400
			alb + α	*alb* + α(+ β)	*alb* + α + β (+ γ)	(*alb*+)α + β + γ	β + γ	γ	−
Exudate A 9747	II	4,000	2,000	4,000	2,800	2,800	2,000	500	
A 9761		4,500	4,500	8,000	4,000	4,500	4,000	200	
Serum B 1679		3,200	≦40	110	640	2,500	5,000	≧20,000	

nonspecific AST increases, a few exudates with high titers and, for comparison, a serum from a scarlet-fever patient, were subjected to electrophoretic separation.

The results are shown in table 5. In the exudates it is seen that the AST of the end fractions had decreased more or less, most clearly so when they were obtained in a comparatively pure state by applying a compensating hydrostatic pressure to the fluid in the electrophoretic cell system. In experiment I the albumin fraction, and in experiment II the γ-globulin fraction, had been made electrophoretically homogeneous by this procedure. The serum from the scarlet-fever patient behaved quite differently in the electrophoresis experiment. The

TABLE 6

The effect of lipid extraction upon AST in sera and exudates

SPECIMEN	AST				
	Before extraction	After extr. acc. to			In serum
		Blix	McFarlane		
			−78 C	+20 C	
Exudates					
A 9907	28,000		≦18	14,000	64
A 9935	11,000	40			64
A 9747	8,000	125			500
A 9905	2,500		40	1,250	90
A 9920	2,200	640			560
A 8299	1,000	450			1,000
A 9949	900		140	4,500	180
A 8726	800	200			500
A 9950	110	32			64
B 59	90	40			56
Sera (hepatitis)					
A 9825	5,000		500	5,000	
B 4063	320,000		80	56,000	
Serum (scarlatina)					
Epid. S.E.	900		560	640	
Serum (normal)					
A 8470	80		80	80	

highest AST value was observed in the γ-globulin fraction. With increasing electrophoretic mobility of the fractions their AST gradually decreased.

AST in sera and exudates after lipid extraction. The lipids may be more or less completely removed from sera (or exudates) without noticeably impairing the proteins by Blix's (1941) acetone-ether extraction method at low temperatures or by McFarlane's (1942) ether extraction at temperatures below −25 C. The changes in AST after lipid extraction according to either of these methods have been studied in several exudates and sera. Typical results are given in tables 6 and 7. It will be seen that pleural exudates and hepatitis sera with elevated AST after lipid extraction as a rule show an almost complete loss of their in-

hibitory power. Exceptions to this rule sometimes may be noted, e.g., in exudates A 9747, A 9940, A 8299, and A 8726. These exudates, however, derived from patients whose sera also had an increased AST, which indicates that the

TABLE 7

The effect of contamination and treatment with acid or alkali upon AST in lipid-extracted exudates and sera

SPECIMEN	AST											
	Before extraction				After extraction							
	Sterile	Infected	Treated with		Sterile		Infected		Treated with			
									HCl		NaOH	
			HCl	NaOH	(a)*	(b)†	(a)	(b)	(a)	(b)	(a)	(b)
Exudates												
A 9907	28,000		22,000	32,000		≦20		≦20		64		≦16
A 9935	11,000				40		40		≦16		≦16	
A 9950	110	25,000	9,000	2,500	50		100		≦16		≦16	
B 59	90	1,800	4,500	3,200	40	≦18	40			≦18		≦18
Sera												
A 8470	125	7,000	10,000	10,000		80		80		≦25		≦25
B 147	220	25,000	1,800	2,800	80	≦18	80			≦18		≦18

* (a) Extracted according to Blix's method.

† (b) Extracted according to McFarlane's method.

TABLE 8

AST in relation to cholesterol changes developing in exudates and sera infected with Pseudomonas fluorescens

SPECIMEN	AST		CHOLESTEROL		
	Titer	Increase	Total	"Free"	"Free"/Total
			mg %	*mg %*	*per cent*
Exudate					
B 59 sterile	90		98	41	42
B 59 infected	1,800	20×	101	43	42
Serum					
A 8470 sterile	125		139	69	50
A 8470 infected	7,000	56×	149	89	60
Serum					
B 147 sterile	220		225	71	32
B 147 infected	25,000	110×	243	147	60

rise was probably due to the presence of specific antistreptolysin. For the extractions performed with specific antisera, such as the scarlet-fever-patient serum (Epid. S.E.), demonstrated that antibody is not removed together with

the lipid fraction. In table 6, attention should be given also to the fact that extraction with ether at room temperature did not result in any conspicuous reduction of the titer, though extraction below −25 C did.

If exudates or sera initially showing strong nonspecific rises in AST, or anyway capable of showing such rises if contaminated or treated with acid or alkali, had been subjected to lipid extraction, no further titer increase could be brought about by the above-mentioned procedures (table 7).

AST and amount of total and "free" serum (or exudate) cholesterol. Numerous investigators (Boyd and Connell, 1938; Epstein and Greenspan, 1936; Greene *et al.*, 1940; Thannhauser and Schaber, 1926) have shown that in hepatitis sera there is a striking decrease in ester cholesterol, the total cholesterol level remaining unchanged. The possibility was therefore taken into consideration that the clue to the origin of the nonspecific rise in AST in hepatitis sera might be found

TABLE 9

AST in relation to cholesterol changes developing in sera treated with HCl or NaOH

SERUM	TREATED WITH	AST	CHOLESTEROL		
			Total	"Free"	"Free"/Total
			mg %	*mg %*	*per cent*
A 8470	NaCl	125	165	60	36
	HCl	10,000	142	55	39
	NaOH	10,000	135	73	54
B 594	NaCl	250	172	74	43
	HCl	7,000	146	42	29
	NaOH	18,000	151	79	52
B 1043	NaCl	640	270	161	60
	HCl	40,000	262	128	49
	NaOH	28,000	250	176	70

in the ensuing increase in "free" serum cholesterol. If so, then corresponding changes in the condition of serum and exudate lipids might by analogy be expected also in other instances of nonspecific AST. For more exact information on this point, a number of cholesterol analyses have been made with sera and exudates. Representative records are given in the following tables:

It was found (table 8) that a 20-fold increase in AST in exudate B 59 after 7 days' incubation (at room temperature) with *Pseudomonas fluorescens* was not associated with any change in the proportions of "free" and ester cholesterol. In serum A 8470, which showed a 56-fold rise in titer after infection, a shift by 10 per cent (from 50 to 60 per cent) from bound to "free" cholesterol was noted, and in serum B 147, where the titer rose 110-fold, the "free" cholesterol had increased from 32 to 60 per cent. Hence, the increase in "free" cholesterol observed in sera A 8470 and, especially, B 147 might possibly have some connection with their rise in AST. However, the absence of such an increase in "free" cholesterol as regards exudate B 59 indicates that the primary cause of

these AST rises apparently is to be sought in some mechanism other than the hydrolysis of ester cholesterol.

This inference is corroborated by the cholesterol analysis of sera treated with 2 N HCl at 56 C for 18 hours (table 9). A slight decrease in the total cholesterol content was noted as the result of this energetic chemical treatment. The amount of "free" cholesterol decreased to the same, or to a still higher, degree. On the other hand, alkali treatment (2 N NaOH at 56 C for 18 hours) did result in a hydrolysis of ester cholesterol. This may, but by no means must, imply that the rise in AST in these sera was due to the increased content of "free" cholesterol.

TABLE 10

AST and cholesterol in pleural exudates

PLEURAL EXUDATE	AST	CHOLESTEROL			PLEURAL EXUDATE	AST	CHOLESTEROL		
		Total	"Free"	"Free"/ Total			Total	"Free"	"Free"/ Total
		mg %	*mg %*	*per cent*			*mg %*	*mg %*	*per cent*
A 8282	70	115	69	60	A 9809	400	64	45	70
B 59	90	108	56	52	A 8338	560	98	64	65
A 7839	100	102	47	46	A 9871	560	146	71	49
A 8105	110	72	34	47	A 9793	640	107	66	62
A 9950	110	101	61	60	A 8726	800	78	36	46
A 8571	140	45	8	18	A 8299	1,100	86	33	38
A 8038	180	92	51	55	A 9775	1,100	128	68	53
A 7871	220	38	19	50	A 9930	1,600	132	38	29
A 9949	220	74	54	73	A 9850	2,500	68	36	53
A 9905	250	87	51	59	A 9907	2,500	74	45	61
A 9803	360	62	43	69	A 9867	2,800	138	83	60
A 8098	400	88	47	53	A 9890	2,800	186	102	55

The cholesterol analyses of pleural exudates (table 10) reveal that no correlation whatever obtains between their AST and their total cholesterol content, nor their levels of "free" and ester cholesterol, respectively.

AST in pleural exudates and the duration of the effusion. When the clinical data pertaining to the pleurisy patients was compiled, an obvious relationship emerged between AST and the duration of the pleural effusion. This is illustrated by figure 1, in which the AST values of 282 exudates from 146 patients are plotted against the duration of their effusions. It will be seen that the AST during the first month of pleural exudation as a rule remained "normal," the average titer being 100 units. Only in 12 cases did the exudate titer exceed 200 units. In 11 of them the serum titer was within 50 per cent of the exudate value. During the following months the titers continuously increased until reaching an average titer of 1,400 units during the fifth month. A considerable scatter was observed of the individual AST values with increasing persistence of the exudate. After the fifth month of exudation, the average titer remained about the same. Possibly there was a slight tendency toward a further increase in titer in cases

whose exudate persisted for years. For comparison a curve representing average serum titers for the same patients is inserted in the figure. It will be seen that the sera show only insignificant increase from approximately 100 units in the first half-year to 140 units subsequently. It should be mentioned that the idiopathic exudates did not differ in any respect from those developing after institution of artificial pneumothorax, or after pneumonolysis.

Finally, it was obvious that the AST increase in the exudates very often coincided with certain characteristic changes in their cellular content. Although

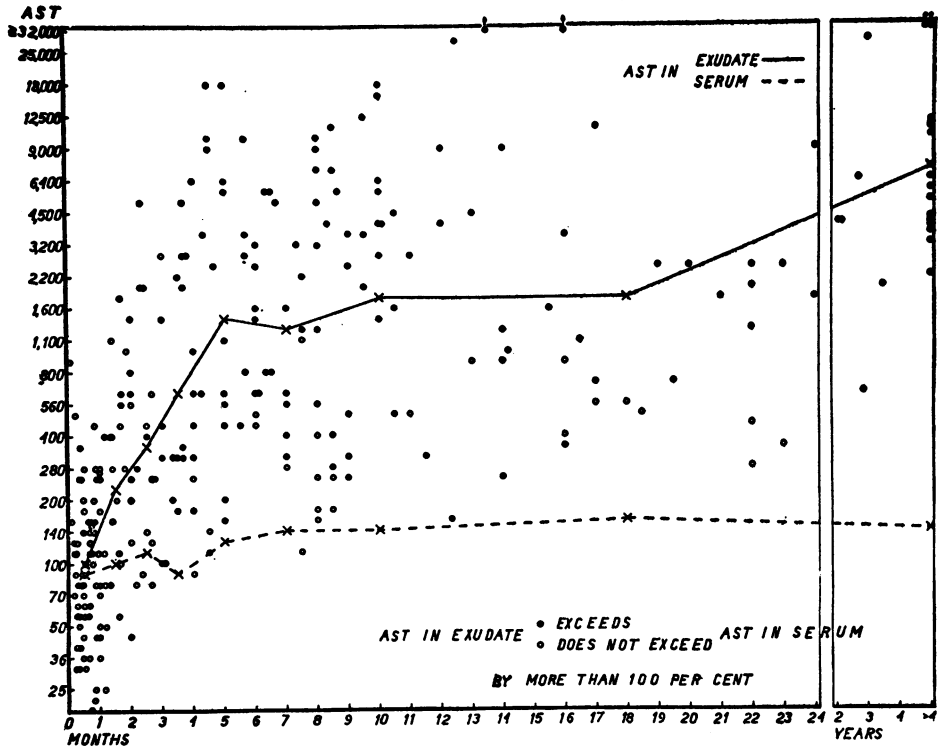

Figure 1. AST in pleural exudates and duration of exudation.

lymphocytes predominated in the fresh exudate, increasing numbers of polynuclear leucocytes entered into the picture when the exudate had persisted for some time, the AST commencing to rise. Unfortunately, detailed figures cannot be presented, as this statement is based on routine examinations, in which the numbers of lymphocytes and leucocytes were recorded only as rough estimates, i.e., "a few," "moderately," "abundantly," or "predominantly."

DISCUSSION

The supposed nonspecific nature of AST rises (1) in sera and pleural exudates after contamination with certain bacteria, (2) after treatment with acid or alkali, (3) in most of the hepatitis sera, and (4) in pleural exudates of some mini-

mum duration has been confirmed, *inter alia*, by the nonabsorbability of the inhibitory factor with specific streptolysin, as well as by the blocking of the inhibition through precursory adsorption of streptolysin to the red cells at 0 C.

In their study of the nonspecific inhibition following bacterial contamination, Hewitt and Todd (1939) found the neutralizing factor to be present in the acetone-soluble fraction of the serum. They assumed the bacteria to "effect some change in the condition of serum lipins, enabling them to neutralize streptolysin O." Since cholesterol was found to inhibit streptolysin hemolysis in high dilutions, the authors suggested that the bacteria liberated cholesterol by their proteolytic action on the serum proteins. However, the mechanism of this "liberation," and consequently the entire problem of the role actually played by the cholesterol in the inhibition of streptolysin hemolysis, remained obscure.

The observation that electrophoresis reduced the nonspecific inhibitory power just in those fractions, viz., the albumin and γ-globulin portions, in which according to Blix *et al.* (1941) the cholesterol content is also reduced seems to be strong evidence in favor of the cholesterol actually being responsible for the inhibition in question. The removability of such an inhibitory power, either actual or potential, by the aid of lipid extraction is then quite a logical sequence.

A hypothesis that an increase in "free," i.e., not esterified, cholesterol, such as observed in hepatitis sera, might be considered the real clue to most, if not all, nonspecific AST rises in sera and exudates could not be confirmed by actual analyses of their "free" and esterified cholesterol content. Only in alkali-treated sera (and exudates) was a hydrolysis of cholesterol ester consistently demonstrated. Possibly the increase in "free" cholesterol observed in some sera after contamination may have contributed to the particularly pronounced rise in AST exhibited by them. Since, however, in other contaminated sera with raised AST the "free" cholesterol level remained unaltered, it is unlikely that the *primary* increase in these sera was attributable to such a change in the condition of their serum cholesterol. If the conception of serum (exudate) cholesterol as the primary factor causing nonspecific AST reactions is still to be maintained, another explanation has to be found of the actual mechanism.

According to present opinion, the bulk of the serum lipids occur as lipoproteins having the character of secondary valence complexes which are held together by forces of the van der Waals type (Chargaff, 1944). The bonds between the lipids and the serum proteins appear in the main to be weak ones (Chargaff, 1944). Under such circumstances the most promising avenue of approach to the problem seems to be the following hypothesis: As long as these lipoprotein complexes are unimpaired, the active OH groups of the cholesterol remain engaged in the linkage to the protein and are not available for the attachment of the streptolysin molecules. However, as soon as the complex is broken up—whether by the action of acid or alkali, bacterial enzymes or enzymes liberated or activated through the disintegration of leucocytes in exudates of long duration, or by the decay of liver cells in hepatitis—the hydroxy groups of the cholesterol molecules are uncovered, and a combination with the sterolaffine groups of the streptolysin is rendered possible. This combination implies a blocking of the necessary

attachment of the streptolysin molecules to the red cell membrane sterols. It should be stressed here that in hepatitis another mechanism is also conceivable: the dysfunction of the liver cells may primarily result in an incomplete synthesis of lipoprotein complexes. But the final outcome is the same, viz., cholesterol molecules with free hydroxy groups in the serum.

The chemical methods available at the present moment for quantitative estimation of the proportion of protein-linked and actually free cholesterol in serum are as yet not quite appropriate, but it is to be hoped that further progress in this field will afford us more conclusive observations concerning our problem.

SUMMARY

Strong increases in antistreptolysin titer, AST, independent of specific stimuli occur (1) in sera (and pleural exudates) contaminated with certain bacteria, or (2) in sera treated with acid or alkali, (3) in most hepatitis sera, and (4) in pleural exudates of some duration.

The nonspecific nature of these rises was confirmed by the nonabsorbability of the inhibitory factor with specific streptolysin and the blocking of the inhibition by precursory adsorption of streptolysin to the red cells at 0 C.

In electrophoretic separation experiments the inhibitory factor was found to remain chiefly in the β- and α-globulin fractions, i.e., just those fractions in which the serum cholesterol accumulates, whereas the antibodies are found in the γ-globulin fraction.

Lipid extraction of sera and exudates abolished nonspecific AST reactions in them, and rendered them simultaneously unable to respond with a rise in AST to bacterial contamination or treatment with acid or alkali. Specific antistreptolysin, on the other hand, remained unaffected by this procedure.

It has not been possible to prove, by cholesterol analyses, that an increase of "free," i.e., not esterified cholesterol—as seen in hepatitis sera—should be considered the consistent explanation of most nonspecific rises in AST. However, the hydrolysis of ester cholesterol may play some part in the origination.

It is suggested that the primary mechanism of the nonspecific AST reactions is a breaking up of the lipoprotein complexes, in which the serum lipids are supposed to occur. The uncovered, active OH groups of the freed cholesterol combine with the streptolysin molecules, thus preventing them from attacking the red cells. The breaking-up process may be brought about, *inter alia*, by the action of acid or alkali, or bacterial enzymes, or enzymes liberated or activated through the disintegration of liver cells in hepatitis, or leucocytes present in exudates of some duration.

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