ON STAPHYLOCOCCAL PRECIPITIN REACTIONS IN CASES OF ACUTE AND CHRONIC INFECTIONS AND ALSO IN SERUM SICKNESS.

By

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INTRODUCTION.

This work was undertaken for the purpose of determining the occurrence and specificity of bacterial precipitins in infections caused by the *Staphylococcus aureus*. The differences in antigenic properties of various strains of orange and white staphylococci employed in the preparation of the filtered antigens and the importance of the presence of peptone in this connection led to numerous experimental observations which had not been anticipated. The presence of strong *S. aureus* precipitins in the blood of an adult suffering from very acute serum sickness following an injection of anti-diphtheritic serum led to an investigation of the blood of patients suffering from serum sickness, the results of which are fully recorded here. The investigation of the blood of normal rabbits revealed the fact that in some of these animals staphylococcal precipitins were present, which led to unexpected difficulties in the study of precipitins in the actively immunised animal.

TECHNIQUE.

The Patients' Blood. Blood was withdrawn always by vein puncture into sterile test tubes, which were slanted until the serum had separated. The serum was centrifugalised until it was quite clear or showed only turbidity due to fats or lipoid substance. It was then pipetted off into sterile test tubes and heated in a water bath at 58° C. for twenty minutes, and tested with the antigens within twelve hours of obtaining the samples of blood. This was the routine procedure, but comparative tests were made on numerous occasions with unheated sera and with sera which had been heated at 55° C. for thirty minutes, and 60° C. for ten minutes.

Rabbits' Blood. Test samples were obtained from a vein of the ear in the usual way and the blood serum was prepared by the same technique as described for human blood. Rabbits which were immunised were bled from the axillary artery and the serum when separated was stored in sterile bottles

undiluted, or diluted with saline. Preservatives such as 0·1 per cent. formalin, or 0·25 per cent. phenol were employed.

The Reaction. Human or rabbit's serum was diluted with fresh sterile saline containing 0·25 per cent. phenol in all these experiments, and the dilutions employed were 1 in 5, 10, 20, 40, 80, 160 and occasionally 1 in 320. We used 1 c.c. of the diluted serum and 0·5 c.c. of one of the clear antigens[1]; control experiments were made by reversing the proportions of antigen and anti-sera, but the former was the routine procedure. At the outset of the work the mixture of antigen and diluted serum was placed in glass tubes ($3\frac{1}{2} \times 3/10$) in a closed water bath. We employed a constant temperature of 37° C. for 48 hours, taking readings at 24 and 48 hours respectively against a black background, but the long incubation at 37° C. in spite of the presence of the small quantity of antiseptic, allowed bacterial contamination in some instances. We, therefore, after full experimental observations abandoned the temperature of 37° C. and employed open water baths at 52° C. for 48 hours entirely for these experiments, although Kraus (1897), Nicolle (1898) and Radziewsky (1900) all found that a temperature of 37° C. was most satisfactory for bacterio-precipitins. The following Table shows the method we considered to be the most satisfactory for these experiments.

Table I.

Antigen (undiluted) (c.c.)	0·5	0·5	0·5	0·5	0·5	0·5	0·5	—	—	0·5
Saline (c.c.)	—	—	—	—	—	—	—	0·5	0·5	1·0
Serum (c.c.)	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	—
Dilution of serum[1]	1 in 5	10	20	40	80	160	320	5	20	—

The precipitate present was recorded in the following manner and is referred to by these abbreviations: T or trace, a granularity remaining in suspension, + a distinct granularity with a deposit, ++ a deposit of much greater intensity so as to completely cover the convex surface of the tube or give a milky turbidity, and finally +++, a still heavier deposit without granularity of the supernatant fluid. 0, signifies no reaction and – that the reaction was not made. The deposit might be granular like very fine sand, or definitely flocculent. Both varieties occurred in reactions of equal intensity, and as a whole the flocculent was more common with immune rabbit sera than with the human, but both varieties of precipitate occurred with normal and immune rabbit sera, and with human sera obtained from cases of serum sickness or *S. aureus* infections.

The deposit from the positive tubes examined microscopically was found to consist of a granular débris.

Control Reactions. Control observations were made with 1 per cent. peptone in normal saline, horse serum, anti-toxic horse serum, beef broth, a 1 per cent. solution of starch. All the reactions were carried out by the routine method already referred to in detail and by various modifications.

[1] In the total volumes the dilutions of serum are therefore 1 in 7·5, 15, 30, 60, etc.

Preparations of Antigens. The various antigens used, except where specially stated to the contrary, consisted of a basis of beef broth to which 1 per cent. of peptone and 0·5 per cent. of sodium chloride were added, and gave a reaction of 7·5 to the universal indicator. Each flask which contained 200 c.c. of the medium was autoclaved at 110° C. for twenty minutes. The peptone employed in the majority of these experiments was obtained from Messrs Allen and Hanbury and is known as "blood peptone," but other samples of peptone obtained from Hopkins and Williams, May and Baker, and the peptone sèche de Chapoteaut appeared to be of equal merit, and the experiments referred to in the section on serum sickness indicate that this belief is correct and that any of the four samples of peptone referred to may be employed. Antigens were also made of horse flesh instead of beef, marmite, and beef broth without the addition of peptone. A limited number of comparative experiments were made with cultures of the *S. aureus* grown in the broth flasks at 37° C. for one week, two weeks and three weeks, but apart from these observations, all our experiments were made with antigens which had been incubated at 37° C. for one month, then filtered through carefully washed Berkefeld candles (of pre-war German make) by the aid of a water exhaustion pump, and to which 0·1 per cent. formalin was added. The sterile filtrates were stored in bottles with paraffined corks in the dark at room temperature, and the necessary amount was readily withdrawn when required. By this method of filtering and storing, absolutely bright and clear antigens were always obtained and were seldom contaminated. We also tried 0·25 per cent. phenol, and toluol, as preservatives, but 0·1 per cent. formalin was found to be the most satisfactory agent. Graham Smith and Sanger (1903) also found that in dilutions below 1 in 100 the addition of formalin did not interfere with serum precipitation.

The flasks of peptone beef broth were inoculated with strains of *S. aureus* obtained from pus, sputum, and by blood culture, but the strain most commonly employed as it always gave an efficient antigen is referred to throughout this communication as "Orpen." This culture of *S. aureus* was isolated from a purulent sample of sputum from a case of bronchitis, but antigens prepared from other strains, however, of *S. aureus* were equally efficient. Antigens were also prepared from cultures of the *S. albus* obtained from a bone abscess, pustular acne, and human milk, from haemolytic and non-haemolytic strains of *Bacillus coli*, from *B. proteus*, *B. typhosus*, *B. paratyphosus* A, B and C, and *B. diphtheriae.*

CASES OF *STAPHYLOCOCCUS AUREUS* INFECTION, AND CONTROL CASES.

Blood sera were examined for the presence of *S. aureus* precipitins in 118 cases of *S. aureus* infection. In all these cases the organism had been isolated from the blood or from the pus, sputum, or other exudates. It was obtained in pure culture in all the cases of furunculosis, carbuncle, osteomyelitis, and septicaemia, and in almost all the cases of abscess formation. In some of the

remaining cases such as those of pulmonary, uterine, and urinary infections other organisms were present, but the number of staphylococci was so great as to indicate that this organism had played an important part in the causation of the infection. The results of these investigations are shown in the following table (II).

Table II.

	Total cases examined	Total negative	Total positive	Positive: Very strong (+++)	Positive: Strong (++)	Positive: Moderate (+)	Positive: Weak (Trace)
Furunculosis	66	34	32	4	5	13	10
Carbuncle	7	2	5	1	0	1	3
Abscess	19	8	11	2	1	5	3
Osteomyelitis	5	3	2	0	1	0	1
Cellulitis	5	1	4	1	0	1	2
Septicaemia	2	1	1	0	0	0	1
Other infections	14	4	10	5	2	1	2
Total	118	53	65	13	9	21	22

A very large number of sera were tested against two, three or more filtrates made from different strains of *S. aureus*, because sera which gave a strong reaction with one filtered antigen might give a weak reaction with another or fail to react. A general review of all the sera tested showed that of the antigens employed the "Orpen" and the "Nauen" (from a case of *S. aureus* pyaemia) antigens gave the best results in most cases. This, however, was not invariably the case, and in a few instances other antigens gave better results than either of the above, and further some sera showed a marked difference in the strengths of the reactions obtained with the "Orpen" and "Nauen" antigens.

In determining the strength of the precipitin reactions it was necessary to make allowance in those cases which, as is so often seen in agglutination experiments, showed zonular reactions, which are referred to by Kolmer. We have noted that many sera which gave zonular reactions at the end of 24 hours' incubation have subsequently undergone marked changes, so that at the end of 48 hours the zones had disappeared. Many zonular reactions can also be avoided if the sera are tested on the same day on which the blood is collected. It has been stated previously that the sera were heated before use at 58° C. for 20 minutes, and it was thought that this procedure might have some tendency towards the production of an inhibition zone, but comparative experiments made with heated and unheated sera showed that there was no appreciable difference. Further, it was noted that whereas a zone might be obtained with an antigen made from one strain of *S. aureus*, with antigens made from other strains no such zone was apparent. This variation in the reaction with antigens made from different strains of *S. aureus* was not infrequent and since, as stated above, many of the sera were tested with two or three different antigens, it was possible to obtain a more accurate estimate of the amount of the precipitate formed with the lower dilutions of the sera.

In a certain number of cases also the sera were examined for precipitins

with antigens prepared from other organisms, *e.g. B. typhosus*, *B. paratyphosus* B, *B. coli* (two or three strains). In one or two cases the presence of a co-existing infection was definitely established and in these cases co-existing precipitins were demonstrated in the sera. The following example may be given: Miss S. suffering from typhoid fever (positive blood culture). Towards the end of the third week septic foci, from which *S. aureus* was recovered, appeared in various parts of the body and numerous colonies of *S. aureus* were isolated from the faeces. This condition persisted for over a fortnight. An examination of the patient's serum showed the following results:

	1/5	1/10	1/20	1/40	1/80	1/160
c̄. *S. aureus* (Nauen) filtered antigen	+ +	+	T*	T	T	0
c̄. *B. typhosus* filtered antigen	+	+ +	+ +	+ +	+ +	T

* T = Trace.

In other cases where there has been no evidence of co-existing infection no reaction has been obtained with antigens other than those prepared from *S. aureus*.

In a number of cases the same sera were tested against antigens prepared from *S. albus* of which four strains were employed. One of these strains was isolated in pure culture from a case of acute infective osteomyelitis and differed from most cultures of *S. albus* in fermenting mannite. Sera which showed positive reactions with *S. aureus* antigens also reacted with this antigen, but no reactions were obtained with *S. albus* antigens prepared from the remaining three strains, which were isolated from two cases of pustular acne and from normal human milk.

It will be seen from Table II that out of 118 sera examined in cases of *S. aureus* infection 53 or 45 per cent. were completely negative; 65 or 55 per cent. were positive, but of these 22 or 18 per cent. showed only weak reactions. Of the 66 cases of furunculosis, many of which were chronic cases with several recurrences, 34 or slightly over 50 per cent. showed no precipitins for *S. aureus*. Nor did we find any evidence that the precipitin content was necessarily increased as the result of vaccine treatment. A number of cases of furunculosis were examined both before and after a course of injections with *S. aureus* vaccine, but apart from two cases which showed a slightly increased reaction no change occurred.

In addition to the preceding a series of 98 sera were examined from cases in which there was no *à priori* reason to suspect the presence of an infection necessarily due to *S. aureus* and this organism was not isolated from any of the patients. The majority of the individuals who furnished these sera were in good health and considered normal. Of these 98 sera 56 or almost 58 per cent. were completely negative, nine showed a very strong reaction, twelve a strong, eleven a moderate and ten a weak. It is impossible however from an examination of the past history to exclude definitely the previous existence of infection caused by *S. aureus*. Further, we wish to draw attention to the frequent presence of *S. aureus* in the intestinal flora. The faeces were examined in

17 cases chosen at random from a medical ward in the hospital, cases in which there was no reason to believe that a staphylococcal infection existed, but in four of these cases colonies of *S. aureus* were obtained. Of the cases in which there is a definite *S. aureus* infection the proportion showing the presence of this organism in the faeces is much greater. It is suggested that in some cases where no other cause can be ascertained the presence of precipitins in the serum might be associated with this infection of the intestinal tract. In addition, as will be noted later, a history of previous serum treatment is of the utmost importance. The following case may be quoted: O.W., complaining of no symptoms and apparently healthy. In April, 1915, he received anti-tetanic serum without reaction. In April, 1916, he received several further doses of anti-tetanic serum. A very marked serum sickness followed with severe urticaria, vomiting, and oedema. His serum tested in November, 1923, showed a very strong precipitin reaction with *S. aureus* (Orpen) filtered antigen. A perusal of the results obtained in cases of serum sickness and given in the subsequent section of this paper shows however that the persistence of this reaction for such a long period of time is unusual.

It would appear also that cases of asthma require special consideration. Walker (1916) has found that the proteins of bacteria commonly found in the sputum of asthmatics may be the primary exciting agents and notably of *S. aureus*. We have examined the sera from six cases of asthma. One which showed a large number of colonies of *S. aureus* in culture from the sputum gave a strong precipitin reaction. Of the remaining five, from none of which *S. aureus* was recovered, one gave a strong, one a moderate, one a weak, and two a completely negative reaction.

Whether precipitins for *S. aureus* are found in the blood of some normal individuals, as in some rabbits, is difficult to determine with certainty, but it is possible that this may be the case. The percentage of sera, from healthy normal individuals and from patients suffering from affections unassociated with *S. aureus*, which give positive precipitin reactions is large. The impossibility, however, of definitely excluding a previous *S. aureus* infection and the importance of other considerations mentioned above render the difficulty apparent.

THE EXAMINATION OF THE BLOOD IN CASES OF SERUM SICKNESS WITH FILTERED *STAPHYLOCOCCUS AUREUS* ANTIGENS.

The venous blood of patients who had received serum treatment was examined for the presence of precipitins with filtered cultures of *S. aureus* grown for one month at 37° C. in peptone beef broth. The details of the preparation of these antigens and of the technique employed has been already described. It may be necessary, however, to emphasise that these reactions were carried out in a water bath at a temperature of 52° C. and readings were taken at intervals of 24 and 48 hours. Comparisons were also made at a temperature of 37° C. and with other antigens to be referred to. The majority

of these patients had been treated with anti-diphtheritic serum, but a few had received anti-tetanic, anti-meningococcic, or anti-streptococcic serum, but each anti-serum was prepared from horses. Within 24 hours of collecting the blood the sera were tested with one or more of the various antigens. In all, 109 cases were investigated, and as already stated the majority of the patients had received anti-diphtheritic horse serum in doses varying from 2000 to 73,000 units subcutaneusly. The blood was collected in most instances in direct relation to the appearance of the rash and other manifestations of serum sickness, but occasions occurred when the blood was collected many days after the rash had faded, and in a few instances within 24 hours before the rash appeared. Some patients whose blood gave a strong reaction were re-examined after convalescence had been established, and it was then found that a negative or only very weak reaction remained. It is interesting to note that Wells (1915) found precipitins for horse serum in the patient's blood occasionally before the onset of serum sickness. He found that these precipitins were present in low concentration during serum sickness, but that the strength of the reaction increased rapidly towards the end of the illness.

In the total of 109 cases, 43 very strong positive reactions, 21 strong, and 22 moderate occurred, in 9 instances a weak reaction and in 14 a negative was recorded. These results give a percentage total of 87·3 per cent. positive of which 79·6 per cent. gave a well-marked reaction. In eight cases which had received anti-diphtheritic serum a positive reaction was obtained and in five of these cases it was well marked, but there was complete absence of clinical evidence of serum sickness. Wyard (1912) made observations on serum precipitation on 51 men who had received one or more injections of horse serum. He was unable to establish any connection between serum sickness and circulating precipitin for horse serum in the patient, as those whose blood contained these antibodies were in no way more likely to suffer from serum sickness than those who were free from them. The blood of 30 patients who gave a positive reaction of varying intensity with *S. aureus* antigens failed to react with *S. aureus* antigens prepared *without the addition of peptone to the medium*; and with *S. albus* antigens made with peptone. In addition many sera from cases of serum sickness which gave marked reactions with *S. aureus* antigens gave completely negative results when tested with antigens similarly prepared from *B. typhosus*, *B. paratyphosus* B, *B. coli*, *B. proteus*, and with diphtheritic toxin—a large number of these sera were also tested with 1 per cent. peptone solution under identical conditions, but negative results were obtained.

In Table III is shown the results of the blood examination of four cases of serum sickness with four beef broth antigens of *S. aureus* (Orpen) prepared from four distinct preparations of peptone.

These experiments are of special interest as they show the effect of preparing the beef broth antigens with four different samples of peptone. The same strain of *S. aureus* (Orpen) was used to inoculate each flask and the

flasks were then incubated at 37° C. for one month. The blood was collected from four patients suffering from serum sickness and each experiment was carried out with the same four antigens and the same technique was employed. The results obtained in each experiment are almost identical, which shows that this strain of *S. aureus* is able to make use of the four samples of peptone to the same extent. Since this observation was made our findings have been confirmed with further samples of sera.

Table III.

No.	Age	Amount of anti-diphtheritic serum injected	Interval between injection of anti-serum and appearance of rash	Interval between injection of anti-serum and blood examination	Result of Blood Examination (Dilution of patient's serum) 1 in 5	10	20	40	80	160	Antigen employed. Staphylococcus aureus (Orpen)
		units	days	days							
1	4½	54,000	13	14	+ + +	+ +	+ +	+	T*	T	1. Beef broth. A. and H.'s blood peptone
					+ + +	+ +	+ +	+	T	T	2. Beef broth. Hopkins and Williams' peptone
					+ + +	+ +	+ +	+	+	T	3. Beef broth. May and Baker's peptone
					+ + +	+ +	+ +	+	T	T	4. Beef broth peptone. Sèche de Chapoteaut
2	4½	9,000	9	10	+ + +	+ +	+ +	+	T	T	Antigens 1–4 employed in same order as above
					+ + +	+ +	+ +	+	T	T	
					+ + +	+ +	+ +	T	T	T	
					+ + +	+ +	+ +	T	T	T	
3	11	9,000	15	16	T	T	T	0	0	0	Antigens 1–4 employed in same order as above
					+	T	T	0	0	0	
					+	T	T	0	0	0	
					+	+	T	0	0	0	
4	21	12,000	9	10	+	T	T	T	T	0	Antigens 1–4 employed in same order as above
					+	T	T	0	0	0	
					T	+	T	0	0	0	
					T	T	T	0	0	0	

* T = Trace.

The blood reactions of six cases of serum sickness were compared as regards our *S. aureus* precipitin reaction and precipitation with antitetanic horse serum. For this reaction 0·5 c.c. of serum obtained from cases of serum sickness was added to each tube of 0·5 c.c. of anti-tetanic horse serum diluted from 1 in 2·5 to 1 in 1000 with carbolised saline, and incubated at 37° C. for one hour, then put in the ice safe overnight, when the readings were taken, which is the method recommended by Longcope and Rackeman (1918). As no precipitation occurred with any of these six samples of blood and anti-tetanic horse serum when incubated for one hour at 37° C., a further incubation was given on the following day at 52° C. for 24 hours, but no reaction occurred. Five positive results out of the total of six cases of serum sickness were obtained, however, by our method with *S. aureus* antigen as recorded in Table IV.

Table IV.

No. of case	Amount of anti-toxin given	Presence of rash	Strength of reaction (dilution of patient's serum) 1 in 5	10	20	40	80	160
1	9,000	Faded	+	+	T*	T	T	0
2	12,000	Onset of rash	+ +	+ +	+	+	T	T
3	27,000	Faded	0	T	T	0	0	0
4	39,000	Present	+ +	+	+	T	T	0
5	45,000	Onset of rash	+ + +	+ + +	+ +	+	+	T
6	15,000	Faded	0	0	0	0	0	0

* T = Trace.

ON THE PRESENCE OF PRECIPITINS IN NORMAL AND IMMUNE RABBITS' BLOOD WITH FILTERED ANTIGENS MADE FROM CULTURES OF *STAPHYLOCOCCUS AUREUS*.

The first observations on this subject were apparently made by Castellani (1902) who immunised rabbits with cultures of *S. aureus* and found that the immune sera precipitated old cultural filtrates of this organism.

It was an unexpected discovery for us to find that the blood of some normal rabbits in perfect health gave a positive reaction with our *S. aureus* precipitin antigens, and that others failed to do so. Reactions varying in degree from strong to feeble occurred, and further, the strength of the reaction was not a constant feature when repeated observations were made at irregular intervals, although the same antigen and identical technique were employed.

These results are shown very clearly in the following table (V):

Table V. *Normal Rabbits' Blood.*

Rabbit	Dilution of blood serum 1 in 5	10	20	40	80	160
1	+ +	+ +	+	T*	0	0
2	+	+	T	T	0	0
3	+ + +	+ +	+	T	0	0
3 Re-tested 13 days later	+	T	T	T	0	0
4	0	T	0	0	0	0
5	+ + +	+ + +	+ +	+	T	0
5 Re-tested 21 days later	+	+	+	T	T	T
5 „ 30 „	T	T	T	0	0	0
5 „ 43 „	+ +	+ +	+	+	+	T
6	T	T	T	T	0	0
7	T	T	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0

* T = Trace.

Various points are clearly demonstrated in the above table, namely the intensity of the reaction among normal rabbits, the variation in the degree of the reaction among individual rabbits, and the alteration in the intensity of this reaction when the rabbits are re-tested at intervals of several days. This last observation is clearly demonstrated in the case of rabbit 5.

Having shown that normal rabbits' blood may give a strong reaction, and that this reaction is subject to wide variation, we were fully able to appreciate our results when rabbits were injected with living cultures, filtered cultures of one month's growth, or vaccines of *S. aureus*.

Table VI.

Inoculated Rabbits. Experiment 1.

Date	Inoculation	Antigen	Results (dilution of serum) 1 in 5	10	20	40	80	160
April 29	—	*S. aureus* (Orpen)	++	++	+	T*	0	0
		S. albus	0	0	0	0	0	—
		Diphtheritic toxin	0	0	0	0	0	—
May 6	50 million *S. aureus* (Orpen) living							
May 12	—	Filtered *S. aureus* (Orpen)	++	++	++	++	+	+
May 13	Death from Myelitis							

* T = Trace.

In the experiment described in Table VI the presence of *S. aureus* precipitin was demonstrated in the blood of the rabbit before it was inoculated, although no reaction occurred with antigens of *S. albus* or with the diphtheritic toxin. A week after obtaining positive results with the uninoculated rabbits' blood, 50 millions of the living emulsion of the same strain of *S. aureus* as furnished in the antigen were injected intravenously. A week later it was found (Table VI) that the strength of the reaction had considerably increased in spite of the fact that the rabbit died on the day following the blood examination from myelitis. Three further experiments are shown to illustrate similar points in the *S. aureus* precipitin reaction (Tables VII, VIII and IX).

Table VII.

The antigen used in this experiment was *S. aureus* (Orpen)

Date	Inoculation	Results (dilution of serum) 1 in 5	10	20	40	80	160	320
July 2	None	T*	T	T	T	0	0	0
,, 5	25 million live *S. aureus* (Orpen) I. V							
,, 9	—	++	++	+	T	T	T	0
,, 16	50 million							
,, 21	—	+	+	T	T	T	0	0
,, 23	Death from myelitis							

Table VIII.

The antigen used in this experiment was *S. aureus* (Nauen)

Date	Inoculation	Results (dilution of serum) 1 in 5	10	20	40	80	160
Feb. 27	50 million live *S. aureus* (Nauen) I. V						
Mar. 1	—	0	0	0	0	0	0
,, 5	200 ,, ,,						
,, 8	—	0	0	0	0	0	0
,, 16	—	++	++	++	+	+	+
,, 21	—	++	++	T	0	0	0
,, 24	400 ,, ,,						
April 5	—	++	++	++	++	T	0
,, 6	Killed						

* T = Trace.

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Table IX.

The antigen used in this experiment was *S. aureus* (Orpen)

Date	Inoculation	Results (dilution of serum) 1 in 5	10	20	40	80	160	320
July 2	—	T	T	0	0	0	0	0
„ 5	25 million live *S. aureus* (Orpen) I. V.							
„ 9	—	+	+	T	T	T	T	0
„ 16	50 „ „							
„ 21	—	+ +	+	+	T	T	T	0
„ 24	2 c.c. of *S. aureus* toxin (Orpen)							
„ 30	—	+ +	+ +	+	+	T	T	0
Aug. 13	—	+	+	+	+	T	0	0

In these three experiments the rise in the reaction following the intravenous inoculation of an emulsion of living *S. aureus* using the same culture for the inoculation as employed for the preparation of the filtered antigen is demonstrated.

It is well, however, to realise that fluctuations in the intensity of this reaction occur with the blood of normal healthy rabbits, so that a rise or fall in the reaction may appear apart from inoculation.

The effect of storing Rabbit Serum on the precipitin reaction. The following experiments illustrate the loss of potency when immune sera are stored, and how this varied with the method of storage adopted and the specificity of the reaction.

Other experiments with immune sera and various antigens of *S. aureus* were tested and gave somewhat similar results. A serum which gave a very strong precipitin reaction with a *S. aureus* antigen even when diluted to 1 in 1000 with normal saline, was divided into two portions to one of which two drops of chloroform were added and the other was diluted 1 in 5 with formol saline. Each sample of serum was stored in the ice-safe. One month later the chloroformed undiluted serum was the stronger, but the end point

Table X.

1. Immune serum obtained from a rabbit which had been inoculated with emulsions of living *S. aureus* (Nauen) isolated from pus. First tested April 9th, 1923.

Antigen	(Dilution of serum) 1 in 5	10	20	40	80	160
S. aureus (Nauen)	+ +	+ +	+ +	+ +	+	0
„ (Todman)	+ +	+ +	+ +	+	T	0
„ (Smith)	+ +	+ +	+ +	+	+	0
1 per cent. peptone	0	0	0	0	0	0
B. typhosus filtered peptone beef broth	0	0	0	0	—	—
B. coli (Dow) „ „	0	0	0	0	—	—
„ (4869) „ „	0	0	0	0	—	—

The rabbit serum was then diluted with formol saline (0·1 per cent.), stored in the ice-safe and re-tested on May 15th, 1923.

S. aureus (Nauen)	T	T	0	0	0	0
and again re-tested December 12th, 1923						
S. aureus (Nauen)	0	0	0	0	0	0

Table XI.

2. Immune serum obtained from a rabbit which had been inoculated with a live emulsion of *S. albus* (osteomyelitis) and tested with filtered antigens prepared from same strain. Serum was collected and divided as follows:
(1) Pure serum containing 0·1 per cent. formalin. (2) Diluted 1 in 5 with 0·1 per cent. formol saline. (3) Heated at 58° for 20 minutes then diluted with 0·1 per cent. formol saline. All these samples of serum were stored in the ice-safe.

		Result Dilution of serum					
Date		1 in 5	10	20	40	80	160
June 20	Immune serum	+ +	+ +	+ +	+ +	+	T*
July 4	Stored immune serum						
	No. 1	+ +	+ +	+	+	T	T
	,, ,, 2	+ +	+ +	+ +	T	T	0
	,, ,, 3	+ +	+	0	0	0	0
July 19	,, ,, 1	+	T	T	T	T	0
	,, ,, 2	0	0	0	0	0	0
	,, ,, 3	0	0	0	0	0	0

* T = Trace.

had fallen from 1 in 2000 to 1 in 80. These immune sera, however, gradually lose potency in the living animal.

Nuttall (1904) states with reference to haematosera that in the majority of cases these anti-sera deteriorate markedly after three or four months, although some had given good reactions after being sealed in a pure state for several months *in vitro*.

CONCLUSIONS.

I. Of methods adopted and of results obtained in staphylococcal infections.

(1) *Staphylococcus aureus* precipitin antigens were prepared with beef broth and 1 per cent. peptone, and were filtered after one month's incubation at 37° C. Filtration was carried out with Berkefeld candles.

(2) Antigens prepared without the addition of peptone to the beef broth were of no value.

(3) All strains of *S. aureus* were found to form efficient antigens, but only one strain of *S. albus* obtained from a case of acute osteomyelitis.

(4) These antigens were satisfactory for a period of at least six months when stored in cupboards at room temperature. The most satisfactory preservative was 0·1 per cent. formalin.

(5) The precipitin reactions were carried out in closed water baths at 37° C. and open water baths at 52° C., but the latter method was preferred, more especially as bacterial contamination was avoided. Readings were taken after 24 and 48 hours.

(6) Heated and unheated sera could be employed, but in most instances the sera were heated at 58° C. for 20 minutes.

(7) Stored sera rapidly deteriorated.

(8) Out of 118 cases of known *S. aureus* infection 43 or 37 per cent. showed a strong or moderate precipitin reaction, 22 or 18 per cent. a weak reaction and 53 or 45 per cent. were negative. The percentage of positive results (55 per cent.) is higher than that found amongst cases where there was no reason to believe that such an infection existed, for amongst this latter group of 98 cases, 42 per cent. were positive. When present, however, this reaction appears to be specific as it was not obtained with filtered antigens made under the same conditions from cultures of *B. coli*, *B. typhosus* and other organisms mentioned except in the presence of co-existing infection.

(9) The blood of many normal rabbits gave a strong or moderate positive reaction with *S. aureus* antigens, but such reactions were found to vary in normal animals and were increased in strength as a result of inoculation of living cultures of *S. aureus*.

II. OF RESULTS IN SERUM SICKNESS.

Of the 109 cases of serum sickness examined 87·3 per cent. gave a positive reaction with *S. aureus* filtered antigen and of these 79·6 per cent. a well-marked reaction. Viewed as a whole there is no doubt that the precipitin reaction for *S. aureus* in serum sickness is considerably stronger than that obtained in known *S. aureus* infections. The reaction appears also to be specific in the sense that antigens prepared from other organisms in an exactly similar way are of no value. The reaction was not obtained with *S. aureus* grown without peptone and peptone alone showed no antigenic effect. The phenomenon appears to be due to some interaction between the precipitin in the patient's serum and some body which *S. aureus* elaborates from the beef broth and peptone. It was thought that this precipitin in the serum of patients suffering from serum sickness might be bound up or even identical with the precipitins for horse serum which have been described by various workers, but we failed to show this correlation by experiment.

In conclusion we wish to offer our sincere thanks to Dr Foord Caiger for permission to obtain samples of blood from cases of serum sickness at the South Western Fever Hospital.

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