ANTI-STREPTOLYSIN TITRES OF HUMAN SERA IN HEALTH AND IN VARIOUS STREPTOCOCCAL INFECTIONS

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The study of *Streptococcus haemolyticus* infection attained new prominence when Todd (1932*a*, *b*) showed that the anti-streptolysin titre of human sera could be determined by titration against a suitably prepared streptolysin and that a definite rise in such titres could be demonstrated following infections with haemolytic streptococci. Rising titres, he contended, could also be found in rheumatic fever and were of very marked importance as indicating the etiology of that condition. Before such a conclusion can be drawn it is essential to consider the anti-streptolysin production in a large series of cases of known streptococcal and other infections with a view to determining the nature, quality, and incidence of that response. In addition, the correlation of anti-streptolysin titres with the severity and character of infection deserves some attention.

The objects of the present investigation are to determine:

(*a*) The variations of anti-streptolysin titres in streptococcal and other infections.

(*b*) The correlation of such titres with the severity and characters of the illnesses.

Todd (1932 *b*) tested a number of sera from normals and from convalescents from haemolytic streptococcus infections, and pointed out that the anti-streptolysin titres of the latter were significantly higher. In three patients with tonsilitis and in one with erysipelas studied during the course of illness he found a marked rise in such titres in from 9 to 17 days after the onset of infection. His rheumatic fever cases, in the acute stage, showed titres much above those of his normals and considerably higher than those of a similar series in the quiescent stage, and he was able to prove in several cases a rise in the amount of circulating anti-streptolysin coincident with the onset of an acute rheumatic condition and a fall to normal on recovery. This seemed to indicate, he contended, a close etiological relationship between *S. haemolyticus* infection and rheumatic fever. Here it seems necessary to state that though the particular investigation to be discussed does not treat directly with rheumatic fever yet the observations may have some indirect bearing on the conclusions of other workers, which are reviewed here. Coburn and Pauli (1932) in their extensive work on the relationship between *S. haemolyticus* and rheumatism

showed that complement fixation and agglutination reactions with sera of their rheumatic fever patients indicated recent streptococcal infection and that this was largely borne out by their findings, in conjunction with Todd, of raised anti-streptolysin titres in such cases. They quoted a series of single titrations in eleven normals and in ten convalescents not from haemolytic streptococcal diseases all with low anti-streptolysin titres and seven cases of scarlet fever, two of which showed respectively high and low titres early in the course of infection and five which had high titres in the third week. They noted that rheumatic fever cases gave what they believed to be an unusual response to throat infections with haemolytic streptococci in that their anti-streptolysin titres did not rise till the onset of the rheumatic process considerably later.

The preceding observers have very largely compared what they considered to be a normal range with a higher range indicating infection with *S. haemolyticus* and have given few examples of how the titre varies in each particular case. Myers and Keefer (1934), however, in a larger series showed that there was considerably more variability in normal titres and in response to infection than had previously been thought. They found in seventy-one scarlet fever convalescents, approximately 4 weeks after the onset of infection, a wide range but generally higher than normal, and in eighteen erysipelas patients investigated during illness "normal or slightly increased titres at the onset of erysipelas, and a gradual rise which was maintained for several weeks". In nineteen cases, however, of acute follicular tonsilitis due to *S. haemolyticus*, they stated that "in some cases an increase in the titre was observed as the disease progressed; in others, this was not apparent". In conclusion they contended that the variability of the anti-streptolysin range, both in normal and in disease conditions, including rheumatic fever, and the fact that an attack of rheumatic fever was often preceded by throat infection raised considerable doubt as to the etiological significance of anti-streptolysin titres in such conditions. Wilson, Wheeler and Leask (1934) in their investigation of the significance of anti-streptolysin titres in children found a very wide range, and pointed out that no significance could be attached to any single determination. They found that only 20 per cent. of their rheumatic fever cases showed a rise above the control period level and came to the conclusion that an increase in circulating anti-haemolysin was of no etiological significance. In a more recent paper the same authors (1935) investigated the relationship of upper respiratory infection to rheumatic fever in children and again observed that the anti-streptolysin titre was correlated rather with respiratory infection than with rheumatic manifestations. They also found that the fluctuations in such titres could not be correlated with the detection of haemolytic streptococci in the throat.

It seems, therefore, that the nature of the anti-streptolysin response to infection with haemolytic streptococci is still under some doubt, and that any additional evidence should be of value.

Methods

Todd (1932 *a*, *b*) prepared a streptococcal haemolysin which was inactivated by serum anti-streptolysin *in vitro*. This haemolysin he and other observers have shown to be group specific. It will be referred to as streptolysin in the remainder of this paper.

Preparation of streptolysin. The technique of Todd and Hewitt (1932) in the preparation of buffered glucose broth was followed exactly except for the substitution of ordinary beef for horse flesh, apparently without any deleterious effects. As will be seen later, not all haemolytic streptococci produce a potent streptolysin in this medium, and the strain used requires careful selection. This is a matter of convenience mainly, since the streptolysin appears to be identical in all cases. Streptolysins from strains isolated from erysipelas, tonsilitis, puerperal fever and meningitis have been found to give identical results in titrations against different immune sera. The volume of filtrate, however, required to produce sufficient haemolysis is inconveniently large in any but "good" strains. Cultures were incubated for 16 hours at 37° C. and then filtered through a Chamberland F candle, the first 80 c.c. of the filtrate being discarded and the remainder tested for haemolytic power. As Todd demonstrated, the haemolytic filtrate is very readily oxidised on exposure to air, and it was therefore reduced with sodium hydrosulphite according to his method immediately before use. The M.H.D. is the smallest amount of freshly reduced filtrate which will cause complete lysis of 0·5 c.c. 5 per cent. washed horse blood cells in 1 hour at 37° C. $2\frac{1}{2}$ M.H.D. is taken as the unit of streptolysin and is made up to 0·5 c.c. volume with normal saline (0·9 per cent.).

Titration of anti-streptolysin. Blood samples were taken from all patients at weekly intervals dating from admission to hospital. The serum was separated aseptically and was stored in the ice-chest till required for use. To ensure correct comparative readings the anti-streptolysin titres of all samples of sera from each individual case were determined at the same time, and each batch of titrations was controlled by the inclusion of previously tested sera whose titres were known. The question of deterioration of the earlier samples in storage was considered, but it was found that sera, tested repeatedly over many months, did not show demonstrable change in the amount of anti-streptolysin present.

The following dilutions of the sera to be tested were made: 1/50, 1/100, 1/200, 1/400, 1/800, 1/1000, 1/2000, 1/4000, 1/8000, 1/10,000 in 0·5 c.c. normal saline. One unit of freshly reduced streptolysin was added to each. The tubes were left in the water bath at 37° C. for 15 min., 0·5 c.c. of 5 per cent. horse blood was then added to each. They were replaced in the water bath and readings were taken after one hour.

Todd expressed his anti-streptolysin titres as the highest serum dilutions which *completely prevented* haemolysis, but I found this rather difficult to judge in the case of certain sera and adopted the first tube showing *complete* haemo-

lysis as the end-point in my series of titrations. Thus the fraction representing the strongest concentration of serum permitting complete haemolysis was the measure of its anti-streptolysin content, and its titre in units of anti-streptolysin was recorded as the reciprocal of this fraction (*e.g.* complete haemolysis in the presence of the 1/800 dilution and upwards equals 800 units of anti-streptolysin). My figures are accordingly considerably higher than those of Todd, but they appear to be quite satisfactory as a measure for comparing the cases examined.

HAEMOLYTIC ACTIVITY OF DIFFERENT STRAINS OF STREPTOCOCCI

Table I shows the very considerable variation encountered in strains from different infections. All were definitely haemolytic on blood-agar plates.

Table I

Strain	M.H.D. in c.c.	Source and clinical condition	
1*	0·01	Pus	Abscess
2*	0·02	Cerebro-spinal fluid	Meningitis
3*	0·04	Urine	Lupus erythematosis
4*	0·04	Pus	Pustule
5*	0·06	Pus	Erysipelas
6	0·06	Throat swab	Tonsilitis
7	0·06	Pus	Abscess
8	0·08	Pus	Abscess
9	0·08	Pus	Abscess
10*	0·08	Blood	Puerperal fever
11*	0·08	Blood	Puerperal fever
12*	0·08	Blood	Puerperal fever
13	0·16	Throat swab	Tonsilitis
14	0·16	Throat swab	Tonsilitis
15	0·25	Throat swab	Tonsilitis
16	0·25	Throat swab	Tonsilitis
17	0·25	Pus	Abscess
18	0·50	Throat swab	Tonsilitis
19*	>0·50	Blood	Otitis media septicaemia
20	>0·50	Pus	Septic arthritis
21	>0·50	Pus	Abscess
22	Much >0·50	Throat swab	Tonsilitis
23	Much >0·50	Pus	Empyema
24	Much >0·50	Pus	Abscess
25	Much >0·50	Throat swab	Tonsilitis
26	Much >0·50	Pus	Abscess
27	Much >0·50	Throat swab	Tonsilitis

* Haemolytic streptococci were found in pure culture in these cases.

Some of this variation might have been expected to depend on the varied periods during which some of the strains had been kept in artificial culture but in actual practice little variation attributable to this cause was noted. All strains were, of course, subcultured daily on blood agar plates for 5 or 6 days before being tested. In general, good streptolysin producers maintained their power over considerable periods and showed little variation; poor streptolysin producers were more variable but were consistently poor.

It is noteworthy, in view of later findings of high anti-streptolysin titres in throat infections, that the throat strains tested did not as a rule produce

very good haemolytic filtrates in this medium. These results, of course, cannot be considered to have any bearing on haemolysin production *in vivo*.

Anti-streptolysin titres in persons of normal health

Twenty sera were examined from persons who were not suffering from any recognised infection. It was of course impossible to eliminate entirely streptococcal infections of more or less distant date since sore throats of streptococcal origin may occasionally be quite mild and be disregarded by the sufferer. Also, as will be seen later, a high titre of circulating anti-streptolysin may persist for over 3 months after the causal infection has subsided. The cases were all adults and showed titres ranging from 200 to 4000 units, but fourteen were under 1000. This range is what one might expect in a normal mixed population and indicates, if anything, the prevalence of streptococcal infections and the varied degree of response to them. It also indicates that no reliance can be placed on single blood examinations to show the activity of *S. haemolyticus* and that a series of examinations should be made on each case starting from the onset of infection.

Anti-streptolysin titres in disease

To facilitate comparison the cases examined have been grouped under the headings of throat infections and uterine infections. Thus, under the former, known streptococcal diseases such as tonsilitis and scarlet fever can be compared with diphtheria, and under the latter puerperal fever of streptococcal origin can be contrasted with puerperal fever due to other organisms.

A small group of streptococcal skin infections—erysipelas—is also included.

Throat infections

A. *Streptococcal*

Fifteen cases of scarlet fever, eight cases of streptococcal tonsilitis and one case of erythema nodosum fall to be considered in this series. Bacteriological proof of streptococcal infection was not considered necessary in the scarlet fever group, but in all the other cases cultures from the throat were made and gave profuse growths of haemolytic streptococci. Twenty-three of the twenty-four cases showed a rise in anti-haemolysin titre during the course of infection. The one exception was a mild case of scarlet fever. Seventeen cases had primary anti-haemolysin titres of 1000 units or under and sixteen of these showed a rise to at least twice the primary titre during the course of their disease. The remaining seven cases with primary titres of more than 1000 units all showed a similar rise. Fourteen patients had the steepest rise in titre in the first 2 weeks of illness and nine reached a maximum titre at that stage; nine cases showed the steepest rise in the third week and twelve a maximum titre at that point. Two cases showed slight further rises at later examinations.

Representative cases, mild and severe, are given in Table II to show the variations encountered.

Table II

Condition	Age	Character of illness	Titre of anti-streptolysin 1st week	2nd week	3rd week
Scarlet fever	7	Mild	200	4,000	10,000
	11	,,	2000	4,000	4,000
Tonsilitis*	19	,,	800	2,000	4,000
	19	,,	200	>10,000	>10,000
Scarlet fever	13	Moderate	80	200	800
	19	,,	2000	2,000	4,000
Tonsilitis	19	,,	2000	4,000	2,000
	19	,,	800	2,000	—
Scarlet fever†	12	Severe	800	>20,000	8,000
	12	,,	800	2,000	2,000
Tonsilitis	18	,,	400	800	2,000

* This case still showed a titre of 4000 units when readmitted to hospital with diphtheria 3 months later.

† This case is dealt with more fully later—No. I, Table V.

B. *Diphtheria*

Eighteen cases, mainly children, were examined. All were bacteriologically proved to be infections with *C. diphtheriae*, but no attempt was made to isolate haemolytic streptococci. Blood was taken immediately after admission to hospital and again after 3 weeks. Primary titres varied between 400 and 4000; nine were under 1000 and nine over. It is of interest that age appears to have little bearing on the circulating anti-streptolysin; in the present series one adult of 30 had a primary titre of 400 and another adult of 19 one of 4000, which was equalled by a child of 6. In sixteen of the eighteen cases the titre of circulating anti-streptolysin was the same in both blood specimens, but in the other two a rise from 800 units to 2000 units was noted. The first of these, aged 9, was a moderately severe case with typical membrane and moderate throat congestion. Diphtheria anti-toxin, 20,000 units, was given and the child recovered without complications. The second case, aged 6, was definitely severe. The clinical features were gross membrane, moderate faucial injection and marked adenitis. Diphtheria anti-toxin, 40,000 units, was given and the patient made a singularly rapid recovery. No explanation can be given for these cases though it might be presumed that haemolytic streptococci were taking some part in the throat infection, though this was not clinically evident. In contrast, one case can be mentioned in which a glandular abscess formed and was evacuated. The pus gave a pure culture of *S. haemolyticus*, yet no rise in anti-streptolysin titre was found.

UTERINE INFECTIONS

A. *Streptococcal*

Ten cases of puerperal fever were examined. All the cases showed evidence of septic endometritis and a general febrile reaction of greater or lesser severity. *S. haemolyticus* was isolated in pure culture from the cervix in each

case, but blood cultures remained sterile. A rise in the titre of circulating anti-streptolysin was demonstrated in only five of these patients. Two cases showed the steepest rise in the first week of infection and both reached their highest titres at that time, while the other three cases showed a later rise with the maximum titre at the end of the second week. The highest titre recorded was 4000 units. The remaining five cases did not show any response. Intra-uterine glycerine douching was employed as a routine treatment and all cases recovered. Table III shows representative cases from this group.

Table III

Age	Character of illness	Titre of anti-streptolysin		
		1st week	2nd week	3rd week
34	Mild	200	2000	200
41	,,	1000	4000	4000
34	,,	2000	2000	2000
24	Moderate	800	800	800
31	,,	400	800	2000
35	Severe	800	800	2000

B. *Non-streptococcal*

In this group there were ten cases, some of considerable severity, due to organisms other than haemolytic streptococci. Certainly in no case was *S. haemolyticus* isolated from freshly taken cervical swabs nor from blood cultures, and in every case other organisms such as coliform bacilli and viridans streptococci were found to justify the clinical diagnosis. Of these ten cases, seven showed no alteration in anti-streptolysin titre during the 3 weeks or so of their hospitalisation; their readings were 200, 400, 800, 800, 800, 2000, and 2000 units respectively. One case showed a fall from a titre of 2000 units on admission to one of 800 units after 3 weeks—a paradox probably explained by an attack of tonsilitis from which she had suffered some little time previously. Another case had a slight inexplicable rise from 400 to 800 units, but only one case showed any rapid and definite ascent. This occurred following a mild pleurisy with effusion in the fourth week of her illness, and it is possible to ascribe this late and atypical rise to some haemolytic toxin elaborated during that disease process. Her anti-streptolysin titre rose from 400 to 4000 units in the succeeding fortnight.

Skin infections

Eleven cases fall under this heading, all erysipelas. Ten cases showed a rise in anti-streptolysin titre to at least double their primary titres and with steep rises in the first 2 weeks of infection. Eight had primary titres of 1000 units or under and two of 2000 units. One case alone showed no response. Only four patients in this series were tested more than twice and, of these, two showed a maximum titre at the second testing, that is, after the first week, and two showed moderate later rises.

Table IV gives representative cases.

Table IV

Age	Character of illness	Titre of anti-streptolysin 1st week	2nd week	3rd week	4th week
68	Mild	400	200	—	—
52	,,	800	—	4000	4000
27	Moderate	400	8000	—	—
52	,,	2000	4000	—	—
47	Severe	200	—	2000	4000
6½	,,	800	2000	>2000	>2000

GENERAL CONSIDERATION OF RESULTS

The anti-streptolysin titres of the ninety-four cases examined in health or at the commencement of known infection varied from 200 to 4000 units. Approximately 64 per cent. were under 1000. Though these figures cannot be compared directly with those of other workers, they show the marked variation which may be encountered and fully support the contention of Myers and Keefer (1934) and of Wilson, Wheeler and Leask (1934) that no single observation can be considered to indicate *Streptococcus haemolyticus* infection.

Increase in circulating anti-streptolysin was practically invariable in throat infections with haemolytic streptococci, and, in contrast to the findings of Myers and Keefer, tonsilitis cases showed a marked response and a high rising curve. On the other hand only two diphtheria cases out of eighteen showed any rise in titre, and this poor response, particularly in a condition where it is so difficult to rule out streptococcal co-infection, indicates that non-specific increase in anti-streptolysin is at least uncommon. Uterine infections, however, were quite inconstant; only five out of ten showed any response. Further two cases not known to be associated with *S. haemolyticus* activity showed a rising anti-streptolysin titre. To summarise one can say that a rising anti-streptolysin titre in throat infection is probably indicative of *S. haemolyticus* activity, but that its significance in other infections has not been proved. The highest rise in circulating anti-streptolysin occurs on the average in the first 2 weeks of illness.

Additional factors to be considered are age and height of pyrexia. Children under ten had generally lower initial titres than adults but the maximum titre obtained did not appear to be significantly related to age, sex, or initial level. Similarly the height and duration of pyrexia did not appear to have any bearing whatsoever on the quality of the anti-streptolysin response.

INFLUENCE OF COMPLICATIONS ON THE DEVELOPMENT OF ANTI-STREPTOLYSIN

Nine cases were encountered with complications of various kinds occurring during the course of streptococcal infections. The respective anti-streptolysin titres are given in Table V and the clinical details immediately following. Of the six throat infections considered four showed no anti-streptolysin response

in the first fortnight of illness, one produced a feeble response and one a very brisk rise followed by a rapid fall. The four cases originally mentioned showed a rise in titre immediately following a secondary throat inflammation and, in one case associated with arthritis, this was followed by recovery: in the others it did not appear to have any influence on the clinical condition. The onset of nephritis in two cases was certainly not prevented by antecedent high levels of circulating anti-streptolysin. The three skin infection cases encountered were quite different; they showed an early and active anti-streptolysin response, yet two developed secondary attacks or relapses of Erysipelas and one had an extensive cellulitis associated with profuse pus formation.

These facts support the recognised clinical findings that immune reactions in conditions such as scarlet fever or tonsilitis and erysipelas are markedly different. Scarlet fever, for instance, generally produces an excellent immunity, while one attack of erysipelas notoriously predisposes to a second attack. And here we find a high level of immunity to the haemolytic toxin to have no influence on the development of relapses or of pyogenic processes in the latter, and it is probable that antitoxic immunity has little bearing on such conditions. The findings in the throat conditions, on the other hand, might suggest that the haemolytic toxin was associated with the development of secondary throat inflammation and nephritis. But a closer examination reveals the lack of correlation between anti-streptolysin response and clinical recovery. It is therefore suggested that the initial poor immune reaction is merely indicative of a general poor response to some other toxin or toxins. It is therefore probable that an antitoxic immunity of some kind is here necessary for recovery. In secondary arthritis alone, cases 4 and 6, is any evidence seen of clinical improvement associated with anti-streptolysin response, but the evidence is much too narrow to be of any significance.

Table V

		Titre of anti-streptolysin				
No.	Condition	1st week	2nd week	3rd week	4th week	5th week
1	Scarlet fever	800	>20,000	8000	>20,000	>20,000
2	,,	2000	2,000	4000	4,000	—
3	,,	1000	1,000	8000	10,000	10,000
4	,,	2000	800	2000	2,000	—
5	Tonsilitis	800	800	2000	2,000	—
6	,,	400	800	—	—	—
7	Erysipelas	800	4,000	4000	4,000	—
8	,,	200	2,000	2000	2,000	4,000
9	,,	1000	8,000	8000	8,000	8,000

Case 1. Here an early high titre corresponded to an apyrexial period of clinical improvement, a fall in titre occurred before the onset of complications starting with a secondary tonsilitis, but the second high wave of circulating anti-streptolysin did not appear to coincide with any amelioration of symptoms nor to influence the onset of further complications. The patient developed in turn acute nephritis with haematuria and haemoglobinuria, severe epistaxis and subcutaneous haemorrhages, and otitis media.

Case 2 showed a high primary titre and a reasonable late antibody response. The maximum titre was reached *before* the onset of a mild nephritis and no further change was noted in relation to that disorder.

Case 3 had a stationary titre coinciding with a period of clinical lack of improvement, but the rapid response to a secondary pyrexia associated with renewed faucial inflammation did not coincide with recovery, and a third relapse occurred before definite improvement was noted.

Case 4 had a high primary titre which fell in the first week of illness but rose again in the second week considerably later than the clinical improvement. The maximum titre was attained before the onset of a secondary tonsilitis and a slight albuminuria, and no further change was noted.

Case 5 is interesting. Here the level of the anti-streptolysin remained constant at 800 during the first 10 days of illness, and it was noticeable that a recurrence of the throat symptoms associated with arthritis occurred very soon after that. This relapse produced, or was associated with, a marked rise in titre and this was quickly followed by recovery.

Case 6 also showed a poor immunological response in the first week of illness and suffered from arthritis. This patient was discharged from hospital early and could not be followed up to see if any further rise in titre occurred with complete clinical recovery.

Case 7. This patient was admitted with a mild facial erysipelas which subsided rapidly but almost immediately after her discharge from hospital she was readmitted with a recurrence of erysipelas over the same area. She showed a high anti-streptolysin titre, due to her original infection, before relapse, and the secondary streptococcal activity did not appear to stimulate any further immunological response. The relapse was, if anything, more severe than the original infection.

Case 8. Here an initial low titre was recorded but a rapid rise in anti-streptolysin noted. This, however, did not appear to have any bearing on the occurrence of a very severe and extensive cellulitis with much pus formation. A slight final rise in titre was, however, recorded on ultimate recovery.

Case 9. A very grave infection associated with delirium and grave toxaemia, showed a fairly high initial titre and a quick response in the first week, when the maximum titre of 8000 units was recorded. Six days later, however, a moderate relapse occurred involving the same area. No further immunological response was noted even on complete recovery.

SUMMARY AND CONCLUSION

1. Streptolysin production by twenty-seven strains of haemolytic streptococci grown in a special buffered glucose serum-free broth has been compared and a very marked variation noted.

2. The anti-streptolysin titres in healthy individuals and in others at the commencement of illness showed a pronounced variation.

3. Throat infections with *S. haemolyticus* usually produced a rise in the level of circulating anti-streptolysin in the patients' blood, and this could be considered diagnostically significant.

4. Throat infections with haemolytic streptococci showed rather more rapid production and higher titres of anti-streptolysin than did skin infections such as erysipelas, and both throat and skin infections were greatly superior to uterine infections in this respect.

5. Neither the level of circulating anti-streptolysin at the commencement of infection nor the rate and height of response could be shown to have any bearing on the severity of the disease, except that a poor anti-streptolysin response was found in certain cases of relapses and toxic complications of throat infections. This appeared, however, to be merely indicative of a general poor antitoxic response and not to have any meaning by itself, except that in certain arthritic cases where the question of its importance was more open. No correlation whatsoever was noted between anti-streptolysin response and relapses and septic complications in erysipelas.

6. In general the anti-streptolysin response in infection appeared to depend more on the individual than on the site or severity of infection.

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