

SUBACUTE INFECTIVE ENDOCARDITIS.

A bacteriological, serological
and histological study.

Ass.

by

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General plan of the investigation.

The investigation herein recorded was undertaken with a view to determining the frequency of the occurrence of streptococcal septicaemia in a series of cases of cardiac valvular disease presenting clinical features suggestive of, or definitely indicating the presence of an infective condition of the endocardium of a subacute type. A large number of similar cases studied in the Cardiological Department of University College Hospital has recently been reported upon by LEWIS and GRANT (1923) and GRANT (1924).

It was observed that cultures made from the blood failed to reveal the presence of streptococci in a great percentage of the cases. These results were in marked contrast to those obtained by workers in other centres (LIBMAN 1920, 1923, MURRAY (1922)).

Failure to isolate an organism from the circulating blood of a patient may be due to several factors.

- (1) The medium employed may be unsatisfactory.
- (2) The presence of the organism in the blood stream may be only intermittent or temporary.
- (3) The organism may not be present in the circulating blood, but may be strictly limited to the site of the lesion, in this case the damaged valve, or alternatively it

may be that the activity of the pathological process has subsided, the organisms have been destroyed and no longer occur either in the circulating blood or the focus of the disease.

In studying the first of these points a comparison has been made of the results obtained in a series of blood cultures by the use of a variety of media and experiments have been carried out to determine the number of organisms which require to be introduced into these media in order that a positive blood culture may be obtained. Experiments were also made with a view to throwing some light upon the nature of the marked delay observed in the growth of bacteria in cultivations made from the blood of a patient with septicaemia.

In most of the investigations made the actual number of organisms in the blood has been estimated in order to permit of the study of the fluctuation in the degree of the septicaemia and so decide the importance of the part played by the alleged intermittency of the bacteraemia in such a series of observations.

The third factor has so far been studied only in part. It has been possible by the courtesy of Sir Thomas Lewis to compare the results of blood cultures made during life with the microscopical findings in the valves after death. The further step of making cultures from the valves after death has not yet been carried out. This is owing to two difficulties. The

first is that a preliminary study of the bacteriology of the heart blood in the cadaver revealed the unreliability of the method of investigation, and the second, that cases of the required kind are infrequent in their occurrence. This essential portion of the investigation has therefore been delayed.

The nature of the organisms isolated from septicaemic cases has been studied. The literature contains many statements to the effect that in this disease a great variety of organisms has been found in the blood stream. Little attention has been paid to the question of the possibility that some of these may be terminal or secondary invaders. In this study attention has been directed to this point and a certain amount of evidence upon it obtained.

The prolonged duration of the bacteriaemia and the relative lack of virulence of the organisms isolated from these patients together with the absence of secondary suppurative lesions in the course of the disease are very striking features and lack a complete explanation. Evidence has therefore been sought as to the occurrence of any peculiarities in the organisms circulating in the blood as compared with those in cultures in laboratory media and also as to the power of the patient's blood to deal with and destroy these organisms. The very high rate of mortality - in the series of cases studied fully in this investigation none has recovered - renders the question of treatment of a specific nature an interesting and important one. Certain observations upon

this subject are recorded.

Experiments have been made upon rabbits with a view to studying the disease process in its evolution and development under definite conditions. The results have not been as satisfactory as had been hoped and much further work remains to be done. Sufficient material has however been available for the comparison of certain features of the experimental disease with those obtaining in the natural disease in man.

The relationship of infective endocarditis to acute rheumatism is not yet accurately determined. This problem has been approached from the histological side (1) by a search for evidence of rheumatic lesions in the myocardium of cases of subacute infective endocarditis. (2) by a study of the lesions in the valves in subacute bacterial endocarditis and a comparison of these with the characteristic lesion in the myocardium in rheumatic fever - the submiliary nodule.

Literature. The very extensive literature upon the subject herein discussed has been examined in detail. A great deal of it is of little value, consisting of records of individual cases or of philosophical speculations without accurate experimental control. It is intended to discuss the literature only in so far as it has a direct bearing upon the points at issue.

General reviews covering the subject of subacute infective endocarditis have been published by SIMONS (1913), DEBRE (1917, I., 1919) and BLUMER (1923). The latter author gives a very complete bibliography up to the date

of his paper. This has been of great assistance in the search for information upon the various problems considered.

Subacute Infective Endocarditis.

Definition.

By this term is intended to be implied a disease characterized during life by evidence (more or less marked) of cardiac valvular disease extending over a period of considerable duration, usually more than three months, and by some or all of the following symptoms and signs:-

Progressive loss of weight and emaciation.

Night sweats.

Pallor or development of a "cafe au lait" tint in the skin.

Splenic enlargement.

Clubbing of the fingers.

Petechial haemorrhages in the skin.

Painful nodes (Osler's nodes) particularly in the fingers and toes.

A secondary anaemia.

Red blood corpuscles in the urine.

Elevation of temperature, varying very considerably in degree and in the frequency of its occurrence, generally low and intermittent but sometimes higher and remittent.

It is almost invariably fatal.

The post mortem findings consist of large vegetations on one or more valves, most frequently on the left side of the heart, usually showing some evidence of chronicity and frequently extending to the mural endocardium or the intima of the aorta (HARBITZ 1899, LIBMAN 1912, GRANT 1924). There is usually no actual destruction of the tissue of the valve or invasion of the myocardium.

Signs of embolism or infarction are common, especially in the spleen, which is usually very large.

In the kidney there is very frequent evidence of a glomerular nephritis. (LOHLEIN 1910, LIBMAN 1912, BAEHR 1912).

Signs of chronic heart failure are commonly to be observed. There may be evidence of septicaemia. Abscess formation does not occur.

The separation of the disease from acute infective endocarditis on the one hand and chronic endocarditis on the other is not definite.

The disease in its fully developed cases is a definite clinical entity with characteristic post mortem appearances.

Material investigated.

The cases of chronic and of subacute infective endocarditis investigated fall into two categories as under:-

(1) Cases in which a clinical diagnosis of subacute infective endocarditis was definitely made.

(2) Cases which had been under observation for a considerable period as out-patients suffering from chronic valvular disease but in whom there were certain individual signs and symptoms raising the suspicion that there may have been a superadded or coincident infective process.

Cases of chronic heart disease with signs suggestive of infection. In this group there were 34 cases. From these there were examined in all 77 specimens of blood and from none of them were streptococci or any other organism isolated.

The number of times individual specimens were examined is indicated below.

Examined once only	11
" twice	7
" three times	12
" four times	4

Of these cases the great majority are still under observation and the long duration of symptoms, in some cases as long as four or five years, renders it improbable that they are really suffering from any active infective process of the heart valves. Four cases have however died in the interval. In three of these the evidence obtained at post mortem was not such as to suggest that they had suffered from subacute infective endocarditis. In the fourth case there was found a very old calcified lesion of the aortic valve which resembled somewhat in its external appearances those which are commonly seen in cases of subacute infective endocarditis and

suggested that it might conceivably have been such a case in which the infective process had subsided and calcification occurred.

In this group then there is so far no evidence to throw doubt on the validity of the results of the blood cultures.

It is unfortunate that the clinical data in the great majority of these cases do not include temperature records. The patients were almost all outpatients which fact renders the obtaining of this information impossible.

Cases definitely diagnosed as subacute infective endocarditis. In this group there fall to be included 16 cases.

Of these, two are still in hospital; three have left hospital and their fate has not been ascertained.

Of the remaining 11 all are dead. In nine cases an autopsy was performed and the clinical diagnosis was amply substantiated. One died at home and no post mortem information is available; the other died in hospital and permission for autopsy was refused.

A brief summary of the clinical details of the cases which were examined post mortem is included in the complete list on p. 179

In view of the suggestion widely made that the septicaemia in these cases is often intermittent cultures were made as frequently as possible. In some cases however circumstances prevented this being done.

The results obtained were as follows:-

TABLE I.

Results of blood cultures in cases of subacute infective endocarditis.

Case.	No. of cultures.	Results.	Remarks.
Ain	1	Pneumococci	P.M. confirmation. *
All	5	Streptococci	No P.M.
Am	9	do.	P.M. confirmation.
At	1	do.	No P.M.
Av	2	do.	No P.M.
Bau	1	Negative	No P.M.
Crudg	6	Negative	P.M. confirmation.
Dud	3	Streptococci	P.M. confirmation.
Jul	2	Negative	do.
Lat	4	Negative	do.
	1	Staph. Albus.	
Mo	2	Streptococci	do.
Mur	2	Streptococci	Still living.
Pet	4	do.	P.M. confirmation.
Ting	1	Negative	No P.M.
Tr	6	Streptococci	Still living.
Wil	2	Streptococci	P.M. confirmation.

Streptococci = non-haemolytic streptococci.
* refers to diagnosis.

The list shows that of the 16 cases examined 10 only showed the presence of streptococci in the blood-stream during life. From the blood of case Ain a pneumococcus was isolated on the day before death - no streptococci. Case Lat after yielding four negative blood cultures gave a growth of staphylococcus albus in the fifth. These two cases will be discussed further in another connection.

The remaining four cases yielded no organism of any kind, two being examined on one occasion only, one on two occasions and the other on six occasions altogether. In the case Crudg the observations

extended over a period of 17 months, the patient being in hospital most of that time and showing most characteristic signs and symptoms of the disease. One of the observations was made by Dr. Embleton and two others by Dr. G. W. Goodhart.

Thus 6 cases failed to show any evidence in blood cultures of the presence of a streptococcal bacteraemia. Two of the cases (those in which only one observation was made on the blood) were not examined at autopsy. In the remaining four the lesions found were characteristic of the condition.

With regard to those cases from which streptococci were obtained it remains here only to be noted that they were isolated with the greatest ease on the medium employed and that in all cases where attempts were made to repeat the original observation this was found to be possible. In case A in this was done on as many as nine occasions.

In dealing with this point I have had an opportunity of consulting the records of many of the cases in Sir T. Lewis's collection. There is a group of 17 cases in whose histories a statement occurred as to the result of a blood culture. It was found that twelve failed to give any growth of streptococci while in five the organisms were obtained.

In the whole 33 cases there were 18 with no evidence of streptococcal bacteraemia and 15 from whose blood streptococci were cultivated.

The percentage incidence of streptococci in all cases here studied is as follows:-

- (1) This series of cultures 10 out of 16 = 62.5%
 (2) Sir T. Lewis's cases 5 out of 17 = 29.4%

Or of all cases of the two groups 15 out of a total of 33 contained definite evidence of streptococci circulating in the blood, which is equal to 45.4 per cent.

In addition there were examined histologically vegetations from 11 cases in which no blood culture appears to have been made. In these cases only one showed definite bacteria suggesting that a blood culture would have been positive. The percentage figure given is therefore probably higher than would actually have been obtained if all cases had been fully investigated.

The blood cultures in group (2) of the above cases were done chiefly by Dr. G. W. Goodhart. His technique differed slightly from that which I adopted. Opportunities have however occurred for comparing results obtained by the two methods and they have been found to agree in each case.

Percentage calculations are perhaps not justifiable in such a relatively small number of cases but the first point which clearly emerges is that a large number - the majority - of the cases here considered failed to show evidence of streptococcal bacteraemia.

Further the incidence of this in the later series (Group (1)) has been greater than in the earlier. It is to be noted that Group (1) is probably more nearly representative of the ordinary hospital cases than Group (2) which consisted very largely of cases arising in pensioners invalided from the army after or during the war.

Altogether three cases occur in which pneumococci were isolated in blood culture and one where staphylococci occurred. These will be considered further in connection with the section on terminal or secondary infection. The streptococci isolated from the other cases were of the non-haemolytic type.

Comparison of results with
those recorded in literature.

The bacteriology of the blood stream in this condition has been widely studied, especially in recent years.

HARBITZ (1899) made a thorough histological study of the heart valves in 26 cases and noted that in 16 of these cocci could be readily demonstrated but in 10 others he failed to find any organisms at all.

SCHOTTMULLER (1903) isolated streptococci from the blood of some cases and drew attention to the fact that the organisms isolated were not capable of producing haemolysis on plates of blood agar, a fact which differentiated them from streptococci associated with suppuration. This observation was soon confirmed and from that time onwards the isolation of such an organism from the blood stream was commonly regarded as a

cardinal point in the diagnosis of the condition.

Further definition of the organism found in the blood of cases of subacute infective endocarditis was made by ROSENOW (1909, 1910 I.) who, like Schottmuller, observed its tendency to produce a green discolouration on blood agar. He referred it to the group of pneumococci rather than that of streptococci. This latter view has not received general acceptance but the green discolouration of the medium has been frequently noted so that the name streptococcus viridans has been commonly employed and the term "viridans sepsis" frequently occurs in American papers on the subject.

LIBMAN & CELLER (1910) communicated observations on 36 cases and stated that they had succeeded in obtaining streptococci from the blood in all but one of these. But in 1913 in a further report on 125 cases, which apparently include those reported in the first paper, LIBMAN (1913) states that of his last 82 cases there were 18 from which no organisms could be obtained. These cases were clinically, and from the point of view of morbid anatomy, similar to others of his series and he described them as being in the "bacteria free" stage of infective endocarditis, thereby suggesting the interesting view that in a certain number of cases the patients became free of the infection but, as he remarks, die of the effects of that infection. In a later paper (LIBMAN 1920) he states that 25 per cent of the cases are in

the bacteria free stage though subsequently (LIBMAN 1923) he modifies this somewhat by saying that more than 90 per cent of cases have a bacteraemia. Of the organisms isolated 95 per cent were non haemolytic streptococci.

In view of our experience of blood culture work in these cases it was interesting to see how far reports from other sources agreed with the figures quoted above. In the literature the statement is frequently found that streptococci were obtained from a certain number of cases out of a group. It is not made clear if blood cultures were made in all cases from which streptococci were not obtained. The following list is given of results reported in the literature. For convenience of subsequent reference a column is included to indicate the methods employed for cultivating the organism.

TABLE II.

Results of blood cultures in
subacute infective endocarditis,
as reported in the literature.

Author.	Date.	Posi- tive	Nega- tive	Total.	Method.
Jochmann	1912	7	0	7	Agar plates.
Major	1912	3	2	5	
Libman	1913	64	18	82	Broth (serum glucose) and glucose agar.
	I				
Kinsella	1917	12	0	12	Flasks of broth Glucose agar Blood haemolys- ed and centri- fuged (Rosenow)
Vaquez	1917	6	3	9	
Kastner	1918	16	0		Agar. Broth flasks.
Starling	1918	1	3	4	
Cotton	1920	9	15	24	
Salus	1920	19	20	39	Broth. Agar.
Nicholl	1921	1	8	9	
Morawitz	1921	3	7	10	
Lampe	1922	6	13	19	Identical with Kastner's.
Curschmann	1922	3	8	11	
Hassencamp	1922	4	29	33	
Bargen	1923	35	9	44	Anaerobic. Rosenow method.
Freund & Berger	1924	19	0	19	Clot separated and inoculated into serum broth.
Krieger & Friedenthal	1924	14	0	14	v. Freund & Berger.
Jungmann	1924	6	94	100	
Gracie	1924	0	10	10	
The results refer to non-haemolytic streptococci only.					

(In addition to the figures recorded in Table II. it may be observed that GESSLER (1921) states that he has had poor results in his 33 cases whereas MURRAY (1922) obtained positive cultures from the blood in 70 per cent of his 200 cases).

Clearly there is a very marked discrepancy between the figures given by various authors, the positive results varying from nil to 100 per cent. Considered broadly the reports fall into four main groups.

(1) The American group, in which the percentage of positive blood cultures is high - 70 to 90 per cent.

(2) A group of German workers (Jochmann, Kastner, Freund and Berger, Krieger and Friedenthal), who in a relatively small number of cases have obtained positive cultures in each case.

(3) A second group of German workers whose results have been much less frequently positive (Salus, Morawitz, Lampe, Hassencamp,, Jungmann). These workers have all reported positive results in less than 50% of their cases, some in a much lower percentage.

(4) A group of workers in this country (Starling, Cotton, Nicholl, Gracie) in whose reports positive blood cultures are rare.

It is difficult to explain the great discrepancies in these figures.

One factor affecting the problem considerably is undoubtedly the matter of diagnosis. If the diagnosis is confined to those cases in which positive blood cultures are obtained the percentage of positive cultures in reported cases is naturally high. That point it is difficult to clear up in many of the reports. The adoption of such an attitude is apparently not warranted by the facts.

Another difficulty is that the number of cases of this disease reported upon by individual writers is small and figures obtained from smaller numbers would naturally be unreliable for the purpose of such estimations as are here being considered. LIBMAN'S cases (1913, I.) amounting to 125 and MURRAY'S (1922) amounting to 200 might be considered representative of the disease in America and the percentages agree well. It is to be noted however that in Libman's first 36 cases only one had a negative blood culture, while of his next 82 there were 18 which had no organism in the blood stream.

A view which finds common expression with regard to these discrepancies is that they are due to differences in the technique employed. The point will be discussed at greater length elsewhere but it should be noted that in American work Rosenow's technique is widely used. He uses a large quantity of blood (30 - 40 c.c.), lyses it in distilled water, centrifuges the fluid and removes the supernatant fluid, adding glucose broth containing ascitic fluid to some tubes, agar to others, and inoculating some broth tubes under a paraffin seal. That is to say he attempts to remove the blood fluids, which are considered inhibitory to growth and cultivates the material in a variety of media, varying particularly in their oxygen tension. Such a technique is claimed to be greatly superior to those more commonly employed though experimental evidence is lacking

or unsatisfactory. KINSELLA (1917) comparing this method with that of inoculating blood direct into broth and into agar could find no advantage in using Rosenow's method. The results were identical in all cases.

FREUND and BERGER (1924) who report positive results in 100 per cent of their 14 cases allowed the blood to clot and then removed the serum and inoculated the clot into 50 c.c. of broth - a form of technique recommended also by FELIX (1924) for use in the case of typhoid fever. But a close scrutiny of their report leaves one in doubt as to the value of their results. In the first place it is not clear that the cases with which they were dealing were really infective endocarditis. The history of the cases is only briefly given but is not such as is usually associated with the disease. Moreover the cultural results were remarkably irregular when the procedure was repeated and further there are many cases given of the isolation of streptococci from the blood in diseases not usually associated with a streptococcal septicaemia. The cases of KRIEGER and FRIEDENTHAL (1924) were apparently in the main the same as those reported by Freund and Berger. It is however to be noted that OILIE, GRAHAM & DETWEILER (1915) reported a series of 26 mild cases of endocarditis with fever from which they were able to cultivate streptococci by Rosenow's method, an observation which is apparently unique.

The other methods employed were really very similar to one another, the main variation being in the amount of blood employed, a factor which must affect the chances of obtaining organisms. It is to be noted however that some writers stress the importance of using solid media while others insist that liquid media should be employed. Most workers appear to use both. DURAND (1921) makes the statement that failure to isolate a streptococcus in a case of this disease is due to faulty technique. His own technique consists in inoculating 20 c.c. of blood into 3 times that amount of glucose broth or glucose ascitic broth, which really does not differ from what is done in most laboratories as a routine procedure.

Finally it is to be noted that KASTNER (1918) reports positive blood cultures from all his 16 cases, whereas LAMPE (1922) whose cases are investigated in the same laboratory by exactly the same technique reports only 6 positive in 19 cases. Here in what is virtually a series of 35 cases the first 16 had a bacteriaemia whereas of the next 19 only 6 had. Lampe himself points out that this discrepancy could not be accounted for by technical differences. Control observations showed that it was not the method which was at fault.

However argumentation of this kind is not conclusive. For that reason the whole question of technique has been carefully investigated and ^{the result} is reported

in the next section.

Before proceeding to that it should be noted that most of the cases with negative blood cultures herein discussed occurred among war pensioners. It is interesting to note that GOW (1920) has remarked on the difficulty of obtaining positive cultures since the war. It is quite possible that the frequency of the bacteriaemia may be greater in one country than in another and also that it may vary from time to time in the same country.

On the Technique of Blood Cultures.

The technique employed in making blood cultures has been variously considered in the literature on the subject. It would appear, however, that very little exact comparative study of the various methods suggested has been carried out. Recommendations of methods have been made on speculative grounds with but little evidence to support the claims advanced. With the very natural result that diametrically opposite conclusions have been reached by different workers with regard to the advantages of different methods, some authors insisting on the importance of using liquid media, while others claim that solid media are more suitable.

Comparisons of the efficiency of different media as judged by obtaining positive results in individual cases or in a series have been made by various authors. LIBMAN (1906) as a result of his experience in a large number of observations on the blood recommends that several media (liquid and solid) should be used and that a series of tubes should be inoculated with varying amounts of blood with a view to avoiding possible inhibition of growth in higher concentrations of blood.

ROSENOW (1909) has stated that growth sometimes occurs in solid media when it fails to occur in broth. This statement is however open to a certain degree of

criticism when used to substantiate a claim that the medium therefore has certain advantages. As KIPNIS (1923) has pointed out and as will be shown later it is in fact a matter of chance whether bacteria will grow in a particular tube of medium inoculated with blood containing organisms. Apart altogether from the qualities of the medium the growth must depend on whether organisms are present in the particular portion of blood inoculated. And as the bacteria are particulate objects suspended in a fluid it is not surprising if they may be unevenly distributed. Further if the number of organisms is less than 1 per c.c. of blood there must be some samples of blood of that size which will contain none. Therefore conclusions based on these occasional observations of the superiority of a given medium are liable to error. SALUS (1920) indeed affirms that exactly the opposite of Rosenow's statement has been his experience.

Two factors in the technique of blood culture which are generally considered of importance are the prevention of coagulation and adequate dilution.

DOUGLAS and COLEBROOK (1916) in an experimental consideration of blood culture technique point out that, in the process of clotting of the blood, the organisms are taken up by the clot in large numbers and that they are then in a less advantageous position as regards destruction by leucocytes. Dilution in their view is useful because of its effect

upon the destructive and inhibitory agents present in the blood. They have experimented with trypsin and shown that in test tube mixtures of bacteria and anti-serum and in specimens of blood from rabbits with experimentally produced septicaemia, broth containing trypsin (5 per cent) has distinct advantages over ordinary broth. The specimens more frequently gave a growth and the growth occurring appeared earlier. Their experimental observations were confirmed by observations on cases of paratyphoid fever and streptococcal infection.

It is noteworthy that in the experiments on ordinary broth no anticoagulant is reported as having been added. The action of the trypsin is explained as due to its preventing clotting and reducing opsonic action and to its "antitryptic" action on the blood, which latter according to WRIGHT (1915) is powerfully inhibitory to growth of organisms and is often more marked in infected than in normal people.

With the object of preventing this antibacterial or growth inhibitory action of the blood various other methods have been suggested. ROSENOW (1914), as indicated above, attempts to separate the bacteria from the fluids of the blood. Freund & Berger and Felix use the clot, after the separation of serum, for their inoculum. On the other hand FINKELSTEIN (1923) uses the serum separated from the clot to obtain her cultures and dilutes well in broth (10 c.c. of serum to 300 c.c. of broth).

WALKER HALL (1922,II.) working first with mixtures of bacteria and appropriate immune whole blood stated that weak concentrations of certain acids (especially lactic acid) showed definite effect in overcoming the inhibitory effect of the whole blood and later in work upon patients with septicaemia found that the improved growth in "acid" broth was an indication of the powers of recovery of the patient. The difference in the two types of media could not be explained by variations in the initial reaction. In a previous paper (1922,I.) it was suggested that the effect of the acids was upon the phase of growth of the organism known as "the logarithmic phase", the actual rate of multiplication being increased.

Still another method or rather a variation of the ordinary method of overcoming the difficulties discussed was used by AUERBACH (1920) who simply adds the blood to half a litre of distilled water and incubates this. He does not discuss the reason for selecting distilled water. STADLER (1914), KASTNER (1918) and HASSENCAMP (1922) all insist on the great importance of inoculating the blood at once into the appropriate medium, claiming that within a very short time (2 - 6 hours) the bacteria are destroyed by the blood in the tube at the ordinary temperature. SALUS (1920) also recommends the same procedure while KAMMERER (1914) says that he has found cultures made at the bedside positive, while those made from blood defibrinated by shaking with glass beads and inoculated later

into medium were sterile. KIPNIS (1923) on the other hand suggests preliminary incubation of the blood for 24 hours before inoculation into media.

Finally there are statements recorded regarding the selection of the time for collecting the sample of blood from the patient. KASTNER (1918) indicates that the blood should be drawn "at the peak of the temperature". LEECHKE (1916) says that the best time is "before the rigor at the height of the fever". After the rigor the organisms had disappeared, "the rigor bring due to destruction of the bacteria". AUERBACH (1920) agrees that the best time is before the rigor and GESSLER (1921) considers that absence of fever may account for failures in isolating the organism. None of the authors give any quantitative observations in support of the various statements.

From this consideration of the literature it is clear that many statements are made which lack experimental substantiation and any procedures based upon these is liable to be unsound. Accordingly the experiments recorded below were carried out with a view to obtaining more precise information upon the various points. They were originally designed to settle certain questions of technique but as the study progressed it seemed possible to extend them so as to include consideration of the organism in the blood and the resistance of the patient to it. The whole group of experiments will be recorded together and the application of

each to the question of technique considered.

The other problems arising out of the experimental results will be considered under the appropriate headings.

Methods employed.

The methods employed in the investigation have been altered slightly as experience on the subject has been obtained.

The first main difficulty has been to ensure the absence of contamination from the skin and air.

It has been found that contamination from the skin was more common in out-patients than in hospital patients confined to bed, but it could be avoided in practically all cases if the skin were first cleansed thoroughly with a weak solution of lysol in warm water, then with alcohol and finally with ether.

In the event of there being any roughening or irregularity of the skin surface even these precautions sometimes failed to avoid contamination.

The most frequent source of trouble in this connection has however proved to be those cases in which difficulty has been experienced in entering the vein. Here it has proved advisable to put a fresh needle on the syringe and make an entirely fresh puncture in the skin.

Air contamination has however been more troublesome. It has been found that this is most easily avoided if the blood with an anticoagulant is placed

in a single large test tube and removed to the laboratory where it is distributed along a series of tubes containing appropriate medium. In this way suitable precautions against air contamination are more easily taken than is usually possible in a hospital ward. Test tubes are easier to manipulate than flasks and less liable to be contaminated. Moreover if contamination does occur it is usually to be found confined to one or a small number of tubes while organisms from the blood are generally, though not always, present in all.

McLEOD & BEVAN BROWN (1918-1919) have pointed out the importance of using in the medium and in the collection of blood only such constituents and apparatus as can be thoroughly sterilized by heat. The instruments have accordingly always been sterilized by boiling for half an hour and all media have been autoclaved, excepting where trypsin has been employed.

Employing these methods it may be interesting to note that some 900 tubes have been inoculated with blood from hospital patients and 13 have been contaminated and of these 7 occurred in one patient who had some irritation of the skin at the site of puncture and 2 others through the use of a specimen of trypsin which was not completely sterile.

The commonest organism found as contaminant was the staphylococcus albus, next to that B. Subtilis and then a diphtheroid bacillus. B. Coli occurred once and Streptothrix albus once.

Media.Citrate Broth.

The basic medium employed has been a broth made from Veal extract with 1% peptone. For routine work 0.5% of sodium citrate was added to this and to the sterilised medium (9 c.c. in a test tube 6" x $\frac{5}{8}$ ") were added varying amounts of patient's blood containing 1% of sodium citrate. The preliminary experiments were all conducted with the medium and blood citrated to this extent. Later slight modifications were made in the details which will be recorded. In most cases 12 tubes were inoculated.

Trypsin Broth.

The preparation of Trypsin recommended by DOUGLAS and COLEBROOK (1916) was not at first available so that the earlier experiments were carried out with Messrs. Allen & Hanbury's preparation of Liq. Trypsin Co. This is stated to be inferior to the other preparation and my results confirm this view. It was tested for sterility before use and added to broth to give a concentration of 5%.

Lactic Acid Broth.

As recommended by WALKER HALL (1922) Lactic Acid was added to broth in a certain number of experiments. (0.25 c.c. of $\frac{N}{I}$ Lactic Acid to 50 c.c. of broth).

Agar.

ROSENOW (1909) has stated that the streptococci responsible for endocarditis are greatly affected by

the oxygen tension of the medium and that blood inoculated into deep tubes of agar often gives growths of organisms when other methods fail. This method was tried but, as noted by LIBMAN (1906) the colour of the tube is so dark that it is often impossible to determine if organisms are present or not.

Agar plates have been constantly employed. 1 c.c. of the citrated blood was placed in a Petri dish and agar, melted and cooled to the neighbourhood of 45° C., poured into it, the whole being thoroughly ~~mixed~~^x. This method serves as a good means of comparison with the liquid media and also enables estimations of the numbers of colonies developing to be made. Three plates were used in each routine culture.

Anaerobic Methods.

The use of anaerobic jars was rather inconvenient when a number of observations was to be made. Experiments showed that when ^{to} the medium employed were added 5 drops of 0.5% Meth.Blue and the mixture was covered with half an inch of soft paraffin and incubated at 37° C. complete decolourization of the Methylene blue occurred within 18 hours. Liquid paraffin seals were much less satisfactory. Accordingly this method was adopted, the blood was added to the medium and the whole was then covered with a soft paraffin (vaseline) seal.

The methods used for comparing media were two in number.

(1) A series of tubes of each medium was inoculated with blood from patients and note taken of the tubes which showed the presence of growth and the time which it took to appear.

(2) Specimens of blood containing known small numbers of organisms were prepared in a series of dilutions and then inoculated into appropriate amounts of the various media. This enabled determination to be made of the number of organisms which needed to be present in order that a positive culture might be obtained.

The determination of the occurrence or failure of growth was usually easily made by naked eye observation. Where necessary confirmation was obtained by microscopic observation or subculture.

The violet discolouration of the blood noted by LEVY (1921) as indicative of the occurrence of growth in blood cultures is valuable in the cases where citrate of sodium or heparin is used as anticoagulant. Where lactic acid or trypsin is used or where the change occurs after three days it may be no indication of bacterial growth.

As regards (1) it may be stated that there has not been a case where growth has occurred in one medium and failed to occur in any of the others. Individual tubes of each or all of the media have failed at times to give a growth but where a series of five tubes of each has

been employed the statement holds good.

Time of appearance of growth. On the whole this did not vary greatly in the different media in the earlier experiments. Later it was found that the growth usually appeared earlier in media containing the trypsin specially prepared for medium making by Allen & Hanbury. The problem will be discussed at greater length elsewhere.

(2) Determination of number of organisms necessary to give positive blood culture on the different media employed.

Experiment 1.

Lactic Acid Broth.

The blood was obtained from patient and citrated to concentration of 1% (Sodium Citrate). 1 c.c. of this was added to each of 4 agar plates and similarly to each of 5 tubes containing 9 c.c. of Lactic Acid broth. A series of dilutions of the blood (1 in 10 to 1 in 1000) was prepared in broth and 1 c.c. of these added to media as indicated in Table III.

TABLE III.

Inoculum 1 c.c. of	Colonies on Agar plates.				Lactic Acid broth.				
	1	2	3	4	1	2	3	4	5
Blood undiluted					1				
do. dil. 1 in 10 [#]	10	9	10	12	-	+48	+48	+48	-
do. dil. 1 in 100 [#]	2	Nil			+72	+72	+72	-	-
do. dil 1 in 1000 [#]					-	-	-	-	-
					-	-	-	-	+48

[#]to the media receiving the higher dilutions of blood 0.1 c.c. of sterile rabbit's blood was added to enrich it to approximately the same extent as the medium receiving blood diluted 1 in 10.

+24 = growth in 24 hours etc. Where no entry occurs no inoculation was made.

In this case the average number of organisms^{*} per c.c. of blood was 10. Growth therefore occurred in this medium in three of the five tubes inoculated when the number could not well have been more than 1 per c.c. of inoculum.

The growth of organisms from the inoculum diluted 1 in 1000 is to be noted and will be referred to later.

^{*}This should more accurately be expressed chains of organisms.

Experiment 2.Ordinary Broth.

Ordinary broth (uncitrated) inoculated with varying amounts of citrated blood (1% Sodium Citrate).

The method employed was the same as in Experiment 1.

TABLE IV.

Inoculum 1 c.c. of	No. of Colonies on Agar plates.			Ordinary broth.				
	1	2	3	1	2	3	4	5
Blood (undiluted)	7	11	14	+24	+48	+72	+48	-
do. dil. 1 in 10	0	1	2	+48	+48	+48	-	broken
do. dil. 1 in 100*				-	-	-	-	-
do. dil 1 in 1000*				-	-	-	-	-

*Rabbit's blood 0.1 c.c. added as in Exprt. I.

As in the previous experiment growth occurs in three out of four tubes where the number of organisms inoculated could not have been much greater than 1.

These two experiments were carried out on different specimens of blood obtained at an interval of three days from the same patient.

The next experiment was carried out a week later upon the blood of the same patient in order to allow a comparison of the different media to be made on one and the same specimen.

Experiment 3.

The details of technique were the same as in Experiments 1 and 2.

TABLE V.

Inoculum 1 c.c. of	Colonies on Agar plates.			Acid Broth.				
	1	2	3	1	2	3	4	5
Blood undiluted	18	17	14					
" dil. 1 in 10	1	3	2	+48	+72	+72	-	-
" dil. 1 in 100*								
" dil. 1 in 1000*								

Inoculum 1 c.c. of	Citrate Broth.				
	1	2	3	4	5
Blood undiluted	+24	+48	+24	+24	+48
" dil. 1 in 10	+96	+48	+24	+48	-
" dil. 1 in 100*	-	-	-	-	-
" dil. 1 in 1000*	-	-	-	-	-

Inoculum 1 c.c. of	Trypsin broth.					Ordinary broth.				
	1	2	3	4	5	1	2	3	4	5
Blood undiluted										
" dil. 1 in 10	+48	+72	+24	+48	+72	+72	+96	+96	-	-
" dil. 1 in 100*										
" dil. 1 in 1000*										

* Rabbit's blood 0.1 c.c. added to each tube of medium as in previous experiments.

Growth occurs here also in all media under conditions such that not more than 2 organisms (chains) were likely to have been present in the inoculum.

It is however to be noted that growth does not occur in every tube. The Trypsin broth and Citrate broth would appear to give the best results but it must be remembered that a very slight and quite possible

unevenness of distribution of the organisms in the blood would quite account for the slight differences observed. There is however a definite tendency for growth to become visible later in the ordinary broth and acid broth than in the other media and this should, I think, be associated with the fact that coagulation is not completely prevented in these media. This undoubtedly has an effect upon the growth of organisms in blood culture.

Effect of Anaerobiosis.

From these experiments it is clear that there is very little difference in the various media employed with regard to their suitability for the obtaining of a positive blood culture. Citrated broth was accordingly adopted as the most suitable medium for carrying out further experiments on growth under anaerobic conditions. The technique was as before, 1 c.c. of various dilutions of blood being inoculated into 9 c.c. of Citrate broth. Half of the tubes were inoculated aerobically. The remainder were covered with a seal of soft paraffin. The result was as follows:-

Experiment 4.TABLE VI.

Inoculum 1 c.c. of	Citrate Broth				
	Aerobic.				
	1	2	3	4	5
Blood undiluted	+40	+40	+40	+40	+64
do. dil. 1 in 10	+64	+64	+88	+64	+64
do. dil. 1 in 100*	+88	+7dys-	-	-	-
do. dil. 1 in 1000	-	-	-	-	-

Inoculum 1 c.c. of	Citrate Broth.				
	Anaerobic.				
	1	2	3	4	5
Blood undiluted	+40	+40	+40	+40	+40
do. dil. 1 in 10	+64	+64	+64	+64	+88
do. dil. 1 in 100*	+88	+7dys-	-	-	-
do. dil. 1 in 1000	-	-	+7 dys	+7 dys	-

Colony counts on Agar (4 plates) averaged 70
per c.c.

* Rabbit's blood 0.1 c.c. added as in previous experiments.

The experiment shows no marked advantage in the use of either the aerobic or anaerobic methods. The occurrence of growth is observed in just the number of tubes in which the probability is that one organism (chain) has been inoculated. The columns marked +7dys are possibly misleading. The other readings all indicate the occurrence of growth visible macroscopically. In the case of these four tubes however it was overlooked until films were examined and subcultures made at the time stated.

That two tubes inoculated with dilution 1 in 1000 showed the occurrence of growth is surprising and will be discussed elsewhere.

The combination of the results of a series of eight experiments of this kind has given figures which are included in the following table.

TABLE VII.

No. of organisms inoculated into each tube.	Results in different media.							
	Citrate broth.		Acid broth.		Trypsin broth.		Ordinary broth.	
	+	-	+	-	+	-	+	-
1 - 10	23	2	14	6	10	0	8	3
11 - 50	12	0	7	0	2	0	4	1
51 - 100	18	0	3	0	3	0		
	53	2	24	6	15	0	12	4

Under + is given the number of tubes in which growth occurred.

Under - the number which failed to show any growth.

A similar compilation of results has been made from the results of all the blood cultures yielding streptococci no matter what medium was used to isolate them. This is recorded in Table VIII.

TABLE VIII.

No. of organisms inoculated per tube.	Number of tubes.	
	Positive.	Negative.
1 - 10	65	16
11 - 50	98	1
51 - 100 or more	149	1*

* This result was obtained in a case in which the streptococcus was extremely delicate and could not be subcultured on media free from blood.

In these experiments the estimation of the number of organisms is based upon the number of colonies observed to grow when 1 c.c. of blood is inoculated into Agar, each of these being assumed to arise from a single organism or chain. It is also assumed that all the organisms present in the blood actually grow on the agar plate. It has not been possible to determine if this assumption is correct but it should be noted that in the cases examined the dilution of blood failing to give growth in broth media is that one which would be expected to do so if the number of bacterial colonies in the agar plate actually represented the number of organisms present in the blood. For example, in Experiment 2. the number of colonies on the agar plates averages 11 per plate. Corresponding to this growth occurs in the broth inoculated with blood diluted 1 in 10 but not in the case of the dilution 1 in 100.

It is to be noted that in no case has there been any discrepancy between the results in solid media (agar) and in liquid and where these have been tested quantitatively there has been good agreement.

It is generally recommended (HISS & ZINSSER p.214) that in making blood cultures a series of tubes should be used and that different amounts of blood should be added to the various tubes. I have been able to find no valid evidence in favour of this procedure, which appears to be based upon the assumption that growth may

occur in the lower concentrations of the blood and fail in the higher on account of the excess of inhibitory substances present in the latter, the effect of these being removed by dilution.

That growth may occur in tubes containing small amounts of blood and not grow in others containing more blood is undoubtedly true but the reason given above does not appear to be correct. Whether growth occurs in a given tube or not is influenced, as KIPNIS (1923) demonstrates in the case of typhoid septicaemia, by the chance distribution of organisms in the various specimens of the inoculum.

In all of the cases of this series, except one, growth has occurred quite regularly in all tubes, regardless of the amount of blood inoculated. In each of the observations made the amount added to 9 c.c. of medium has varied from 0.5 c.c. to 1.5 c.c. and in some cases to 3.5 c.c. Within these limits the time of appearance of growth and the actual occurrence of a positive culture does not appear to have been influenced by the size of the inoculum.

It is generally considered also that if growth occurs in only one or two tubes of a series it is probably due to contamination. This is no doubt frequently true but not always. In one case in this series a Pneumococcus was isolated from the blood, growing in only four tubes out of thirteen which were inoculated. The number of organisms was not estimated. That the organism

was not a contamination was demonstrated at post mortem.

In Experiments 1 and 4 there are two good instances showing that in the cases where the number of organisms in the inoculum has been greatly reduced the distribution of tubes showing growth is apparently haphazard.

The possibility must also be borne in mind that in the course of the manipulation chains consisting of a number of organisms might be broken up into a number of smaller chains.

The chance of a positive blood culture being obtained is naturally increased if the quantity of blood examined is large. For this reason it has been my custom to examine always at least 20 c.c. and to repeat the examination.

Summary of these Experimental Results.

The experiments recorded above would appear to indicate that in any one of the media employed, liquid or solid, aerobic or anaerobic, growth is capable of occurring if even one organism is in the blood which is introduced into it.

Where the number of organisms is small the factor of chance assumes importance in determining whether growth shall occur or not in any given tube. This may be overcome in part by the use of large amounts of blood and inoculation of several tubes.

When the number of organisms is larger (10 to 100 per c.c.) practically every tube inoculated will give

a growth of organisms.

The difference in efficiency between the different media is slight but tends to suggest that media containing an anticoagulant are rather better than those which do not and that trypsinized media have a slight superiority over citrated media.

There is however just a possibility that these conclusions might not apply in toto to some peculiarly delicate strains.

It is unlikely that anyone working with any or all of the media here employed would fail to cultivate a streptococcus from the blood of a patient if that streptococcus were present in the specimen collected.

On the delay in appearance
of growth in blood culture.

It has been frequently noted that in cultures from the blood of cases of subacute infective endocarditis growth does not usually appear until after the lapse of more than 24 hours and it is customary to incubate blood cultures for at least a week before rejecting them as negative. In the present investigation the standard of appearance of growth has been the appearance of an amount visible to the naked eye. Before complete rejection microscopic examination of films and preparation of subcultures have been made.

The majority of tubes inoculated have failed to show visible growth in 24 hours but in some there has been obvious growth within this period. In one case it was possible to detect growth within 24 hours in all tubes of the three cultures done. Variation in time of appearance of growth has occurred in successive cultures from the same patient as well as in different tubes of the same culture. Tubes not showing evidence of growth within 3 days rarely showed any at a later date.

In the earlier experiments there seemed no evidence of the effect of any particular medium in shortening the period of delay though later experiments have shown more definite results.

There appeared to be no connection between rate of growth of the bacteria in culture and subsequent duration of life of the patient. In a case in which death occurred

three days after the culture was taken growth first appeared in 48 hours. In another where death occurred six months afterwards growth appeared in 24 hours. In a third taken a week before death growth occurred also in 24 hours.

Moreover where a series of cultures was made from the same patient there was no evidence that the delay was shorter in the later cultures than in the earlier.

DOUGLAS & COLEBROOK (1916) dealing with the occurrence of the same phenomenon in other infections attribute it to the effect of inhibiting substances in the blood of the patient.

A detailed analysis however appears to be lacking. Experimentation upon the subject has been confined to artificial mixtures of organisms and immune blood (WALKER HALL 1922) to the septicaemia of an animal inoculated with laboratory cultures (DOUGLAS & COLEBROOK 1916) and to mixtures of organisms from laboratory media and patient's serum (DOUGLAS & COLEBROOK 1916). None of these appears exactly to represent the state of affairs in a culture from a patient with septicaemia and the experiments recorded have therefore been carried out upon the septicaemic whole blood of the patient.

The experiments recorded earlier have shown that the medium employed (Citrate broth) has been perfectly satisfactory for the routine investigation of the blood. The concentration of Sodium Citrate actually present in the tube of medium inoculated with blood was 0.6% and this amount had been somewhat arbitrarily selected with

a view to ensuring that coagulation of the blood should be prevented completely. In view of H. C. BROWN'S work (1921) experiments were necessary to determine whether this might not be the cause of the delay.

The first point requiring elucidation was the stage in the growth cycle of the organism at which delay occurred. A preliminary experiment showed that the main cause of delay in growth was a prolongation of the latent period.

Experiment 5.

1 c.c. of citrated blood from patient Al containing 40 streptococci per c.c. was added to 4 c.c. of broth containing 0.5% of sodium Citrate. (Final concentration of Sodium Citrate 0.7%). This was incubated at 37° C. and specimens drawn off and plated at various intervals to determine the number of organisms present. The result was as follows:-

Colonies developing from 1 c.c. of culture.

At once.	After 5½ hrs. at 37° C.	After 24 hrs. at 37° C.	After 48 hrs. at 37° C.
8	10	13	∞

∞ = too many to count.

Clearly then growth does not occur, that is there is no increase in the number of viable bacterial units, in the medium until after the lapse of 24 hours.

A similar experiment on the blood of patient Tr containing some 40 streptococci per c.c. gave the following results. The technique was the same except that 2 c.c. of blood were inoculated into 8 c.c. of citrate broth. Concentration of Sodium Citrate = 0.7%.

Experiment 6.

No. of colonies developing from 0.5 c.c. of culture.

Immediate	After 7 hrs. at 37° C.	24 hrs. at 37° C.	48 hrs. at 37° C.
6	4	5	2

Curve of growth of Streptococcus Al.

The delay then being due to increase in the length of the latent period it became necessary to determine whether this long latency is a characteristic of the organism as grown on laboratory media. This seemed unlikely as subcultures on broth when made from 24 hour cultures were usually fully developed in 24 hours.

Experiment 7.

1 loopful of broth culture of Streptococcus Al. (22 hrs.) was inoculated into 10 c.c. of broth containing no citrate, and specimens drawn off at intervals and plated in blood agar. The colonies were counted after incubation for 48 hours.. The following figures were obtained:-

No. of colonies developing on plate.

At beginning of expt.	630,000
After 2 hrs. at 37° C.	920,000
After 4 hrs. at 37° C.	600,000
After 6 hrs. at 37° C.	2,130,000
After 8 hrs.	5,000,000
After 19 hrs.	700,000,000

The figures here recorded have been used to construct a logarithmic curve illustrating the growth of the organism. From this it is clear that the latent period of this streptococcus under laboratory conditions

is between 4 and 6 hours, probably more nearly four hours, a figure which agrees well with those observed by CHESNEY (1916) for Pneumococcus and B. coli, and also with that obtained for Pneumococcus on the same medium as that here employed.

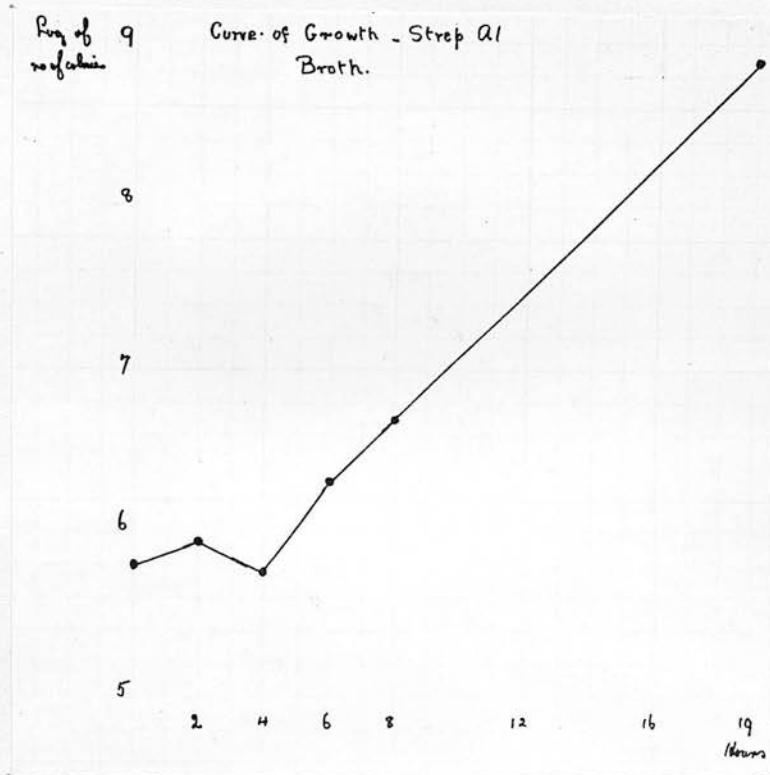


Chart I.

Logarithmic curve of growth of a 24 hour broth culture of streptococcus A1.

The delay in growth on blood cultures cannot therefore be regarded as a characteristic of the organism.

The experiment does not however exactly reproduce the condition of affairs existing in septicaemic blood. In the first place the number of organisms in each cubic centimetre of blood is usually small. Moreover it is quite possible that many of the organisms in such a sample of blood are damaged and less capable of multiplication than those used in the experiment.

A study of growth curves was therefore made with a view to imitating these conditions with the laboratory culture.

Effect of number of organisms inoculated
upon the subsequent latent period.

Experiment 8.

For this purpose a loopful of a 22 hour broth culture of Strep. Al. was placed in 10 c.c. of broth and of this one loopful was inoculated into broth as before and incubated, samples being removed at intervals and plated in blood agar. The following figures were obtained.

Colonies developing on plate.

At beginning of experiment	400
After 2 hrs. at 37° C.	400
After 4 hrs. " "	500
After 6 hrs. " "	2,500
After 8 hrs. " "	10,000
After 19 hrs. " "	400,000,000

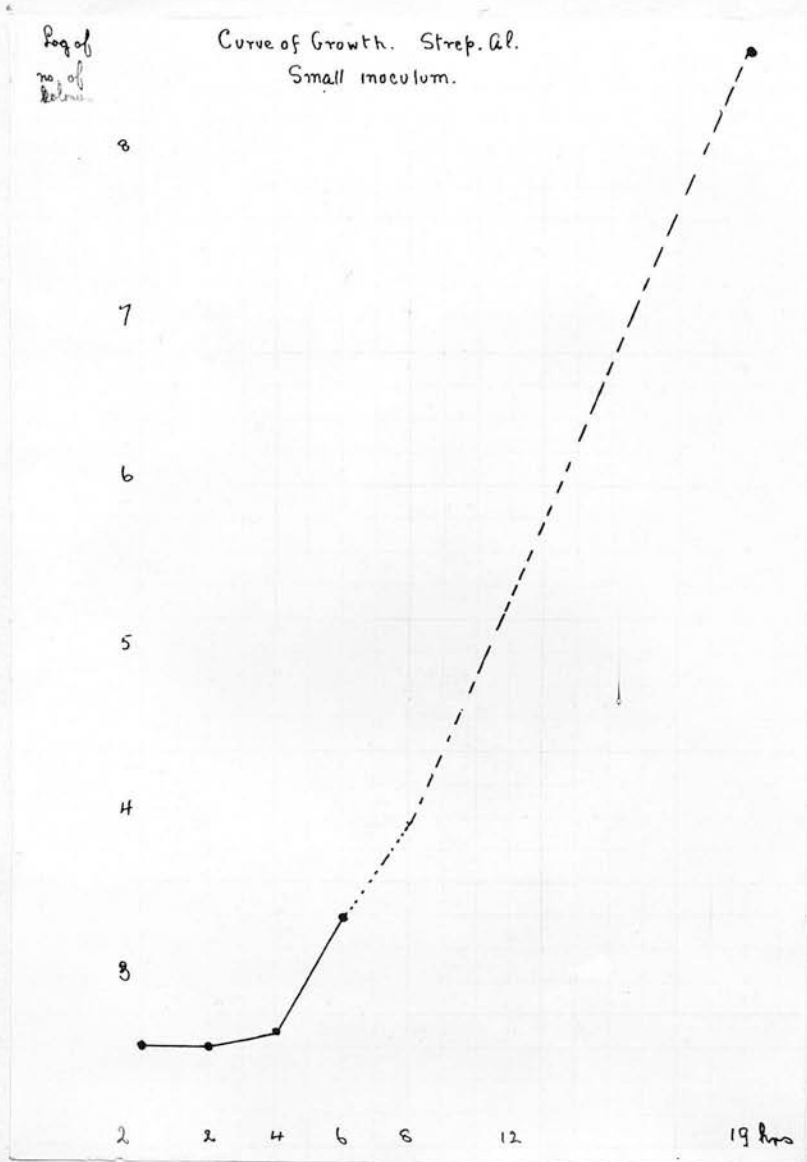


Chart II.

Logarithmic curve of growth of small amount of *Streptococcus Al* (24 hour culture) inoculated into broth.

Chart II. illustrates the points revealed by this experiment the chief one being that the duration of the latent period is not affected by the size of the inoculum, which is in agreement with the findings reported by MCKENDRICK & KESAVA PAI (1911) for B. coli. Here as with the larger inoculum the latent period is in the neighbourhood of 4 hours.

Effect of age of culture
on latent period.

It has been almost constantly observed in the course of making routine subcultures on broth of the streptococci isolated from cases of infective endocarditis that growth frequently fails to appear in 24 hours if the inoculum is taken from a culture more than three or four days old. Accordingly an experiment was made to see if this were due to prolongation of the latent period.

Experiment 9.

A loopful of a culture of Strep. A1 which had been incubated for four days and then kept for 3 days at room temperature was inoculated into 10 c.c. of broth and samples plated at intervals.

No. of colonies on plate.

At beginning of experiment	2,000
After 2 hrs. at 37° C.	1,800
After 4 hrs. " "	1,500
After 6 hrs. " "	1,700
After 8 hrs. " "	1,000
After 19 hrs. " "	10,000,000

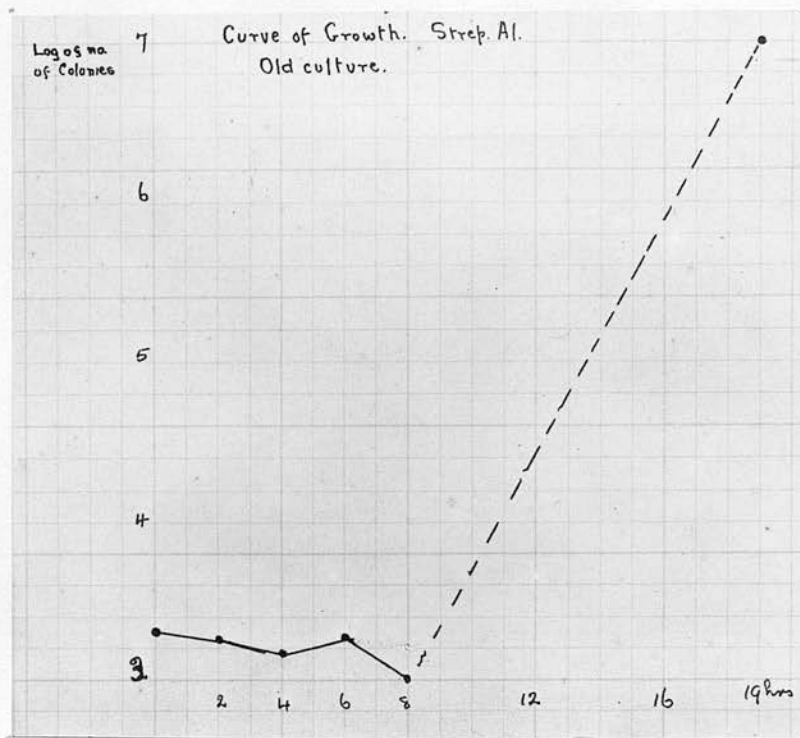


Chart III.

Logarithmic curve of growth of 7-day culture of *Streptococcus* A1 inoculated into broth.

It will be seen from the figures and from Chart III. that there is a definite increase in the latent period which is here at least eight hours. Now CHESNEY (1916) attributes latency to an alteration to or damage of the organism resulting from its environment. He points out that latency is always observed when the medium is inoculated from a culture which is itself in a phase of latency, but not if the organisms are in the "logarithmic" phase of growth. They may in other words be regarded as damaged organisms and the experiment goes to show that the longer the exposure the greater the damage and the longer the latent period when they are inoculated into fresh medium.

From these experiments it is clear that the prolonged latent period observed in blood cultures cannot be attributed to either (1) peculiarities of the streptococci;

or (2) the small number of organisms present.

If however the organisms present were "damaged" they would be expected to have a long latent period.



Effect of Sodium Citrate on
the growth of Bacteria.

In view of the amount of citrate present in the medium it was next attempted to determine the effect of this upon the latent period.

Experiment 10.

1 loopful of a 22-hour culture of Strep.Al. was inoculated into the same medium as was employed in Experiments 7 to 9 with the exception that Sodium Citrate was added to a concentration of 0.6 per cent. Specimens were removed and plated at intervals as before.

The following figures were obtained:-

No. of colonies on plate.

At commencement of Expt.	350,000
After 2 hours at 37 ⁰ C.	320,000
After 4 hours " "	390,000
After 6 hours " "	300,000
After 8 hours " "	540,000
After 19 hours " "	40,000,000

The logarithmic curve of growth is recorded in Chart IV.

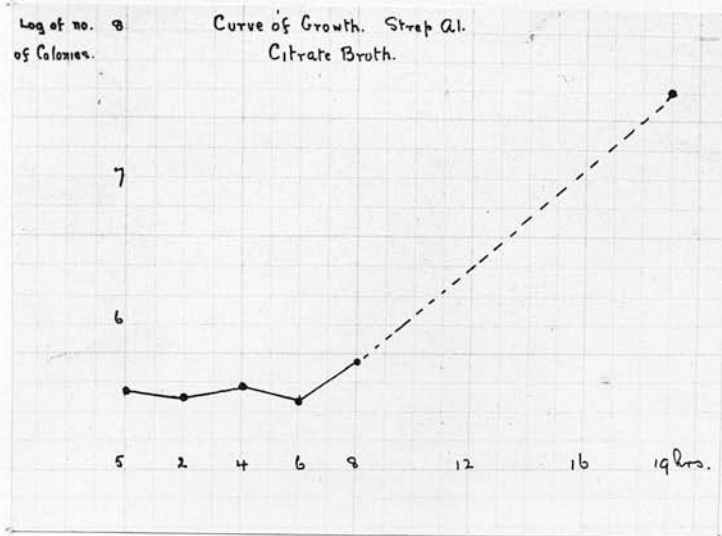


Chart IV.

Logarithmic curve of growth of Streptococcus A1 in broth containing sodium citrate.

From this it would appear that citrate of sodium in a concentration of 0.6 per cent causes a prolongation of the latent period. Whether there is also slight diminution of the rate of multiplication is not quite clear from this experiment. The chief point however is that latency is prolonged.

In these experiments it was observed that in all cases excepting in the case of the inoculation from the old culture growth was visible macroscopically in the tubes in 24 hours. The degree of inhibition of growth was not then so great as in the case of blood cultures.

It now seemed desirable to work out more accurately the concentration of Sodium Citrate which was inhibitory to growth of these streptococci in order to determine the amount of this substance that might be employed most usefully in preventing clotting of the blood and to determine whether there would be any advantage in using Ammonium oxalate as the decalcifying agent.

Complete curves of growth were not made in these experiments, the criterion of the occurrence of growth being production of a visible opacity in the medium.

Inhibition of growth of 24 hr.
culture of streptococcus A1. by
Citrate of Sodium.

Experiment 11.

A loopful of 24-hour broth culture of Streptococcus A1 was inoculated into a series of tubes containing 5 c.c. of broth and varying concentrations of

sodium citrate as recorded in Table IX. The incidence of growth was recorded after intervals as indicated.

TABLE IX.

Sodium citrate per cent.

	Nil		0.1		0.2		0.3		0.5		0.75		1		2		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
24 hrs.	+	+	+	+	+	+	+	+	+	*	*	*	*	-	-	-	-
48 hrs.									+	+	+	+	+	+	-	-	
72 hrs.																	-

+ = visible growth.

- = growth not visible.

* = growth settled out to the bottom of the tube. Elsewhere it was evenly distributed throughout the medium.

Under the conditions of this experiment the inhibitory effect of the sodium citrate is exerted in a concentration of 0.5 per cent, this preventing appearance of growth in 24 hours. It is not sufficient however to prevent growth occurring later and in 48 hours growth is fully developed in tubes containing 1 per cent of the salt. The reaction of the medium (Ph. 7.6) was unaffected by the addition of the salt.

Inhibition of growth of old culture of streptococcus by Sodium Citrate.

Experiment 12.

In this case the inoculum consisted of a loopful of a culture of the same streptococcus as that used in Experiment 11. which had been incubated for a week.

TABLE X.
Sodium citrate per cent

	Nil		0.1		0.2		0.3		0.5		0.75		1	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
24 hrs.	+	+	-	-	-	-	-	-	-	-	-	-	-	-
48 hrs.	+	+	+	+	+	+	+	+	+	+	-	-	-	-
72 hrs.									+	+	-	-	-	-
96 hrs.									+	+	-	-	-	-

+ = visible growth.
- = no growth visible.

Here as in Experiments 11 and 13 the figures 1 & 2 under each strength of citrate indicate merely that the experiment was carried out in duplicate throughout.

This experiment indicates that inhibition of growth in the case of the older culture is effected by 0.5% of the salt, about half of the amount which is effective for the 24 hours culture.

SHERMAN and ALBUS (1924) have shown a difference in regard to resistance to the destructive action of sodium chloride between organisms in the lag phase and those in the actively growing phase. The former are

less susceptible. Here however as between two sets of organisms in the lag phase that which has been in that condition longer is more susceptible to inhibitory salt action than the one which has been latent for a shorter period.

Inhibition of growth of streptococcus by Ammonium Oxalate.

Experiment 13.

The technique employed was the same as that for experiment 11, a 24 hr. culture of Streptococcus A1 being employed; one loopful was inoculated into a series of tubes of broth containing varying concentrations of Ammonium oxalate.

TABLE XI.

Ammonium oxalate per cent

	Nil		0.05		0.1		0.15		0.25		0.4		0.5		0.75		1		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
24 hrs	+	+	+	+	+	+	+	+	+	*	*	*	*	-	-	-	-	-	-
48 hrs									+	+	+	+	-	-	-	-	-	-	-
72 hrs													+	-	-	-	-	-	-
96 hrs													+	-	-	-	-	-	-

* = in these tubes the growth when fully developed was not evenly distributed throughout the tubes but settled in a mass at the bottom.
Elsewhere growth occurred evenly throughout the medium.

Ammonium oxalate then is inhibitory to growth, preventing its appearance in 24 hours in a concentration above 0.25% while total inhibition occurs above 0.5%. The molecular weight of the substance (124)

is about half that of sodium citrate (258) so that the inhibitory concentration (0.25%) is of about the same molar concentration as that of sodium citrate (0.5%). It would appear therefore that there would be no advantage to be gained by substituting this salt for sodium citrate as the anticoagulant in routine blood culture work.

These experimental results would appear to indicate that the concentration of sodium citrate employed for blood culture work was rather high (0.6%). But it is certain that a good deal of the citrate is used up in the presence of blood, probably much more than in the medium employed in these experiments, which was alkaline and contained sodium phosphate (Na_2HPO_4) in a concentration of 0.2 per cent.

The following experiment was therefore undertaken to determine whether the same degree of inhibition occurred when rabbit's blood was added to the medium in the presence of the same concentration of sodium citrate.

Experiment 14.

A normal stock rabbit was bled from the ear vein with aseptic precautions, 9 c.c. being received into 1 c.c. of sodium chloride solution (0.85%) containing 10 per cent of sodium citrate.

Of this citrated blood 1 c.c. was added to 9 c.c. of broth containing 0.5 per cent of sodium citrate the final concentration of the citrate in the blood broth being thus 0.6 per cent.

Into this was inoculated 1 loopful of a 24 hour broth culture of Streptococcus Al. Controls were included to show the amount of growth of the organism in the broth alone without any addition of blood both with and without Sodium Citrate. The experiment was carried out in duplicate. The result was as follows:-

TABLE XII.

Medium.	Amount of growth in			
	24 hrs.		48 hrs.	
	1	2	1	2
Broth - no citrate	+	+	+	+
Broth + 0.5% citrate	?+	?+	+	+
do. + 0.6% citrate	?+	?+	+	+
do. + Blood (0.6% citrate)	+	+	+	+
do. + Plasma (" ")	+	+	+	+
do. + *cells (" ")	+	+	+	+

*These were obtained by centrifuging and making up to original bulk with sodium chloride solution containing 1 per cent of sodium citrate.

+ = growth visible macroscopically.

?+ = visible microscopically, not macroscopically.

Control tubes inoculated with blood constituents were sterile.

Here as before in plain broth the inhibitory effect of the citrate was manifested in a concentration of 0.5% to a certain extent.

On the other hand in the presence of blood (10%) this inhibition is abolished and growth was actually more profuse in the blood tubes than in the citrate free broth.

It may be noted in passing that the rabbit's blood was used here because the same phenomenon of delay in growth had been observed in the case of the organisms

in the blood of a rabbit with experimental endocarditis and septicaemia.

Now the patient suffering from subacute infective endocarditis is to a certain extent in the position of an immunized animal, that is there is a demonstrable amount of antibody in his serum. The same also was true of the rabbit suffering from experimental endocarditis upon which the growth experiment was carried out. It seemed possible that the explanation of the failure of the citrate to inhibit growth in the above experiment with citrated blood might be due to the fact that the citrate had been largely used up by the calcium of the rabbit's blood and so the actual amount of free citrate was small. Possibly in the immunized animal the inhibitory power of some blood constituent might be enhanced and this with the same amount of citrate might delay growth.

Experiment 15.

A rabbit (No.126) was available which had been immunized against streptococcus A1 for some time and its serum agglutinated this organisms in a dilution of 1 in 1280 in 2 hours at 55°C. An experiment was performed using the blood of this animal in exactly the same way as that of the normal rabbit in Experiment 14. This was done at the same time and with inocula from the same culture.

TABLE XIII.

Medium.	Amount of growth in			
	24 hrs.		48 hrs.	
	1	2	1	2
Broth - no citrate	+	+	+	+
Broth + 0.6% citrate	?+	?+	+	+
Broth + Immune blood (0.6% citrate)	+	+	+	+
Broth + Plasma*	+	+	+	+
Broth + Cells*	+	+	+	+

* Obtained as in the preceding experiment.

In this experiment as in that on normal rabbit's blood there is no evidence of any inhibitory effect produced by citrated blood from an immune rabbit under the conditions of the experiment. Not only so but the amount of growth appeared actually to be greater than in the control tubes.

Experiment 16.

The number of organisms present in the inoculum in Experiment 15 would be of the order of a million as judged by other experiments. Accordingly a further experiment was carried out in which the number of organisms was much smaller. A broth culture of Streptococcus A1 (24 hours old) was diluted 1 in 1000 in broth and one loopful of this latter dilution used as inoculum.

TABLE XIV.

Medium	Amount of growth in			
	18 hrs.		24 hrs.	
	1	2	1	2
Broth + Citrate 0.6%	-	-	-	-
Broth + Blood (immune rabbit) (Citrate 0.6%)	+	+	++	++*

Control. Immunized blood 1 c.c. in 9 c.c. broth - two tubes - no growth.

* - judged by opacity.

Here it is clear that the broth to which immune blood has been added is a very much better medium than the broth without this addition. There is no evidence of any inhibition of growth.

Experiment 17.

A further experiment was carried out upon the effect of the serum of the same immune rabbit (No. 126) in producing inhibition of growth both alone and in the presence of sodium citrate. The inoculum was a loopful of a 24 hours growth of Streptococcus Al. in broth.

TABLE XV.

Medium.	Amount of growth in					
	17 hrs.		24 hrs.		48 hrs.	
	1	2	1	2	1	2
Broth - no citrate	++	++	++	++	++	++
Broth + 0.6% citrate	+	?+	++	+	++	++
do. + serum 1 in 25 & citrate 0.6%	-	?+	++	++	++	++
do + Serum 1 in 25	++	++	++	++	++	++

Serum control - sterile.

The serum in this concentration does not inhibit growth nor does it completely remove the inhibitory effect of the citrate. The concentration is of course much less than in the case of the experiments with blood.

It may be noticed in passing that in all cases where growth occurred in the presence of serum or blood from an immune animal it was aggregated at the bottom of the tube and not evenly distributed through the medium as in the case of media containing no blood or blood from a normal animal. Similar appearances

were noted where late growth occurred in media containing a large amount of sodium citrate or ammonium oxalate. In the case of cultures from the blood of cases of subacute infective endocarditis the tendency is for the growth to occur in masses or balls at the surface of the blood cells at the bottom of the tube.

A similar phenomenon was observed by WASHBOURN (1895) in the case of the pneumococcus and by AINLEY WALKER (1922) in the case of *B. typhosus*. It has also been noted by KINSELLA (1917) in the case of streptococci grown in the presence of serum from patients with infective endocarditis.

The general conclusions to be drawn from these experiments may be summarized as follows:-

(1) Delay in growth in blood cultures is due to prolongation of the latent period.

(2) This prolongation is not found to occur in the case of cultures made from 24 hour growths of the same organism on laboratory media.

(3) The latent period is increased when the inoculum is taken from an old culture (seven days).

(4) The size of the inoculum does not affect the length of the period of latency.

(5) Sodium citrate and ammonium oxalate delay appearance of growth in about the same molar concentration.

(6) The amount of Sodium citrate present in the

routine media employed in the earlier experiments (0.6%) is very close to the concentration of that salt which delays growth in ordinary laboratory media.

(7) The delaying action upon growth produced by sodium citrate in this concentration is due to prolongation of the latent period.

(8) The inhibitory effect of sodium citrate (0.6%) is not manifested upon a large or small inoculum of a 24 hour laboratory culture of the organism in the presence of blood from a normal or from an immunized rabbit.

The information obtained by the experiments described above was valid for cultures of streptococci on laboratory media. It did not however seem warranted to assume that it also held good for the organisms circulating in the patient's blood. Accordingly a certain number of experiments has been carried out upon the patient's blood containing bacteria.

The difficulty which first required clearing up was the effect of the anticoagulant upon the rate of growth. That some such method of maintaining the blood in a liquid condition was necessary was shown by an observation upon defibrinated blood, the fibrin being removed by shaking with glass fragments. Over 90 per cent of the organisms were removed in the process. This would of itself explain the observation of KAMMERER (1914) recorded above in which the inferiority of defibrinated blood as inoculum is mentioned. The first experiments were therefore directed to determining the effect of varying the amount of citrate used.

Experiment 18.

A preliminary observation was made upon the patient Pt. Blood (9 c.c.) was drawn into 1 c.c. of sodium citrate solution (10%). Of this citrated blood (1% of sodium citrate) 1 c.c. was added to each of two tubes of media (1) containing 0.5 per cent of sodium citrate; (2) containing 0.1% of the same. The final concentration of sodium citrate in each of these media was thus 0.6 per cent in (1) and 0.2 per cent in (2). These were incubated and specimens

drawn off at intervals and plated.

TABLE XVI.

Medium.	No. of colonies on plates inoculated after incubation.		
	Nil	5 hrs. at 37° C.	24 hrs. at 37° C.
0.6% Citrate broth	7	7	++
0.2% citrate broth	9	13	+++

In this case there was no growth in five hours but unlike the other cases up to this date there was definite growth in 24 hours in both media, too great a number of colonies being present to permit of counting. There was however much heavier growth in the presence of the weaker strength of sodium citrate.

In conjunction with other observations it shows that the period of delay varies from one patient to another as has been indicated elsewhere. Further, delay in the presence of 0.6 per cent of sodium citrate is greater than in the case of a lower concentration (0.2 per cent).

The latter point was further amplified in a subsequent experiment and at the same time the effect of addition of trypsin to the blood also determined.

Experiment 19.

Patient Pt. Blood (4 c.c.) was added to 1 c.c. of trypsin (Allen & Hanbury's "Azoule" preparation for trypsinizing media). Of this 1 c.c. was added to 9 c.c. of broth containing 0.3 c.c. of trypsin, the final concentration thus being five per cent of trypsin.

Blood was also collected in sodium citrate solution and added to citrated broth so that there were two sets of broth containing 9 c.c. of broth and 1 c.c. of blood, in (2) the concentration of sodium citrate being 0.6 per cent, and in (3) 0.2 per cent.

Samples of 0.5 c.c. were drawn off at intervals and plated, counts of colonies giving the figures in Table XVII.

TABLE XVII.

Anticoagulant.	Colony count after incubation at 37°C.					
	Nil	5 hrs.	7 hrs.	9 hrs.	11 hrs.	24 hrs.
Trypsin	6	7	12	153	656	+++
Citrate 0.2 per cent	7	9	14	154	600	++
Citrate 0.6 per cent	3	4	4	4	15	+

In this experiment the inhibition of growth extends to 7 hrs. in the case of trypsin and the weaker citrate concentration but in the case of the stronger concentration of citrate delay in commencement of growth is longer. There is no marked difference in the time of commencement of growth between the trypsin and the weakly citrated medium. The amount of growth was however undoubtedly greater in the trypsinized medium at the end of 24 hours.

A further observation upon this point was made on the blood of the same patient 10 days before his death.

Experiment 20.

Blood was drawn into Trypsin and also into citrate solution and added to broth so that the concentration of Trypsin was five per cent and of sodium citrate

0.2 per cent as before. Colony counts were made as in previous experiments.

TABLE XVIII.

Anticoagulant.	Colony count from material incubated at 37° C.					
	Nil	5 hrs	7hrs	9 hrs	11 hrs	24 hrs.
Trypsin	7	12	91	275	2277	++++
Citrate 0.2 per cent	6	4	7	10	24	2 million.

In this case there is undoubted advantage in the use of trypsin. The growth in twenty four hours was visible macroscopically in the trypsinized medium and only on microscopic observation in the citrated broth. The latent period in the former case was between 5 and 7 hours while in the latter it was much longer.

Here then there is evidence of a variation in the period of latency in two different samples of the same patient's blood. The medium employed in both experiments was the same.

An observation made previous to these experiments indicated that his serum agglutinated the streptococcus isolated from his blood weakly in a dilution of 1 in 10, control sera being without effect.

Similar observations have been made upon the blood of the patient Tr. Two preliminary experiments indicated that with one sample of blood in 0.6 per cent citrate broth no increase in the number of colonies occurred in 24 hours. A second sample in the presence of 0.2 per cent sodium citrate did show increase in that time.

The substance heparin having been found to have no effect on the growth of laboratory cultures of streptococcus Tr the following experiment was done with a view to eliminating the effect of sodium citrate altogether.

Experiment 21.

Blood was drawn off without addition of any anti-coagulant and quantities added to the three anticoagulants trypsin, sodium citrate and heparin, and the treated blood (1 c.c.) was then added to broth so that the concentration of the various substances was as follows:-

- (1) Trypsin 5 per cent.
- (2) Sodium Citrate (0.2 per cent).
- (3) Sodium Citrate (0.6 per cent).
- (4) Heparin (0.02 per cent).

Coagulation was avoided in all tubes.

Colony counts were made from specimens in the usual way.

TABLE XIX.

Anticoagulant.	Colonies on plates inoculated after incubation at 37°C.						
	Nil	4 hrs	6 hrs	8 hrs	11 hrs	24 hrs	48 hrs.
Trypsin	6	6	4	5	8	3600	*** [*]
Citrate 0.2 per cent	6	6	5	5	8	81	9 million
Citrate 0.6 per cent	8	6	6	6	5	0	0
Heparin	5	5	6	7	12	104	8 million.

*Growth visible macroscopically.

Here then is a very marked degree of delay in the commencement of growth in all media and complete failure in the case of the stronger concentration of sodium citrate.

The observation that one tube may fail to show growth in a series inoculated with organisms in comparatively large numbers has been made elsewhere. Here the number actually introduced was about 130. Clearly inhibition is less marked in the case of the trypsinized medium than in the others and in the case of Heparin it is practically identical with that in the presence of a concentration of 0.2% sodium citrate. There is also a suggestion that the rate of multiplication in the trypsinized media is greater once growth has started but that has not been studied sufficiently.

In this experiment as in all others it has been found that delay in growth was also to be seen in plates of agar inoculated with blood. No colonies appeared in 24 hours in this case and only few in 48, a complete count not being possible until 72 hours had elapsed. Usually no alteration in the count was noticeable between 48 and 72 hours. Here it was very marked. Not only so but the colonies were very small even at that period and agar inoculated from the trypsinized blood showed colonies before that inoculated with blood which had been taken into citrate solution. Moreover a small portion of the citrated blood used for this experiment had been inoculated with a small number (60) streptococci from an actively growing 18 hour laboratory culture of the same strain. The sample so inoculated showed fully developed colonies in 24 hours similar in number to what would have been expected if they had all come from the culture inoculated.

The agglutination titre of this patient's serum has been observed on two occasions against her own strain of streptococcus. It has been the same on both occasions and agglutination has occurred in a dilution of 1 in 10, control serum having no effect.

The close similarity in the degree of inhibition in heparin and in 0.2 per cent sodium citrate would seem to suggest that part of this inhibition is really due to something other than the anticoagulant, and the failure of the trypsin completely to overcome this in the present instance coupled with the variability noted at different examinations and the fact that the medium is constant suggests that it is to the organism itself that we have to look for the explanation. Complete solution of this point is however difficult. A preliminary observation is however of interest.

Experiment 22.

As heparin is apparently not inhibitory to growth in the concentration used (0.01 per cent) an experiment was carried out to determine the amount of sodium citrate required to prevent growth of the organisms in blood in which coagulation was prevented by the use of heparin. Blood (9 c.c.) was drawn off and added to heparin (1 per cent). Of this 1 c.c. was added to a series of tubes containing 9 c.c. of blood and varying concentrations of sodium citrate. Occurrence of growth was observed macroscopically.

TABLE XX.

Amount of growth at various intervals in broth containing citrate of sodium as under.							
	Nil	0.1%	0.2%	0.4%	0.6%	1%	2%
24 hrs.	-	-	-	-	-	-	-
48 hrs.	+ +	+ +	+ +	+ +	+	-	-
72 hrs.	+ +	+ +	+++	-++	+ +	-	-

The failure of growth up to 24 hours is to be ascribed to conditions in the blood. Any failure thereafter is to be considered as due to the added citrate. It would appear then that a certain amount of inhibition is produced by addition to the blood of 0.6 per cent of sodium citrate and complete inhibition by larger amounts. The concentration of free sodium citrate in the presence of the blood added would of course be less than that if broth alone were present, a certain amount of the salt being combined with the calcium of the blood. Accordingly it would seem that the effect of sodium citrate is rather greater in inhibiting growth of the organisms forming part of a streptococcal septicaemia than upon organisms of a twenty-four hour laboratory culture in the presence of blood and more comparable with the effect of that salt upon an 'old laboratory culture.

Direct comparison is however desirable and such a comparison is recorded in another section.

From the point of view of technique these experiments lead to the conclusion that delay in the commencement of growth is observed in the presence of heparin and

in the presence of a concentration of sodium citrate which is not itself inhibitory. The latent period is reduced by the use of trypsin in a concentration of five per cent.

The concentration of sodium citrate which inhibits growth appears to vary with the specimen of blood examined but is in the neighbourhood of 0.6 per cent. Coagulation being effectively prevented by this salt in a concentration of 0.2 per cent, it would appear inadvisable and indeed undesirable to use more than this amount in attempting to cultivate organisms from the blood.

On the destructive action of blood
from cases of subacute infective
endocarditis upon the streptococci
within it.

In connection with the discussion of blood culture technique three German writers lay great emphasis upon the necessity of inoculating the medium at the bedside HASSENCAMP (1922), STADLER (1914), KASTNER (1918). They stress the fact that the bacteria within the blood are killed off within a short space of time, 2 hours, (STADLER), "at any rate after 6 hours" (KASTNER). The evidence upon which these statements are made is not given. In view of the experimental work required for this study it was desirable to see if the blood could be conveyed to the laboratory and inoculated there without serious alteration in the bacterial content.

Experiment 23.

A preliminary experiment was therefore made upon the blood of the patient A1. Blood was drawn off (18 c.c.) into 2 c.c. of sodium citrate (10%) in the syringe. The citrated blood was placed in a large tube and conveyed to the laboratory where it was inoculated at once (within five minutes of drawing from the patient). Measured quantities (1 c.c.) were inoculated ^{into} ~~with~~ agar and plated cultures prepared. The tube was then allowed to stand at laboratory temperature for $6\frac{1}{2}$ hours when a similar series of plates was inoculated and incubated. The colonies developing were counted after 48 hours' incubation at 37° C.

The resulting figures were as recorded below.

Colonies developing from plates
inoculated.

At once	After 6½ hours.
34	80

(The numbers represent averages of three plates in each case). There is here an apparent increase. Subsequent experiments showed that this was probably not real but due to breaking up of the chains in manipulation or to uneven distribution in the blood. At any rate there is no diminution in the number of organisms on standing 6½ hrs. at ordinary laboratory temperature. So that inoculation of media at the bedside, as ordinarily recommended is not essential.

Experiment 24.

At the end of this experiment the tube of blood containing bacteria was placed in the incubator at 37°C. and after 19 hours' incubation samples drawn off and plated.

There then grew only 2 colonies from each c.c. of blood.

Incubation therefore for 19 hours had almost sterilized the blood.

Experiment 25.

It seemed necessary to verify this observation at length for it pointed to the conclusion that the blood of this patient, who had a pronounced septicaemia, was capable of dealing with the organisms present in the circulating blood.

This was accordingly done, with a similar result.

TABLE XXI.

Blood of patient A1.

No. of colonies developing from 1 c.c. of blood.	
Inoculated at once	40 (average of 3 plates)
After 5½ hrs. at 37°C.	21
After 24 hrs. at 37°C.	5
After 48 hrs. at 37°C.	0

Further observations have been made upon the blood of other patients and in each case the result has been similar.

Experiment 26.

A similar observation was also made upon the blood of a rabbit with experimental endocarditis, produced by the inoculation of Streptococcus A1. on several occasions.

TABLE XXII.

Blood of Rabbit 130. Strep.A1.

No of colonies developing from 1 c.c. of citrated blood.	
Inoculated immediately	2643
do. after 4½ hrs. at 37°C.	2248
do. after 21 hrs. at 37°C.	78

Effect of dilution upon
bactericidal action of blood.

Experiment 27.

In this case the blood was diluted in (1) distilled water, (2) 0.85% sodium chloride, sufficient sodium citrate being added to maintain a constant concentration of 0.5% in each case.

Diluent.	Dilution	No. of colonies developing on plates.			
		Immediate	After 5½ hrs.	24 hrs.	48 hrs.
Nil		40	21	5	0
Aq. Dest. (citrate)	1:2	32	2	0	0
	1:5	6	1	0	0
Saline (citrate)	1:2	16	4	1	0
	1:5	9	2	0	0
Broth (citrate)	1:5	8	10	13	∞

The times refer to the period of incubation at 37°C. prior to planting of a sample in an agar plate.

∞ = too many to count. Growth visible macroscopically.

From this it would appear that dilution, far from preventing the action of the blood upon bacteria seems rather to hasten it. Further evidence upon this point is discussed elsewhere.

It was important to consider the effect of the anti-coagulant upon the destructive action of the citrated blood upon the bacteria.

Experiment 28.

Four specimens of blood were prepared. 20 c.c. of blood were drawn into a syringe without addition of any anticoagulant. Of this 4 c.c. were added to each of four tubes containing solutions as under:-

(1) Sodium Citrate (5%) in sodium chloride solution (0.85%). - The final concentration of sodium citrate in this specimen of blood was thus 1 per cent.

(2) Sodium citrate (1%) in sodium chloride solution (0.85%), this giving a final concentration of sodium citrate of 0.2 per cent.

(3) Heparin - a substance prepared by extraction of liver* - 1 per cent. This preparation is readily soluble and actively anticoagulant. It can be boiled without affecting its anticoagulant action and has no effect upon the growth of bacteria in the concentration employed.

(4) Trypsin - the "Azoule" preparation for medium making supplied by Messrs. Allen & Hanbury. Of this 1 c.c. was placed in a test tube and to it were added 4 c.c. of blood - the amount recommended by DOUGLAS and COLEBROOK (1916) for this purpose.

These four tubes were then incubated and quantities (0.5 c.c.) drawn off and plated at intervals, the colonies developing being counted after 48 hours' incubation.

* Prepared by Messrs. Hyson; Westcott and Danning,
(Baltimore).

TABLE XXIII.

Blood of patient Pt.

Anticoagulant.	No. of colonies developing on planting in agar after incubation at 37°C.		
	Nil	24 hrs.	48 hrs.
Sodium citrate 1%	90	3	0
do. 0.2%	104	1	0
Heparin 0.2%	111	0	0
Trypsin 20%	104	α*	α*

[* It has been found that errors are here likely to arise from the fact that when transplanted on to agar plates there is, on incubation, an apparent lack of growth the medium being discoloured but the individual colonies invisible. That growth had actually occurred was proved however by the observation of films and the inoculation into broth. The organisms were visible in the case of the trypsinized blood at the end of 24 hours but not in the other cases; similarly growth in broth was found on inoculation from the trypsin medium after 48 hours but not from the others.]

From this then it would appear that this destruction of bacteria in citrated blood and in blood treated with Heparin is to be regarded not as due to the anticoagulant but to some constituent of the blood. The difference between the stronger and the weaker concentrations of sodium citrate is negligible. It is unfortunate that the experiment cannot be carried out upon blood which has not been submitted to any anticoagulant.

An attempt was made to carry out such an experiment as follows:-

Experiment 29.

Blood of patient Pt.

Blood (20 c.c.) was drawn into a syringe without addition of any substance to prevent clotting. This was then distributed over 3 tubes as follows:

(1) into Trypsin (final concentration 20%);

(2) into citrate (final concentration 1%);

(3) into a tube in which were some sterile fragments of glass. This was thoroughly agitated till the fibrin had separated out on the glass fragments. The fibrin free blood was then removed into a fresh tube and used for the experiment.

Specimens of 0.5 c.c. were taken off at intervals and plated in agar, and the colonies counted after incubation for 48 hours.

TABLE XXIV.

Blood treated with	Colonies developing on plating after incubation at 37°C. for		
	Nil	5 hrs.	24 hrs.
Trypsin	57	109	∅*
Sodium citrate 1%	54	37	5
Glass beads	5	2	0

* There was the same difficulty here regarding determination of presence of growth as in the preceding experiment.

The interesting point here is the great discrepancy between the bacterial counts where an anticoagulant is used and where defibrination is carried out.

It would seem that at least 90% of the organisms in this sample of blood were removed from the fluid in the process of fibrin formation.

It is interesting to note however that there was no evidence of multiplication on the part of these organisms in the defibrinated blood. So far as it goes it supports the view that destruction is really due to some of the blood constituents and is consonant with the view that the streptococcus concerned was not capable of multiplying in the blood stream. It certainly was not resistant to destruction by the blood elements which has been suggested by ROSENOW (1910) as the reason for its presence in the blood during life.

An observation was made upon the latent period of the organisms growing in blood in the presence of trypsin as under.

Experiment 30.

Patient Tr.

Blood (4 c.c.) was added to 1 c.c. of trypsin and incubated at 37°C., specimens being drawn off for plating at intervals.

Period of incubation before plating.	No of colonies developing.
Nil	29
5 hrs.	33
8 hrs.	47
10 hrs.	128
24 hrs.	+++*

*Visible in microscopic film from incubated blood.

The period elapsing before commencement of growth then is between 8 and 10 hours. Citrated broth tubes inoculated with the same specimen showed no appearance of growth until after 48 hours had elapsed.

From the point of view of technique these experiments throw light upon certain points at issue.

The blood prepared for the cultivation of organisms in the usual way is definitely bactericidal. This activity of the blood is invoked by many to explain negative cultures as mentioned above. This is apparently not likely to be the case for the action is not exhibited in $6\frac{1}{2}$ hours at room temperature.

On the other hand the practice advocated by KIPNIS (1923) as probably advantageous, namely, incubating the blood for 24 hours before plating should not be adopted as the number of bacteria is definitely reduced and frequently the specimen is sterile at the end of that time. It should be remarked that Kipnis worked with *B.typhosus* while these experiments refer only to streptococci.

A very good method of doing away with the bactericidal action of blood is by addition of Trypsin to a concentration of 20% as recommended by Douglas & Colebrook. This should be a most satisfactory way of treating blood to be sent a distance for examination.

This bacteriolytic action may be well studied in blood treated with sodium citrate or with heparin. In the case of the former a concentration of 0.2 per cent has proved an efficient anticoagulant for at least 24 hours at 37°C. Lower concentrations have been somewhat uncertain in their action.

On the effect of dilution on
the bactericidal action of
the blood.

Dilution of the blood used as inoculum is frequently referred to as desirable in making blood cultures. The effect of dilution upon the bactericidal activity has not, so far as I can find, been studied in any detail. It is however a curious and interesting fact that, amid all the statements as to the importance of using an appropriate technique and a suitable medium, AUERBACH (1920) should make the observation that the best method to use for cultivating streptococci from the blood is to inoculate the blood into 500 c.c. of distilled water. An experiment described above showed that the destructive action was not removed by dilution of the blood five times with saline or distilled water containing 0.6 per cent of sodium citrate. The following experiment was carried out to test the effect of various diluents on this property of the blood.

Experiment 31.

Blood Tr.

A series of diluents was prepared as indicated below and into each of these blood (1 c.c.) containing no anti-coagulant was placed. Observations were made at the intervals noted upon the occurrence of growth. This was determined both macroscopically and by the examination of films. In addition subcultures were made at certain times. In this way it could be determined

- (1) whether multiplication had occurred;
- (2) whether in those tubes in which multiplication

had not occurred the organisms had been destroyed.

TABLE XXV.

Diluent.	Dilution.	Occurrence of growth.				Growth* on sub-culture		Clotting in diluted blood.	Remarks.
		24 hrs.	48 hrs.	72 hrs.	8 dys.	72 hrs.	7 dys.		
Distilled water	1 in 2	-	-	-	+	++		+	
Do.	1 in 5	-	+	+				No solid clot.	
Do.	1 in 10	-	+	+				Flocculi.	
Do.+sod.citr 0.2%	1 in 10	-	-	-	+	++		Fine flocculi.	
Do.+sod.citr 0.6%	1 in 10	-	-	?	+	++		No clot or flocculi.	
Do.+sod.citr 1%	1 in 10	-	-	-	-	--	-	Do.	PH after 48 hrs. 7.6
NaCl 0.9%	1 in 10	-	-	-	-	-	-	Do.	Do. 7.8
Do.	1 in 20	-	-	-	-	-	-	Do.	Do. 7.8
Do.	1 in 50	-	-	-	-	-	-	Do.	Do. 7.6
Do.+sod.citr 0.2%	1 in 10	-	-	-	-	-	-	Do.	Do. 7.8
Do.+do. 0.6%	1 in 10	-	-	-	-	-	-	Do.	Do. 7.8
Do.+do. 1%	1 in 10	-	-	-	-	-	-	Do.	Do. 7.8
Do.+Na ₂ HPO ₄ (0.15%) +Gelatin (0.1%)	1 in 10	-	-	-	-	++	++	Do.	Do. 7.6
Do.+Trypsin (5%)	1 in 10	-	-	-	+	++	++	Do.	Do. 7
Ringer's solution	1 in 10	-	-	-	-	-	-	++	
Broth + 0.2% sod.citr.	1 in 10	-	-	++		++		No clot or flocculi	Do. 7.2
Do.+0.6% Sod.citr.	1 in 10	-	++			++		Do.	Do. 6.6 - 6.8
Do.+ 1% sod.citr.	1 in 10	-	-	++		--		Do.	Do. 7.2

- = no organisms in films & no growth.

+ = organisms in films.

++ = growth visible macroscopically.

* The times mentioned under this heading refer to the age of the original culture at the time when the subculture was made.

It is clear that the diluent used has a very great effect upon the multiplication and survival of the organism.

When distilled water is the diluent growth occurs readily in the dilutions 1 in 5 and 1 in 10. In the dilution 1 in 2 growth is much delayed but ultimately occurs. The amount is usually small. Addition of sodium citrate up to 0.6% does not prevent growth occurring though it is rather more delayed.

If normal saline (0.9% sodium chloride) be used the organism does not multiply nor does it apparently survive and the same is evident when citrate is added to the solution. If however Phosphate and gelatine are added to the saline, growth fails to occur but some at least of the organisms survive for eight days. If trypsin be added slight growth occurs late.

In the presence of Ringer's solution clotting is marked and the organisms are destroyed.

The reaction of ^{the} fluids in the different tubes is not sufficient to explain the failure to grow, for this organism has been grown constantly upon medium of the reaction (PH 7.8)

Apparently if the salt content is diminished to half of that in the blood growth will occur. If however unbalanced sodium chloride is used dilution even to 1 in 50 will not prevent destruction of the organisms. The experiment with Ringer's solution is not satisfactory owing to the clotting which occurs in the blood.

The possibility arises that part of the destructive effect in sodium chloride solution is due to the sodium ion.

ROBERTSON, SIA & WOO (1924) have pointed out that this

destructive action of the sodium ion can be overcome by addition of phosphate and gelatin to the mixture. It is interesting that in such a fluid these streptococci survive without multiplying. The only saline mixture allowing of growth is the one in which trypsin is used and there as in the case of the dilution with distilled water the amount is slight and in no way to be compared with the amount of growth in broth.

Multiplication or failure to multiply will presumably depend in such experiments upon two factors. These are (1) the sum of all destructive agencies present and (2) the amount of food supply available. The chance of the former coming into play will of course be diminished by dilution.

But the diluent itself may not be inert. Solution of sodium chloride probably causes some destruction which may be offset by addition of phosphate and gelatin. Distilled water apparently throws out of action the destructive factors, either because of the tonicity being too low to allow the leucocytes to act or because the destructive agents in the plasma are inactivated by alteration of the state of the proteins. The trypsin in the salt solution may act by some inhibition of the destructive agencies but the possibility cannot be excluded that it provides additional nutriment by acting upon the proteins present.

The marked superiority of the broth is almost certainly due to additional foodstuffs provided.

From the point of view of technique it would appear that while growth can occur in distilled water this is inferior to broth as a routine medium.

The consistency of the results
of blood cultures from cases of
infective endocarditis.

The experiments recorded above would appear to indicate that the technique employed was adequate to isolate streptococci from the blood if they were present in the inoculum. It may be objected that this is not necessarily true of all streptococci. It is undoubtedly true that there is considerable variation in the ease with which these organisms are maintained in subculture. In the case of one strain it has been found impossible to cultivate it on media not containing blood. But even with so delicate an organism there was no difficulty in obtaining primary cultures from the patient's blood.

There yet remains the possibility that the organisms might be present in the blood only at certain times or in such small numbers that they might be absent from the specimen examined.

Statements to this effect occur in the literature. DEBRE (1917 II.) records a case in which blood culture on 12.1.13 was negative, that on 23.1.13 positive and that on 10.2.13 again negative. STADLER (1914) had a case from which a positive culture was obtained at the first attempt and a negative one later. KRIEGER & FRIEDENTHAL (1924) report similar irregularities in cases of this disease as under

	1st culture	2nd culture	3rd culture.
I.	-	+	-
II.	-	+	-
III.	-	+	-
IV.	+	+	-

LOREY (1912) records two cases in the first of which a positive culture was obtained on the day before death in liquid media, cultures in solid media being negative. Previous cultures had given no organisms. In the second a negative culture was succeeded by a positive and the patient was later said to have recovered. It is very doubtful indeed if this was a case of the disease here discussed.

MORAWITZ (1921) describes one case in which four negative cultures were succeeded by a positive and two others where a negative culture was obtained at the first attempt and a positive at the second. LIBMAN (1913 II.) mentions a case of infective endocarditis (subacute) from which influenza bacilli (B. Pfeiffer) were isolated at first but which later became bacteria free. HEMSTED (1913) and LATHAM & HUNT (1911) state that in their cases (one each) a long series of positive cultures was followed by a series of negatives. LOSSEN (1913) obtained a preliminary negative culture and then two successive positives. In addition to these definite statements there occur many more general ones to the effect that repeated cultures may reveal organisms when individual ones fail.

It is conceivable that this may be true. At the beginning of the disease and in case of transition to the bacteria free stage there would certainly be a time at which the result of blood culture could, or might, alter.

The observations of Libman, Hemsted, Latham & Hunt and of Lossen seem to be well substantiated. Those of Debre, Krieger & Friedenthal and Morawitz are less certain. The occurrence of an isolated positive culture in the midst of a series of negatives would unquestionably meet with justifiable criticism unless supported by further evidence. Lorey's case falls into another category. The isolation of an organism on the day before death is very liable indeed to be due to the fact that there has been a terminal invasion of the blood stream.

In this series of cases no such irregularities have occurred. If the streptococcus has once been isolated from the blood stream it has been isolated on each occasion that blood has been examined - nine times in one case and six times in another. On the other hand if one culture has failed to give a growth of streptococci, no growth has been obtained subsequently. A list of the findings will be found at the end of the next section. There it will be noted that in case Lat. four negative cultures were succeeded by one positive - but in this case a staphylococcus albus was obtained. The finding was confirmed at the post mortem. The morning after the blood was taken for cultivation the patient developed definite signs of septicaemia of which he died two days later. In another case, Ain, pneumococci were isolated from the blood on the day before death. There were no streptococci in the blood, or in the valves after death, but the patient died of lobar pneumonia. There had been

no previous culture made. These two cases were undoubtedly instances of terminal invasion by another organism.

In order to see the degree of variation in the bacteriaemia in a series of cultures from well established cases a measured quantity of blood (1 c.c.) has been inoculated into three agar plates on each occasion. The average number of colonies developing on these plates has been taken to represent the number of organisms per c.c. of blood. The blood was placed in a Petri dish and the cooled liquid agar poured on it and mixed by agitating and rotating the plate. This seemed more satisfactory than to add the blood to a tube of agar and then mix and pour it into a plate. In the latter method quite a considerable amount of blood is apt to be left in the tube. The degree of accuracy of these counts is probably not great. The variation from the mean was usually $\pm 20\%$ in the individual plates but sometimes was much greater. On the whole this method agreed very well in its results with those obtained by estimating the lowest dilution in broth in which growth occurred.

Estimations of the numbers of organisms in the blood in cases of subacute infective endocarditis have not been very frequently reported. HORDER (1907) records 50 colonies of Pfeiffer's bacillus as growing from 1 c.c. of blood, SCHONE (1912) 230 colonies in one case and 90 in another from a streptococcus endocarditis; MOORHEAD & STOKES (1914) 201 colonies; STADLER (1914) 25 colonies from 2 c.c.; KINSELLA (1917) 5 to 1200 organisms per c.c.

in different cases. He states they tend to increase gradually as the disease progresses; but the number of organisms in the blood cannot be used as an aid to prognosis. LIBMAN & CELLER (1910) found in one case two colonies in 25 c.c.. The maximum number observed by them was 500 in 1 c.c.

LENHARTZ (1901) has recorded observations on three cases in which the numbers vary from 6 to 48 per c.c. in one case, 98 to 286 in the second, and 25 to 133 in the third. The progressive increase noted by Kinsella was not observed in these cases. The numbers varied from time to time during the observation over periods of two months at intervals of varying length. Lenhartz also records an enumeration in a case of acute pneumococcal endocarditis where 1000 colonies grew from 1 c.c. of blood.

The only series of extensive observations upon the number of organisms in different types of septicaemia is that of WARREN & HERRICK (1916). In septicaemias due to non-haemolytic streptococci there were from 1 to 300 organisms per c.c. They also record the following figures in septicaemias due to the following organisms: *S. haemolyticus* from 1 to 360; *staphylococcus aureus* from 1 to 300, 1 case "innumerable"; *Pneumococci* 2, 4 and innumerable.

It has been suggested that the failure to obtain a growth of streptococci from the blood of these cases has been due to the fact that the time chosen for taking the blood has been unsuitable. Kastner and Gessler lay

stress on the importance of taking the blood at the time when the temperature is elevated, while Leschke and Auerbach state that the best time is "before the rigor" - a somewhat difficult occurrence to foretell, especially as rigors appear to be by no means constant in these cases. Leschke considers the rigor to be due to destruction of bacteria, which disappear from the blood after it has occurred. No evidence is adduced for this statement which is opposed to the more general view that the rigor coincides with the throwing out of a flood of organisms into the blood stream. For this statement also I have found no evidence.

The following table gives the results bearing on these points obtained in this series of cases. The observations upon the temperature are not sufficiently complete to be conclusive but are included as of some value.

TABLE XXVI.

Colonies developing from 1 c.c. of blood (average of 3 plates.)									
Patient	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Am Died 29-8-24	+	2/11 +	9/11 27	30/11 10	3/12 11 Temp. high	10/12 16 Temp. high	2/1 97	12/2 70	19/2 118 Temp. low
Av	+	16/5 26							
Al	+	17/5 17 T.99	28/5 34 T.99	12/6 41 T.104 after rigor	25/7 370 T.100	30/7 287 T.100+			
Dud Died 11-6-24	21/4 603 T.99	28/4 399 T. 99.4	28/5 1141 T. 98.8						
Lat Died 12-11-23	20/9 Neg.	23/10 Neg.	29/10 Neg. Temp. high	1/11 Neg. Temp. slight	9/11 Staph. Temp. low				
Mo Died 2-12-23	+	28/11 2553							
Mur	+	28/11 228 Temp. 98.6	19/12 200 Temp. 100.6						
Pt	25/11 55 T.98	28/11 70 T.99.2	9/12 108	31/12 204					
Tr	+	+	21/10 45 Temp. high	28/10 42 Temp. high	26/11 41 Temp. high	6/12 70 Temp. high	6/1 58	28/1 134 Temp. high	
Wi Died 19-2-24	14/1 50	13/2 122 Temp. high							
Ai Died 25-8-23	22/8 Pneumo.								

It would appear from this table that there may be fluctuations quite naturally in the course of the bacteriaemia. There does not seem to be a close relationship between the number of organisms in the blood stream and the temperature.

The number of organisms in the venous blood is in most cases surprisingly low. As the disease progresses the grade of bacteraemia tends to increase but the increase is not regular. The number frequently remains steady for a long period and may actually diminish. There has been no opportunity of comparing the numbers in arterial and venous blood which might quite well be very different. The degree of the fluctuation might be quite sufficient to account for varying results if the number of organisms were very small. It has not given rise to these difficulties in this series.

It was possible in one case to examine the blood at the time of the rise of temperature immediately after a rigor (Case Al.cult.IV). The blood was collected within twenty minutes of the occurrence of the rigor. It will be noted that there were organisms in the blood and the number corresponded with those observed at other times. There is here no definite evidence for or against the view expressed above as a count immediately before the rigor is not available for comparison.

The nature of the organisms isolated.

LEINHARTZ (1901) pointed out in a series of 16 cases of "chronic septic endocarditis" that streptococci were to be found in the blood and also that there might be absence of suppuration even in the presence of septicaemia.

SCHOTTMULLER (1903) first pointed out the peculiarity of the streptococcus associated with these lesions in that they failed to produce haemolysis on blood agar but tended to cause a green discolouration of the medium. He also pointed out that so-called streptococcus mitis seu viridans might produce haemolysis if the amount of blood in the medium was small. The production of this green discolouration he claimed was a definite and constant character. This observation has been amply confirmed.

KINSELLA (1917) has however pointed out that green production does not always occur and LIBMAN (1920) refers to the organism as being non-haemolytic. Of the cases with which he has dealt he notes that 95 per cent of the bacteraemias were due to the streptococci of this group.

HORDER (1909) applying fermentation tests found that the streptococci belonged to the two groups *S. faecalis*, and *S. salivarius*.

ROSENOW (1901 I.) found the fermentation reactions to vary, especially in the case of inulin. He describes the organisms isolated as pneumococci and reinforces his view by evidence that the patient's serum agglutinates pneumococci but not streptococci. In view of the now well established serological heterogeneity of the pneumococcus and streptococcus group this evidence cannot be

accepted particularly as MAJOR (1912) found the opposite of this to be true in regard to complement fixation.

ROSENOW (1909) had previously stated that inulin fermentation tended to be positive when the organism was freshly isolated but later to become negative. He did not apply the test of bile solubility. MAJOR (1912) found inulin fermentation to be absent in his tests and the organism to be insoluble in bile.

KINSELLA (1917) observes that all his strains were insoluble in bile.

There appears now to be general acceptance of the statement that the organisms is not of the pneumococcus group.

BEATTIE & YATES (1911), AINLEY WALKER (1911) JOHN RITCHIE (1908) have suggested that fermentation tests are unreliable in the case of the streptococcus. Conclusions based upon the recovery from an experimental animal (Beattie & Yates) of an organism with different fermentation reactions from those of the organism inoculated are open to objection. It may be that a different organism has been recovered. Variations in laboratory cultures are in a different category. BROADHURST (1915) and THRO (1914) point out the great importance of the environment of the organism in the test medium. Slight variations in this may give rise to notable differences in the result of the test which are not legitimate evidence of variation. Moreover as Thro points out the question of purity of the chemicals employed is a factor of primary

importance. HOLMAN (1916) claims that if due precautions are taken variation in the fermenting power of laboratory cultures is confined to very few strains and KINSELLA (1917) found in his series of organisms from cases of infective endocarditis that fermentation reactions were constant over a period of eighteen months with the exception of one strain. As to variations within the animal body we are without sound evidence. On the whole it would appear that a satisfactory classification on the basis of fermentation reactions is not yet generally accepted though those of ANDREWES & HORDER (1906) and of HOLMAN (1916) are of some practical use.

Similarly from the point of view of serology the attempts made to classify these organisms have not met with success as regards the non-haemolytic organisms. MAR-MOREK'S original view (1902) of the essential unity of the group is no longer tenable. Use of complement fixation tests (HOWELL (1918) and KINSELLA & SWIFT (1917)) and agglutination technique (GORDON (1922) and NORTON (1923)) have shown that while there may be relationships existing between individual strains any serological classification shows a marked individuality of the organism and there is no agreement between a serological classification and one based on biological properties.

KINSELLA (1917) and KINSELLA & SWIFT (1917) find in the case of organisms from endocarditis more interrelationship if complement fixation tests are used than if agglutination methods are employed.

STONE (1923) finds the organisms from malignant endocarditis fall into two groups - salivarius and faecalis. The organisms however were serologically distinct as judged by the agglutination and the complement fixation tests.

The organisms isolated from this series of cases have been examined in regard to their fermentation reactions. The medium employed has been peptone water (de Fresne's peptone) with 0.5 or 1% of the carbohydrate concerned. To avoid alteration of the carbohydrates in sterilization they have been autoclaved in concentrated solution and subsequently added aseptically to the peptone water. Finally rabbit serum (1 per cent) has been added in a sterile condition. Such a medium has in my experience proved more satisfactory than Hiss's serum water. The indicator used was Andrade's reduced fuchsin which is undoubtedly more satisfactory than litmus. Table XXVII. records the results of these tests.

TABLE XXVII.

Character of organisms isolated in blood culture.

Strain.	Virulence for mice.	Bile Solubility.	Lactose	Mannite	Salicin.	Raffinose.	Inulin	Haemolysis	Green production on blood agar.	
An	-	-	+	-	-	+	-	-	+	
At	-	-	+	+	+	+	+	-	-	
Av	-	-	+	-	-	+	-	-	+	
Al	-	-	+	-	+	+	-	-	+	
Dud	-	-	-	-	-	+	+	-	+	
Mo	-	-	+	-	-	?+	?+	-	-	Varied (see below)
Pt	-	-	+	-	+	+	+	-	+	
Tr	-	-	+	-	+	-	-	-	+	
Wi	-	-	+	+	+	+	+	-	+	
Mur	could not be tested.									

Most of the reactions were fully developed in 24 hours but some (Mo and Dud) failed sometimes to show any fermentation until several days or even a week had elapsed. Negative results were not accepted until after a fortnight's incubation.

The reactions of streptococci obtained at different times were identical except in the case of the fourth culture from Pt and the culture obtained post mortem. The first three cultures isolated fermented inulin after three days' incubation. The last cultures failed to do so in 14 days. The difficulty here is that the standard of purity of inulin is unsatisfactory and one has found different reactions to occur with one and the same streptococcus culture inoculated into specimens of inulin, alleged to be pure, obtained from different sources.

Observations on the stability of the reactions of the organisms have been made after the lapse of periods up to one year. These have not varied in the slightest except in one case Mo, a delicate streptococcus which has never grown vigorously. In this case variations in the action upon Raffinose, inulin and lactose have been observed. It has not been possible to exclude slight variation in purity of the carbohydrates employed.

Two strains At and Wi resemble in their reactions the organism described by CLARKE (1924) as being associated with dental caries and called by him *Streptococcus mutans*. Dr. Clarke kindly provided me with some serum prepared against one of his strains. Strain Wi was

agglutinated to full titre and strain At to about one fourth of the titre for the homologous organism.

None of the organisms isolated, except a pneumococcus not included in the above list, were soluble in bile. Nor did 1 c.c. of a 24 hours broth culture have any effect upon white mice when inoculated intraperitoneally. So that no evidence was obtainable in support of the statement that the organisms causing this type of endocarditis are pneumococci.

None of the organisms isolated during the life of the patient produced frank haemolysis in 24 hours in a blood agar plate. J. H. BROWN (1919) has pointed out that all green producing streptococci have in addition to this power of altering blood a certain slight haemolytic power (α type) which manifests itself later than in the case of the frankly haemolytic organisms (β type). He also points out the importance of the medium as a factor in determining whether this shall appear or not and especially the concentration of blood and conditions of temperature. He also points out (confirming HOLMAN (1914)) the tendency for haemolytic streptococci to overshadow and often conceal the presence of non-haemolytic streptococci if present together with them on the plate.

authors

Various have made reference to the fact that they have isolated streptococci of a haemolytic type from cases of subacute infective endocarditis. KASTNER (1918) states that the power of organisms isolated to haemolyse blood cells varied from time to time. He states that it

"appears in organisms collected near the time of death". FREUND & BERGER (1924) consider that the haemolytic and non-haemolytic forms of streptococci may appear at various times in the same case and regards them as merely being variants of the same strain, the change occurring as a result of variations in the state of immunity of the patient. SCHNITZER & MUNTER (1921), MORGENROTH & ABRAHAM (1921) and MORGENROTH, BIBERSTEIN & SCHNITZER (1920) claim to have realized this sort of transformation in mice the first named showing that in the transition there is a change of virulence. This may be true but if it is it must be of rare occurrence. CLAWSON (1924) comments on the fact that in the statement of bacteriological findings on his cases there appear eight haemolytic streptococci and remarks that seven of these were obtained at post mortem. MURRAY (1922) records that in 70% of his cases with bacteraemia the organism was a streptococcus which was non-haemolytic. In the remainder the organisms were almost all haemolytic streptococci. GESSLER (1921) comments upon the fact that a variety of organisms may be found at autopsy in the heart blood and thinks many of these are unimportant.

In this connection must be considered the possibility that a patient dying of infection with one type of organism may, in the later days of the disease, be infected by an additional one or that a patient without bacteraemia may in the course of his illness develop a terminal infection of the blood stream. Evidence upon this point has

been discussed above and the matter will be further considered in another section. The conclusion which seems valid at present is that the view that either the pneumococcus or the haemolytic streptococcus or the staphylococci can produce subacute infective endocarditis is not substantiated. The possibility of the occurrence of terminal invasion by these organisms has not been excluded in those cases in which these organisms have been obtained from the blood stream.

There seems no ground for doubt however that the cases of influenzal endocarditis recorded by HORDER (1906, 1907) and LIBMAN (1912, 1920) and of that due to the haemophilic and haemolytic organism described by MILLER & BRANCH (1923) are well substantiated. The information recorded by these authors is quite sufficient to permit of the exclusion of any terminal invasion of the blood stream.

The significance of organisms
isolated from the heart blood
after death.

The appearance of a haemolytic streptococcus in the blood has been noted by certain workers in cases of this disease. Clawson finds that this is commoner in observations upon the blood collected post mortem than in the blood collected during the life of the patient. LIBMAN (1913) states that the bacteria may not be found during life in the blood but may be present in masses on the valves when examined histologically. He does not say whether in these cases any observation was made upon the blood in the last few hours before death. LUBARSCH (1923) records that cultures from the crushed valves after death have in all cases of this disease given cultures of streptococcus, while cultures made from the surface of the vegetation may fail.

The contention here raised is that cultivations made from the heart blood or valves after death must be regarded with very grave suspicion unless very carefully controlled. In support of this contention are instanced cases in which the result of bacteriological examinations made from the blood post mortem differed from those obtained during life under such conditions that it appeared clear that such a terminal invasion of the blood stream had occurred.

Case Lat was one in which four examinations of the blood prior to Nov. 9th 1923 had revealed no organism although the case was clinically one of this disease. On the evening of that date a fourth specimen was collected and in each of 12 tubes of broth inoculated with this a staphylococcus albus was present. (No pigmentation occurred in a week). The patient was not any worse at this time than he had been previously. This was thought to be a contamination and on the morning of November 12th a visit to the ward was made to make a further observation. Unfortunately the patient had just died. On the morning of November 10th (the day after the staphylococcus had been obtained) petechial haemorrhages had begun to appear, rapidly increasing in number and extent, accompanied by haemorrhages from the mucous membranes, a very marked rise of temperature and marked deterioration in the patient's condition. At autopsy four hours after death the same organism was obtained from the heart blood and in sections of the tissues examined by Dr. W. G. Barnard multiple abscesses were found - a condition not occurring in the

ordinary case of subacute infective endocarditis. The organisms were also present in the vegetations on the heart valves. In this case a single observation in the last three days of life or at autopsy or an observation of the blood made previously to November 9th accompanied by one post mortem might have led to an erroneous conclusion. The explanation which seems most likely to be correct is that the patient had suffered a terminal invasion by the staphylococcus albus. The patient's serum did not agglutinate this staphylococcus.

Case Dud showed a somewhat similar phenomenon. From the blood during life a non-haemolytic streptococcus had been isolated on three occasions. It was characterized by its relatively poor growth, and by the following fermentation reactions.

Lactose Mannite Salicin - not fermented.

Raffinose Inulin - late fermentation.

At the post mortem blood was collected from the left ventricle, the pericardium being cut through and turned back, and the surface thoroughly seared with a red hot iron. A sterile glass pipette fitted with rubber teat was passed through the seared area into the ventricle and the blood aspirated. Contamination could be excluded as a source of any organism present. In the sample of blood thus obtained there were two strains of streptococci. One of these was identical with that isolated during life. The second grew very vigorously, was actively haemolytic and fermented carbohydrates as indicated.

Lactose and Salicin - fermented.

Mannite, Raffinose, Inulin - not fermented.

No observation had been made upon the blood for 19 days before death. It would require a considerable degree of mutation to account for a change of this kind. The organisms differ not only in regard to haemolysis but also most markedly in other directions. It would appear more likely that the haemolytic coccus had invaded a patient suffering from a septicaemia due to another organism.

In a third case El. a blood culture made by Dr. Goodhart seven hours before death revealed no organism. Up to this time the patient showed signs of heart failure, but none of septicaemia. During the afternoon however marked haemorrhages appeared in different parts of the body, a particularly large one in the sternomastoid muscle. At the post mortem 18 hours later the signs were such as to suggest to the pathologist that there was a septicaemia. Cultures from the heart blood revealed a haemolytic streptococcus fermenting lactose, mannite and salicin but not raffinose or inulin. There were no organisms in the sections of the very chronic lesions on the heart valves.

In the case of Ju. a patient from whom two specimens of blood had been examined during life with negative results, a fragment of vegetation was washed in broth and crushed and streptococci of a non-haemolytic type were isolated. (In this case contamination could not be excluded). A culture from the femoral vein collected aseptically contained both a haemolytic and a non-haemolytic streptococcus with entirely different fermenting capacity for carbohydrates. No cocci could be found in sections of the vegetations.

Finally in case Ai a pneumococcus was isolated from the blood three days before death. The patient was very ill at the time and no other organism was present in the culture. At the post mortem there was found to be a definite pneumonic lesion and in sections undoubted pneumococci are to be found in the vegetation, chiefly in a superficial position. A pneumococcus was also isolated from the lung lesion.

These cases alone would seem to indicate that conclusions based upon cultures made in the last few days of the patient's life are liable to error and throw considerable doubt on the view that the haemolytic streptococcus and the pneumococcus may be etiological factors in this disease. It may be noted in passing that in cases where the invasion is very late the organisms may not be seen in sections or, if present, tend to occur in the loose cellular clot on the surface of the vegetation. If they have been present some days they may be seen in spaces in the vegetation or even in vessels in the valve proper or heart muscle. Frequently there is evidence of a polymorphic reaction in these situations. The location on the valve tissue and the acute reaction tend to differentiate these cases from the ordinary uncomplicated cases.

Findings of this type and also the fact that investigation of the valves in non-bacterial cases seemed to be the next step in the investigation led to the preliminary study of the bacterial content of the blood obtained at autopsy in cases dying from diseases other than the one being studied.

Accordingly observations were made upon an unselected series of 100 cases. The blood was collected from the left ventricle by means of a capillary pipette after searing the surface thoroughly with a red hot iron, the pericardium having been opened and drawn back. About 2 c.c. of blood were collected and incubated in broth both aerobically and anaerobically. The blood was collected as soon as the chest had been opened and before any manipulation of adjacent viscera had occurred, the latter precaution being taken to avoid displacement of organisms into the heart blood. The left ventricle was chosen as the observations on infective endocarditis would naturally be most frequently directed to this chamber.

Of the hundred cases 36 had undergone operations shortly before death and of these operations 29 had been upon abdominal viscera.

The full details of these findings are out of place in this discussion and therefore only the important points will be discussed. Of the 100 cases examined 37 contained bacteria of various kinds. In the cases which had undergone operation 44 per cent had bacteria in the blood while of the medical and unoperated cases 21 or 33 per cent contained bacteria. The percentage of positive findings was highest (55 per cent) in cases which had undergone recent abdominal operations.

The following table demonstrates the nature of the organisms isolated and the frequency with which they occurred.

TABLE XXVIII.

Organisms.	Times isolated	In pure culture.	Remarks.
Streptococcus haemolyticus	12	6	
do. non-haemolyticus	8	0	
do (undifferentiated)	2	0	
Coliform bacilli	14	5	8 cases of abdominal operation.
Anaerobic bacilli	8	5	5 cases intestinal disease.
			1 case gas gangrene
			7 cases Welchii
Staphylococci	3	2	
Diphtheroid	4	1	
Pneumococcus	1	0	

The length of time between death and autopsy seemed to have little effect on the percentage of positive results obtained. There appeared to be greater frequency after 12 hours but the number of cases examined before that period had elapsed is too small to permit of detailed examination. Nor did the nature of the bacteria appear to vary with the time that had elapsed since death. The bodies were placed in a cool chamber during this interval. In only 14 cases was there good evidence that the organism or organisms isolated could be considered as the cause of the disease which led to the death of the patient.

From the point of view of this study the very striking fact is the frequency of the occurrence of streptococci with or without other organisms, on 22 occasions altogether, and the predominance of the haemolytic type.

It is not possible to compare these results directly with others recorded in the literature because of the fact that they are not obtained on the same or even similar groups of cases. It is interesting however to note the

results of other workers.

STRAUCH (1910) records results obtained in the study of blood from the right ventricle in 2000 cases. He found bacteria in 1002. The streptococcus occurred in 548 cases, *E. coli* in 187, staphylococci in 138. He considers that organisms found within 24 to 48 hours after death in a well preserved body may be considered to have circulated during life in the blood stream.

GAY & SOUTHARD (1910) in 100 cases found the blood to contain bacteria in 59 (streptococci in eight). They think invasion occurred before death.

GRADWOHL (1904) in 50 cases examined within eleven hours of death found bacteria in 39, the streptococcus occurring in 23. RICHEY & GOEHRING (1918) took blood from an arm vein in 206 cases within ten minutes of death and found organisms in 76 (36.8 per cent). In their series the pneumococcus predominates (37 cases) but streptococci occurred on 30 occasions. In 20 cases the cultures so taken agreed with the antemortem findings, 4 showed additional organisms. Comparing these findings with those at autopsy (1-24 hours after) 27 were identical and 18 differed, additional organisms, chiefly cocci, being found. In 45 cases examined 71 per cent were positive.

GWYN & HARRIS (1905) comparing antemortem and post-mortem findings, the blood at post mortem being taken from the right auricle, found agreement in 7 cases and disagreement in 7. The amount of blood used in the observations after death was very small.

GIORDANO & BARNES (1922) compared antemortem and post

mortem results in 22 cases and obtained different results at post mortem in five. No indication in this or the other papers is given of the interval elapsing between the time of taking the blood culture in life and the death of the patient. They do not think lapse of time up to 13 hours after death causes increase in the number of positive cultures obtained. Post mortem invasion in this time is not marked. In 213 cases the heart blood was found to contain bacteria on 80 occasions (= 38 per cent). In cases of disease of or operation on the intestinal tract the percentage of positive results was found to be 69 (26 cases). The bodies in this series were not refrigerated.

FREDETTE (1916) took the blood from the cephalic vein within 10 minutes of death. In 119 cases he obtained growth of bacteria 42 times (=35 per cent). Here again the predominating organism is a streptococcus. In 5 cases out of 14 the findings differed in some respect from those recorded during life. Comparing results with those obtained at autopsy 1 - 16 hours later, 16 gave the same results and 3 different. In the last three cases the organisms additional were intestinal in type. He concludes that streptococci are by far the commonest terminal invader of the body.

It is clear therefore that independent observers in different countries have found that the streptococci commonly exist in the blood after death, more commonly than any other organism. There is also much evidence to show that they are not merely post mortem invaders. Obviously

then, any conclusion based upon the isolation of such organisms at post mortems must be subject to very serious objection unless there is clear evidence that this terminal or agonal invasion, if it be such, has not occurred. The isolation of such an organism as *B. typhosus* from the heart blood would be strong evidence that the patient had had typhoid fever. It is not found at post mortem except in cases of this disease. But to isolate a streptococcus from the cadaver is, like the isolation of such an organism from the throat secretions, far from conclusive evidence that it is aetiologically related to the particular disease from which the patient suffered. Yet it is to be noted that it is largely upon such evidence that is based the view that acute rheumatism is caused by streptococci. From this point of view all such evidence must be suspect.

As regards subacute infective endocarditis these facts suggest that, like cultures obtained during the terminal period of the disease, cultures obtained at autopsy may lead to erroneous conclusions. They are of little value in determining the aetiology of the disease unless supported by observations upon the blood during life or by actual discovery of the organisms within the vegetations. Further isolation of organisms at autopsy after failure to do so during life does not discredit the latter findings. Each observation is to be considered on its own merits.

Antibodies in the serum
of cases of infective
endocarditis.

ROSENOW (1909) in describing the peculiarities of the organisms isolated by him from the blood of cases of subacute infective endocarditis says that they grow in clumps in broth in the primary cultures. He gives evidence that the continuance of the infection is not due to lack of opsonins. He states that the patient's leucocytes however do not phagocyte the coccus so well in the presence of his own serum as in the presence of normal serum. In another paper (1910 I., II.) he notes that the granular appearance of the growth in the original cultures is lost on subculture. When this property has been lost it can be restored by growing the organism in the presence of the patient's serum. There is diminished phagocytosis in the patient's blood. He remarks however that even if the phagocytosis be increased it may be accompanied by lack of destruction in the leucocytes.

MAJOR (1912) found that an antigen made from the cocci isolated, fixed complement with the patient's serum as also did an antigen made from streptococcus pyogenes while one made from pneumococci did not.

FOX & LYNCH (1917) found the opsonins to be subnormal in some cases, in others increased. The phagocytic activity of the cells was low in the patient's serum but high in normal. The bacteria resisted phagocytosis when freshly isolated but the blood components having to do with phagocytosis were not necessarily reduced. They considered

the defect is in the cells. They found no evidence of the presence of antibodies by the complement fixation method.

KINSELLA (1917) found, on the other hand, that there were present in all cases agglutinins and complement fixing antibodies as demonstrated by the interaction of the patient's serum and the organism isolated from the blood. It is interesting to note that he finds the best method for detecting agglutinins to be the "thread reaction" used by DENNEMARK (1911) following MANDELBAUM (1910) for the diagnosis of old enteric infection. The organism is grown in the presence of the patient's serum and the organisms form very long chains and tend to settle to the bottom of the tube. The agglutinin reaction he found to be limited very closely to individual strains. The complement fixation test seemed rather more catholic.

Finally STONE (1923) showed that the patient's serum did definitely contain complement fixing bodies which reacted with the organism isolated. He has moreover shown that in individual cases from which no bacteria could be isolated a complement fixation test may occasionally be positive with an individual strain of streptococcus from another case. For this test to be of practical use it would seem necessary for the antigen to consist of a very large number of strains.

In the series of cases here discussed the agglutinin test has been carried out upon the serum of the patient in all cases but one. The organism used was always the one isolated from the patient's blood. Cross tests in a

small number of cases have been carried out but with constantly negative results.

The technique of the agglutination test in its application to the group of streptococci has met with the difficulty that the organism tends to be autoagglutinable and so unreliable as an antigen. Various devices have been suggested for overcoming this difficulty having as their basis the reduction of the hydrogen ion concentration or the salt content of the menstruum in which the test is performed. Cultures upon solid media have proved unsatisfactory in my hands. It has been possible however to obtain even suspensions of all streptococci isolated up to the present from these cases by growing them in broth made from veal infusion to which has been added 0.2 per cent of Disodium hydrogen phosphate and 0.1 per cent of glucose with 1 per cent of de Fresne's peptone. No sodium chloride is added. The reaction of the medium has been adjusted to that indicated by PH 7.6 to 7.8. In this medium profuse growth occurs from an inoculum of a loopful in 24 hours. After subculture for a week after isolation the growth becomes evenly distributed through the medium and, diluted to the required opacity, gives a stable suspension quite suitable for use in agglutination tests.

The test has been carried out macroscopically in the water bath at 55°C. The standard time adopted has been two hours at this temperature. It is rare to find the titre altered by longer incubation at this temperature. The results of the test applied in this way are quite as

clearly read as with *B. typhosus*.

Normal serum controls have been put up in each case and none has so far been observed to produce any agglutination in a dilution of 1 in 2 or in higher dilutions. The agglutination titre of the patient's serum is very variable. All examined have produced agglutination of the coccus from the blood stream in a dilution of 1 in 10. One case however did so in a dilution of 1 in 640.

Repetitions of the observation have been made in only two cases. In one case the second observation, made a month after the first, showed no substantial increase in the titre.

In another case the first examination revealed agglutination by the serum in a dilution of 1 in 10; about a month later there was no alteration in the titre. But at a third examination, two months afterwards, the agglutination was complete in serum diluted 1 in 32 and marked in a dilution of 1 in 64. The bacteraemia had in the meantime also increased.

The following table gives a resumé of the findings as regards agglutination.

TABLE XXIX.

Antibodies in serum of patients suffering from subacute infective endocarditis.

Patient.	Dilution of serum in which agglutination occurred.	Remarks.
Am	1 in 160 +++, 1 in 320+	Staphylococcus isolated near death.
Av	1 in 320	
Al	1 in 160	
Dud	1 in 20 ++, 1 in 40+	
Lat	Neg	
Pt	1 in 10	Opsonic index 1.2
Tr	28/10 1 in 10	
	6/12 1 in 10	
	6/2 1 in 64	
Wi	1 in 320	

One observation only has been made upon the opsonic action of the serum. In that case the opsonic index was 1.2.

The bactericidal action of the whole blood has been investigated in three of the patients. In the technique usually employed (COLEBROOK & STORER 1923) the blood of the patient is inoculated with a small quantity of laboratory culture and observations made as to the effect of this blood in preventing the growth of colonies. In the first place this method does not clearly distinguish between destruction of the bacteria and inhibition of their growth. Moreover it assumes that the behaviour of the blood towards a laboratory culture is identical with its behaviour towards the bacteria circulating in the blood. The observations upon this subject have been recorded at some length in discussing technique. The blood is prevented from clotting by addition of heparin (up to 0.1%) or sodium citrate (up to 0.2 per cent) and incubated in a test tube. Specimens are withdrawn and plated in agar at any desired time and the number of colonies developing is compared with the number which developed from the same amount of blood at the beginning of the experiment. From such experiments it has become clear that the organisms in the blood are almost all destroyed after 24 hours' incubation and this power of destroying the organisms is not due to the substances used to prevent clotting. A curious and interesting point arising in the course of these observations has been that there is practically no reduction in the number of organisms during

the first five hours' incubation. The explanation of this is not yet clear. It has not yet been possible to compare the action of the patient's blood upon a laboratory culture with that upon the organism present in the blood stream, nor to study the bactericidal action ^{in comparison} with that of the normal individual. Experiments of this kind await the arrival of a suitable case of the disease.

From these facts it may be concluded that the presence of the septicaemia is not due to failure on the part of the patient to produce antibodies. Moreover the blood of ~~at any rate~~ all the patients examined has proved capable of sterilizing itself when in the test tube so that there appears no valid evidence for assuming that the septicaemia is due to a failure of the defensive factors of the blood stream, if we may take what occurs in the test tube as representative of what occurs within the blood stream. On the contrary from the point of view of the antibodies in the serum the patient suffering from subacute infective endocarditis is in a better position than the normal individual for dealing with the organisms. He is however frequently at the disadvantage of suffering from a degree of leucopenia.

Specific Treatment.

Closely related to the question of the patient's specific resistance to the organism is the question of specific treatment.

The common experience of all those who have studied the disease is that the mortality from it is very high. Whether recovery has ever occurred is a matter for discussion. LIBMAN (1920) states that he has seen four recoveries in 300 cases. No particulars are given. He also has seen cases which have ceased to have a septicaemia but these have died soon after. Disappearance of the septicaemia is not necessarily indicative of cure of the disease. HASSENCAMP (1920) states that he has never seen a certain case of cure. MORAWITZ (1921) records a case of the bacteria free type which he thinks recovered but the evidence is not convincing. MURRAY (1922) states recoveries occurred in two of his cases and BOYD (1921) that he saw one recover. NICHOLL (1921) thinks one of his "bacteria free" cases recovered but the later history is not given. LAMPE (1922) saw one case which he thought was probably cured. It appears therefore a possibility, though a very rare occurrence, that natural recovery may follow the disease. KRIEGER & FRIEDENTHAL (1924) report that five of their cases left hospital "gebessert". It is not at all clear from their reports that the patients suffered from infective endocarditis.

Moreover there are occasionally seen at autopsy old calcified lesions of the valves which might very well be healed lesions resulting from this disease. GLYNN (1903)

draws attention to a group of such cases. Evidence of a conclusive nature has however not yet been produced.

Most clinicians have found treatment of no avail. LOREY (1912) claims to have cured a case with Arsacetin and Salvarsan. But again the diagnosis does not appear certain. GILMAN THOMPSON (1909) records a series of cases of endocarditis which recurred as a result of vaccine treatment. The same doubt attaches to the diagnosis as in Lorey's case. LATHAM & HUNT (1911) consider their case to have recovered as a result of the oral administration of a vaccine. That the septicaemia disappeared seems evident. The view that the treatment was responsible for this occurrence is based on the observation of agglutinins in the serum after the first dose of the vaccine. This is not convincing seeing that in so many cases of the disease the patient's serum has this effect without vaccine treatment. No observation is recorded of the condition of the serum before treatment.

HENSTED (1913) records in full a case where the infective process was present in a heart with congenital defects. Here the bacteriaemia disappeared and the patient completely recovered. The treatment employed was that of injections of serum prepared by immunizing a horse against the coccus isolated from the patient and of autogenous vaccine. The case would appear to be well authenticated.

WORDLEY (1924) reports distinct improvement in a bacteria free case as a result of repeated immuno-

transfusions.

HORDER (1904) found a serum prepared specially against the coccus in the patient's blood of no avail therapeutically.

BILLINGS (1909) tried the effect of whole blood transfusion but could find no improvement in the patient.

KINSELLA (1917) observed a temporary disappearance of the septicaemia following transfusion of whole blood but also noticed the same effect after injection of saline. The effect was not produced unless the injections caused a rise of temperature in the patient.

Considering the relatively high state of immunization of the majority of the patients who suffer from infective endocarditis it is not surprising that measures such as those outlined above should fail. Through the courtesy of Dr. Bolton, F.R.S., and of Professor T. R. Elliott, F.R.S., the opportunity has occurred of trying similar methods in three cases. Dr. R.A. O'Brien was good enough to prepare serum by immunizing horses against the strain of streptococcus obtained from the patients. Unfortunately one of the patients died before a sufficiently active serum was available. In the other two cases intravenous administration of small doses (10 c.c.) at intervals of three days was carried out by Dr. J. W. McNee and Dr. K. S. Hetzell until serious signs of hypersensitiveness caused cessation of the treatment. There were no signs of any benefit to the patient arising out of the treatment.

A fourth case was treated in a different way. The brother of the patient was inoculated at frequent intervals with a vaccine over a period of two months, the vaccine having been prepared from a culture of the organism obtained from the patient. The dose of vaccine ultimately employed was one thousand million cocci. At the end of this period the serum of the brother showed definite evidence of agglutination of the streptococci in a dilution of 1 in 10, normal sera having no effect. Mr. Hunter then transfused 500 c.c. of blood from the brother to the patient. There was no benefit observable. Three days after the operation the patient suffered from a cerebral embolus, the relationship of this to the operation being undetermined.

In each case the blood was examined to see if there was any effect produced by the treatment upon the bacteraemia. No significant effect could be observed as will be seen from the following table.

TABLE XXX.

Effect of specific treatment upon
the bacteriaemia.

Patient.	No. of bacteria per c.c. of blood		Remarks.
	before treatment	after treatment	
Am (serum)	9.11.23	27	1st dose serum 30.11.23 Urticaria followed dose on 9.12 Marked signs of ana- phylaxis after dose on 30.12.24 Treatment stopped.
	10.11.23	10	
		3.12.23 11	
		10.12.23 16	
		2. 1.24 97	
Tr (serum)		12. 2.24 70	Severe signs of ana- phylaxis on 26.11.24. Treatment stopped.
		19.3.24 118	
	21.10.24	45	
	28.10.24	42	
		26.11.24 41	
Al (Trans- fusion)		6.12.24 70	Transfusion done on 28.7.24
		6. 1.25 58	
		28. 1.25 134	
	17.5.24	17	
	28.5.24	34	
	12.6.24 40		
	25.7.24 370	30. 7.24 287	

The slight diminution in the number of organisms after the transfusion in case Al cannot with certainty be attributed to the treatment, inasmuch as it does not exceed the degree of variation that may be seen in untreated cases. An interesting observation upon the blood cells was made for me by Dr. G. W. Goodhart in this case. A blood count was done before transfusion and two others 24 hours, and 48 hours after, respectively. The results were as follows:-

	28.7.24 (before transfusion)	29.7.24 (24 hours after)	
Red corpuscles	3,500,000	4,040,000	3,200,000
Haemoglobin	50 per cent	58%	
Colour index	0.71	0.72	
White cells	5000	18200	10,200
Polymorphs	81% = 4050	93% = 16926	
Mononuclears	17% = 850	5% = 910	
Eosinophils	2% = 100	1% = 182	
Basiphils	Nil	1% = 182	

The increase in the polymorphonuclear leucocytes is very striking and can apparently only be explained as a reaction of the blood forming organs to the injection. And indeed it would appear to indicate that in these patients the bone marrow is capable of a very marked reaction if stimulated. Yet one generally finds a leucopenia in the blood stream and a very marked absence of leucocytes in the lesions on the valve cusps.

These results do not, of course, justify the conclusions that serum treatment is valueless. They do however indicate that the method of administration was faulty. The chief difficulty in serum treatment results from the very great serological individuality which characterises the streptococci. A stock antistreptococcal serum may not contain antibodies which will act upon a given strain that has been isolated. It is possible to ascertain this by an agglutination test. In doing so it is to be noted that normal horse serum may contain agglutinins for streptococci to a marked degree. In one case serum from a normal horse partially agglutinated one particular strain in a dilution

of 1 in 32. A sample of stock serum prepared against cocci from cases of endocarditis, which like the normal horse serum was very kindly placed at my disposal by Dr. O'Brien, agglutinated the coccus from the patient completely in a dilution of 1 in 64. After the course of immunization the titre was 1 in 640. So that we have in the agglutination test some sort of measure of the activity of the serum. Applying this in another case it was found that no significant rise in the titre of the horse's serum occurred until after a month of immunization. In view of the low degree of virulence of these streptococci for laboratory animals no test of the protective power of the serum is available.

Before, then, the failure of serum therapy can be asserted it is necessary to ascertain that the serum was really an efficient one. If stock serum is to be used it should have a high agglutination titre for the particular strain of streptococcus - one would suggest tentatively agglutination in a dilution of 1 in 100. If the serum is specially prepared and time is available, as it frequently is, similar evidence of activity should be sought, the best way of doing so being to compare the agglutination titre of the unimmunized animal's serum with that of specimens taken at various stages of the immunizing process.

Intravenous administration appears in our experience to be accompanied by definite risk arising from the development of hypersensitiveness after the tenth day, even if injections are given every three days. Consequently if this route be employed one would prefer to give larger doses

within a short period. It is proposed later, if possible, to try both this method and that of repeated subcutaneous injections.

Whatever method be employed the standard of cure must be a clinical one. Mere reduction in the number of bacteria in the blood stream or even their disappearance is not enough. Both of these results may apparently occur in untreated cases and the symptoms persist until the patient dies of the disease.

It has been suggested from time to time that tissue sensitization plays a part in determining the onset of streptococcal endocarditis. ROSENOW (1909) states that preliminary small doses of streptococci apparently sensitize guinea pigs and rabbits to a subsequent injection. Later (1912) he reported that guinea pigs inoculated with a dose of killed or living streptococci died immediately with signs of anaphylaxis on receiving a second injection a fortnight later.

HERRY (1914) reported that in his experiments preliminary injection of filtrates of cultures of streptococci rendered rabbits very susceptible to joint infection when they received a subsequent injection of living streptococci. FABER (1915) obtained similar results both when the preliminary injection was intravenous and when it was made locally into the periarticular tissues, the second injection being made into the vein of the ear. If for the preliminary injection an organism was used differing from that employed for the second, arthritis did not occur. He suggests that sensitization of tissue cells is a necessary preliminary to definite and marked tissue reaction.

SWIFT & BOOTS (1923) repeating these experiments were unable to find any evidence pointing to a specific sensitization of tissues. They considered any tendency for organisms to localize in a previously inoculated tissue might quite well be explained as a result of the trauma produced by the first injection.

FOX & LYNCH (1917) claim to have demonstrated the presence of anaphylatoxin as a result of treating streptococci with patient's serum and guinea pig's complement for 1 hour at 37°C., injection of such mixtures killing guinea pigs with symptoms like those of anaphylaxis. It is to be observed that a control in which normal serum was used had a similar effect. The evidence is not convincing. They consider anaphylaxis to play an important part in the pathogenesis of streptococcal endocarditis.

Observations to determine if there is any evidence of hypersensitiveness to streptococci in the patients suffering from streptococcal endocarditis have not apparently been recorded. MCKENDRICK (1923) has observed reactions in cases of typhoid fever following the intracutaneous injection of a small amount of formalinized cultures of *B. typhosus* which he interprets as indicating a degree of sensitiveness to the products of the organism which does not exist in normal individuals. In one case in this series a similar test has been applied. A broth culture of the organism isolated from the patient was grown for twenty four hours and then killed by addition of one drop of formalin and incubation for a further period of twenty four hours. This was diluted with saline till the bacterial content, as measured by opacity, was about 100 million organisms per cubic centimetre. Of this diluted culture 0.2 c.c. was inoculated into the patient intracutaneously. Control inoculations at the same level in the forearm were made with another strain of streptococcus similarly treated and with the nutrient

broth used as medium for the growth of the organism. The sites of inoculation were carefully watched for a fortnight for signs of reaction but no evidence of any was obtained. It may be noted that the observation was made some six weeks before the death of the patient.

There was therefore no evidence of any hypersensitivity in this patient to the products of the organism responsible for the infective condition in his heart valves.

Peculiarities in organisms isolated
from the blood of cases of infective
endocarditis.

ROSENOW (1909) observed certain peculiarities of growth in his primary cultures from the blood. The streptococci were clumped together and tended to stick to the surface of agar slopes. On subculture the properties disappeared but they could be restored by growing the organism in the presence of the patient's serum. He does not note whether the colonies were rough or smooth in appearance.

COWAN (1923 & 1924) reports the separation from a culture of haemolytic streptococci of two types of organism. One of these grew in rough colonies and the other in smooth, similar to those described by ARKWRIGHT (1921) in the case of organisms of the coli typhoid group. She noted that the rough colonies contained relatively avirulent organisms and the smooth more virulent and that they showed certain antigenic differences.

GRIFFITH (1923) obtained very similar results in the case of the pneumococcus which was modified in a similar way by growth in the presence of immune serum.

It is not possible to decide if the organisms described by Rosenow were really modifications of this kind. But as indicated elsewhere the appearance he describes is the sort of appearance which is well known to occur if organisms are grown in agglutinating serum or plasma. Similar observations have been made in the course of this work and could

apparently be explained quite well as an effect of agglutinating substances. But Rosenow also advances the idea that the organisms are "immunized against the antibodies in the blood". It has been pointed out above that destruction of these bacteria can occur in the patient's blood treated with heparin or with weak citrate solution. Observations upon the relative resistance of these organisms as compared with laboratory cultures have not yet been satisfactorily made. It has also been noted above that delay in growth is a very marked feature in the primary cultures from the blood. An experiment was undertaken to determine whether this delay also occurred in the case where a small number of organisms isolated previously from the same case and kept growing on laboratory media was added to broth containing blood from the patient.

Experiment 32.

The blood of patient Tr was drawn off and 18 c.c. added to 2 c.c. of heparin (1 per cent solution). Tubes of broth were inoculated each with 1 c.c. of the blood. Two of these were incubated at 37°C. Two others received each an inoculum of about 40,000 streptococci from a 24 hours' broth culture. This was obtained by adding 1 loopful of the broth culture to 7 c.c. of broth and inoculating 0.1 c.c. of this into the broth containing blood. Specimens from each tube were taken off at intervals and inoculated into blood agar plates, dilutions being made where necessary to permit of counts being made. Colonies were counted after 72 hours' incubation.

TABLE XXXI.

	No. of colonies developing from 0.5 c.c. of Broth + Blood.	Broth + Blood + inoculum of laboratory culture.
No incubation	13	2,000
6 hours	9	10,000
8½ hours	12	63,000
10½ hours	8	220,000
24 hours	2	90,000,000
48 hours	+++	

In the case of the blood only there is evidence of considerable delay before growth occurs. There is no evidence of increase in the number of colonies until after the lapse of 24 hours. Where however an inoculum of a laboratory culture is added to this, marked growth has already occurred in 6 hours. This would appear to indicate that the organism in the blood either has difficulty in commencing to grow because of some peculiarity, morbid or otherwise, in itself or because it is affected by some inhibitory factor in the blood which cannot influence the organism from the laboratory culture. It is in other words at a disadvantage from the point of view of growth as compared with the latter.

A second specimen of blood was centrifuged and the plasma removed as completely as possible. The removal was not complete however and the deposit was not washed with saline. The deposit was made up with broth to the original amount (10 c.c.) and of this 1 c.c. was inoculated into each of four tubes of broth. Two tubes were inoculated without further treatment and to two others an inoculum of laboratory culture was added as in the preceding experiment. The result was almost identical. The tubes receiving the additional bacteria

showed commencement of growth in 5 hours while the others showed no increase at all in 24 hours. In this case the effect of the fluids of the blood had been greatly diminished - but not entirely removed. This would suggest that the peculiar delay in growth is to be referred to the organism itself rather than the blood fluids.

When the delay is so marked as it was in this case there appears to be a diminution in the number of colonies appearing in the specimen taken after 24 hours' incubation. In one tube this actually went on to complete disappearance and no growth resulted. This would give an explanation of the occasional failure of an individual tube inoculated with infected blood to show any growth of the organism.

The above result might be explained as being due to the fact that the organism within the body is not adapted to growth on laboratory media whereas the laboratory culture is. In that case one would expect the organism within the blood to be adapted to grow either in the tissues or in the blood. So far no instance has been observed of any multiplication of these organisms in blood outside the body nor does there appear to have been reported any evidence in favour of this view.

Experimental production of endocarditis.

Evidence has been sought by a number of workers for an explanation of many of the phenomena of this disease by experiments upon animals. The great difficulty has always been to obtain a method of experimentation which would give constant and regular results.

POYNTON & PAYNE (1900) were apparently the first to produce endocarditis experimentally in the rabbit without having recourse to damaging the valves. They used large doses of a diplostreptococcus isolated at autopsy from a case of Rheumatic fever. In their work they refer frequently to the tendency for this organism to lose its power to produce lesions when subcultured, a fact which they attribute to diminution of virulence. Their view appears to be that this diplococcus is a particular species with a very marked tendency to attack the joints and the heart valves. BEATON & AINLEY WALKER (1903) failed to produce endocarditis with their strains though SHAW (1904) later succeeded in doing so. FRITZ MEYER (1902) worked with cultures of streptococci obtained from the throats of cases of acute rheumatism and produced endocarditis in 21 animals out of 100. BEATTIE (1904) succeeded in producing experimental endocarditis in some of his animals as also did BRACHT & WACHTER (1909). BEATTIE (1910) considered as a result of his experiments that the size of dose used had little effect on the result, large doses often failing to produce effects when smaller ones did. All these authors

considered they were dealing with the specific cause of acute rheumatism and that the lesions produced in the joints and heart valves were to be regarded as evidence that this particular coccus had a definite predilection for settling down at the sites named. BEATTIE & YATES (1912) elaborate this point still further in their communication upon the bacteria obtained from rheumatic lesions post mortem. The organisms from other diseases according to them tend to produce a septicaemia without the definite localization in the tissues.

COLE (1904) however studied the effect upon rabbits of the inoculation of streptococci obtained from various sources. His conclusion was that the organisms varied only in virulence, that is in the amount of a culture required to produce a given result. Description of a special type on the basis of its effect upon rabbits was, he thought, not justified. ROTHSCHILD & THALHIMER (1914) came to a similar conclusion. TOPLEY & WEIR (1912) made a similar study upon streptococci from various sources and found no evidence that organisms from a particular lesion tended to attack that tissue in the experimental animal.

ROSENOW (1909) observed that organisms freshly isolated from cases of streptococcal endocarditis produced endocarditis on inoculation into rabbits but subcultures failed to do so. He first correlated this with the peculiarities of growth observed in the primary cultures. Later (1912) he begins to consider this as due to a particular localizing tendency

which is present in the organisms in primary cultures. In 1913 he definitely enunciated the theory that this elective localization of the organisms is the determining factor in producing lesions in the experimental animal. He also states that the same property is to be observed in the cocci isolated from the tonsils in cases of rheumatic disorders. Further (1914) he shows in the case of a streptococcus isolated from a case of rheumatic fever that this elective affinity for tissues is soon lost in the ordinary subcultures. In a paper published in 1915 this idea is greatly extended. It is pointed out that the "tropic" tendency may be preserved for months by keeping suspensions of organisms in the presence of sterile tissue at low temperature. It tends however to disappear on subculture and on passage. Moreover organisms grown in extracts of organs are said to acquire a tendency to localize in those organs. Finally (1919) it is suggested that the preservation of this property is influenced by the oxygen tension of the medium in which it is grown. Accordingly media are employed containing glucose and animal fluids with a fragment of brain tissue at the bottom of the tube. The brain tissue is submitted to sterilizing in the medium at high pressure. It is then considered that in virtue of its reducing power it provides a varied series of tensions of oxygen at various levels in the fluid or semi-liquid medium thus providing the necessary conditions for the growth of the organism and retention of its peculiar properties.

These claims have been much elaborated more recently and applied to a really impressive list of diseases. On the

whole Rosenow's own reports do not appear to be convincing. The evidence upon which the claim is based seems somewhat slender. It is largely founded upon the evidence afforded by inspection of the body of the animal killed with chloroform two days after inoculation and the detection of minute haemorrhages which according to Rosenow are the precursors of a pathological process.

MOODY (1916) after experiments with streptococci obtained from chronic alveolar abscesses could find no difference in localization depending on the presence of systemic disease in the patient from whom the organism was obtained. He considers that the question of elective localization is an open one. DETWEILER & ROBINSON (1916) applying Rosenow's technique considered there was a definite tendency for streptococci isolated from cases of endocarditis to cause cardiac lesions. It is clear from their own experiments that there was a similar tendency on the part of streptococci from the teeth and tonsils of students in a good state of health. The doses of organisms used by these workers were enormous and just short of the lethal dose. Later DETWEILER & MAITLAND (1918) using the same technique found a curious tendency for certain strains to localize themselves constantly in certain organs but they could not convince themselves that the site of localization was in any way related to the source from which the organism was obtained.

DAVIS (1912) thought that his experiments upon the streptococci isolated from the deeper parts of the tonsils supported Rosenow's views, and BARGEN (1923) working with strains isolated from cases of infective endocarditis which

were grown in Rosenow's medium and, for inoculation, on blood agar, obtained a high percentage of endocarditis in his experimental animals.

That local damage may tend to favour the production of an infective process is well known. KINSELLA & SHERBURNE (1923) showed in experiments on dogs that preliminary damage of the valves followed by an inoculation of streptococci may give rise to lesions which, if the animal survives long enough, closely resemble those found in man.

WADSWORTH (1919) working with horses in the preparation of anti-pneumococcus serum sometimes observed a septicaemia to be present in an animal showing a high degree of immunity. In eight horses he noted the presence of endocarditis which from the description appears to have been very like that observed in man.

MAIR (1923) reported the development of endocarditis in seven out of 10 rabbits which had been immunized with killed cultures of pneumococci over a period of 12 weeks. They had then been allowed to rest for 2 months and inoculated weekly with increasing doses of living organisms. Some had a septicaemia and at the same time the serum showed a well marked agglutinating effect upon the organism. In one case the protective power of the serum was high for mice. The organism isolated from the blood in this case was much less virulent than the one used for the inoculations. Animals not showing endocarditis had lost a good deal of weight.

COWAN (1924) working with haemolytic streptococci

which usually produced death from acute septicaemia noted that some immunized animals survived a dose which was lethal for normal animals and in these arthritic and endocardial lesions were found frequently. The finding is attributed to the fact that these lesions occur when the balance between the virulence of the organism and the resistance of the animal is slightly upset to the disadvantage of the animal.

Experiments with streptococci.

The literature quoted above clearly shows that endocarditis can be produced by the inoculation of very large doses. The method most commonly used has been to grow the streptococcus upon agar or blood agar and inoculate the growth from several slopes into the ear vein. In the majority of the experiments here recorded the inoculum has consisted of 5 c.c. of a broth culture, 24 hours old, the bacteria being evenly distributed throughout the medium.

The strains used were, in the main, those which had been isolated from cases of streptococcal endocarditis. One strain however was placed at my disposal by Dr. F.J. Poynton having been obtained from the pericardial fluid of a case of acute rheumatism. It apparently belonged to the streptococcus faecalis group, being non-haemolytic and fermenting lactose mannite and salicin, but not raffinose or inulin. It grew very luxuriantly in all media.

In only one case did endocarditis develop in a rabbit which had received only one injection of streptococci. The details of the experiment were as follows:-

Rabbit 79. Weight 1700 gms.

21/1/24. 5 c.c.² of culture of strep. Poynton inoculated into the ear vein. The animal rapidly lost weight until on 25/1/24 it weighed only 1020 gms. It died on 28/1/24 and at the autopsy there was found very extensive endocarditis of the mitral valve, both cusps being extensively involved with fibrinous vegetations. There were no signs of

infarction or emboli in the organs and no abscess formation. The joints were not involved.

This result is of interest in that it has enabled a definite statement to be made as to the time taken for extensive involvement of the rabbit's valves to occur. Within seven days the lesion may become so large as to cause almost complete obstruction of the blood flow.

It should be stated here that only those lesions are considered as being endocarditis experimentally produced which are unmistakably the result of the injection. TOPLEY & WEIR (1921) have drawn attention to the small roughenings sometimes found on the valves of rabbit's hearts even after injections of such substances as red blood corpuscles. The vegetation experimentally produced is large and extensive and in sections the organisms are clearly seen.

In view of Rosenow's experiences it was thought advisable to make inoculations of streptococci as soon after isolation from the patient as possible.

The blood culture from patient Mo as soon as it had grown sufficiently (after 48 hours' incubation) was used as inoculum. Of this 5 c.c. were inoculated intravenously into rabbit No. 59 which weighed 1950 gms. The animal was observed for ten weeks during which time it increased in weight and at the end of that time was killed. There was no lesion to be found in any part.

Rabbit No. 61 had developed endocarditis as a result of repeated inoculations with a streptococcus from patient Am. This animal died on 3/3/24. From the heart blood a pure culture of streptococci was obtained after 48 hours' growth. Of this primary culture 5 c.c. were inoculated into two rabbits intravenously, No. 89 (weight 2000 grammes) and No. 90 (weight 1600 grammes). When killed at the end of a period of five weeks no lesions of any kind could be found.

Rabbit No. 81 had also developed endocarditis after inoculation with streptococcus Poynton on three occasions. It died on 4/3/24 and a pure culture of the organism was recovered from the heart blood. This had grown on 5/3/24 and 5 c.c. of the primary culture ^{were} inoculated intravenously into rabbit No. 91 (1800 grammes) and No. 92 (1850 grammes). Both animals lost some 300 gms. in weight during the next 6 weeks but at autopsy on 14/5/24 no lesions were found. Blood cultures were made from each rabbit (blood from the ear vein) on several occasions. Rabbit No. 91 gave a

positive culture on 10/3/24, five days after the inoculation, but all subsequent specimens were sterile. All specimens from rabbit No. 92 were sterile.

There was then no evidence in these experiments of any extraordinary tendency on the part of the streptococci freshly isolated from a human being or a rabbit suffering from endocarditis to produce endocarditis on inoculation into rabbits. Neither was there any evidence of increase of virulence on passage.

It seemed possible that although single injections of cultures of streptococci had failed in most instances to produce the experimental condition desired repeated doses might do so.

Two rabbits, No. 60 (1970 grammes) and No. 61 (1850 grammes) received intravenously 5 c.c. of a broth culture of a streptococcus isolated from patient Am on December 7th 1923 and each week/thereafter until February 3rd 1924. On this date rabbit No. 60 weighed 1700 grammes (a loss of 270 grammes) and No. 61 weighed 1570 grammes (a loss of 280 grammes). On 11/2/24, eight days after the last injection specimens of serum were obtained and tested for agglutinins. The titres of the sera were respectively

Rabbit No. 60 1 in 5120 +++

Rabbit No. 61 1 in 2560 +++ 1 in 5120 +

On 17/2/24 a further dose of 5 c.c. of broth culture was inoculated into each rabbit. The weight of both rabbits had risen slightly in the meanwhile but after injection while No. 60 steadily improved No. 61 gradually declined and died on

3/3/24. At the post mortem examination there was found well marked aortic endocarditis with congestion of the spleen and congestion and fatty degeneration of the liver. There were streptococci in the heart blood and these were obtained in pure culture and definitely identified as identical with the organism inoculated.

The interesting point here is the development of a fatal disease caused by an organism against which the animal, to judge by serological standards, was in a highly immune condition. It is not possible to be quite certain as to when the disease actually began in the valve but in all probability it resulted from the last injection. Up to that date there is little to distinguish between the charts of the two rabbits, (Chart V.). Both show that the preliminary injections although not causing any apparent disease process had had some effect upon the general health of the animals as evidenced by some loss of weight. They would both appear to be well qualified to deal with bacteria present in the general circulation. What determined the localization of these bacteria in the valve of one animal and not in the other is not clear. There was no evidence of general hypersensitiveness to the bacteria at the time of the last injection, no sign in fact of anaphylactic shock. The possibility of local hypersensitiveness cannot be excluded nor can it be determined whether the preliminary injections in one case produced slight local trauma and not in the other. Such a trauma would give rise to a locus minoris resistentiae upon which the cocci could settle and set up the lesion.

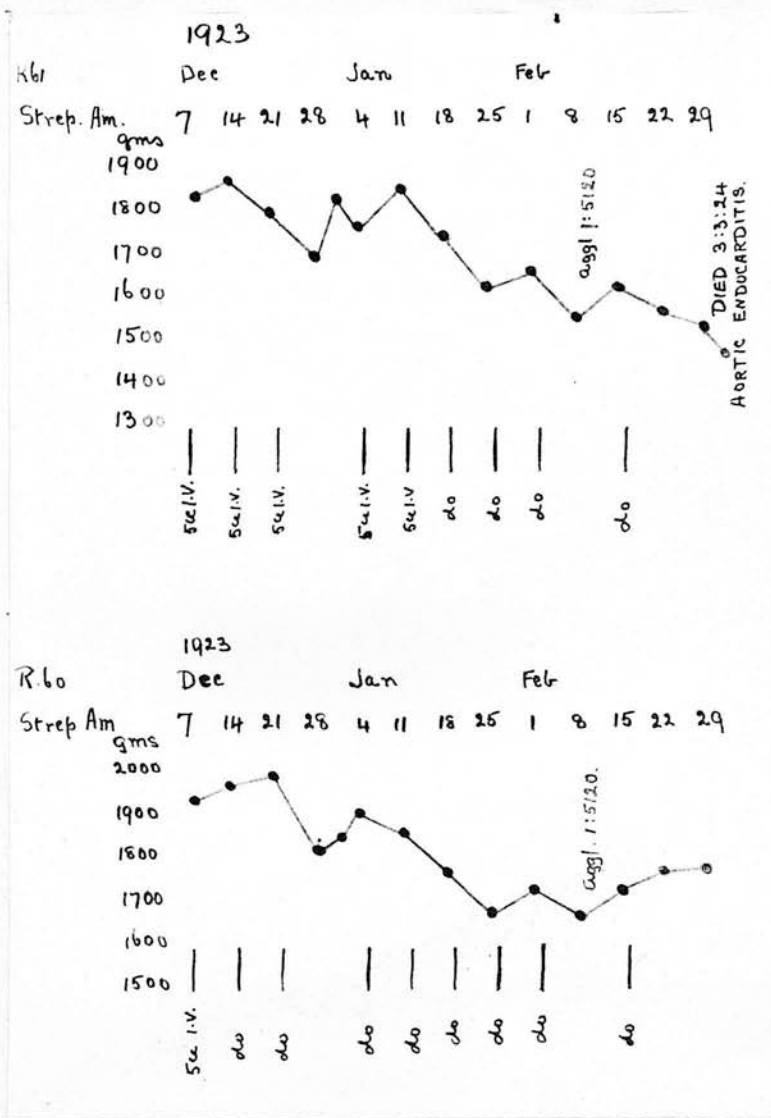


Chart V.

Curves of weight of rabbits Nos. 60 & 61, showing details of injections of streptococci made into the ear veins.

A somewhat similar result was obtained in the case of rabbit No. 81 (weight 1910 grammes). Two injections of 5 c.c. of a 24 hour broth culture on 28/1/24 and 13/2/24 had no effect. A third injection on 19/2/24 was followed at first by a slight rise in weight succeeded by a fall. Serum collected on 27/2/24 agglutinated the culture used in a dilution of 1 in 5120. Yet on 4/3/24 the animal died with massive mitral and tricuspid vegetations and evidence of heart failure. The streptococcus was cultivated from the heart blood in a pure condition.

Rabbit No. 130 (weight 2400 grammes) was inoculated with a series of injections of streptococci from patient A1. The injections consisted of 5 c.c. of an aerobic broth culture intravenously inoculated and were given as indicated in Chart VI. The weight dropped rapidly at first and later more steadily. From the beginning the blood was examined regularly for evidence of bacteraemia. A chart of the findings ^{is appended,} (Chart VII.). It will be seen that the temporary bacteraemia disappears in the course of the first few hours after inoculation but, contrary to the common experience with animals inoculated with these streptococci of low virulence, there is a return of the bacteraemia which does not finally disappear until 27/6/24. Then following renewed injections the bacteraemia returns and gradually, quite slowly, yet progressively increases until the death of the animal on 18/7/24. At the post mortem examination of this animal there were found a marked degree of arthritis in the left knee and an extensive endocarditis involving both cusps of

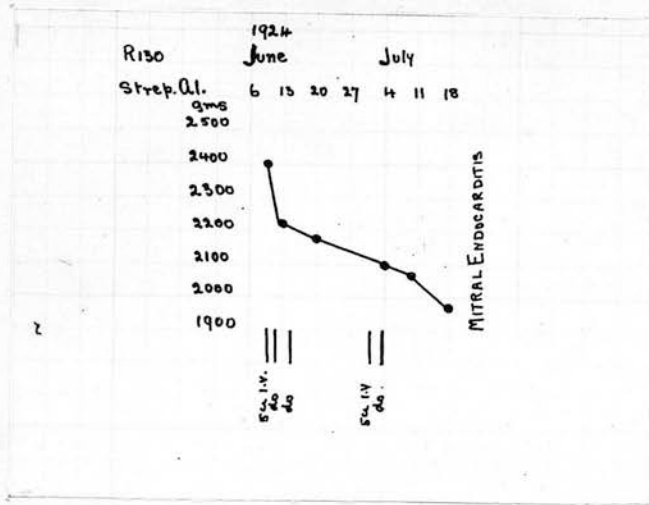


Chart VI.

Showing weight curve and course of injections of streptococci in the case of rabbit No. 130.

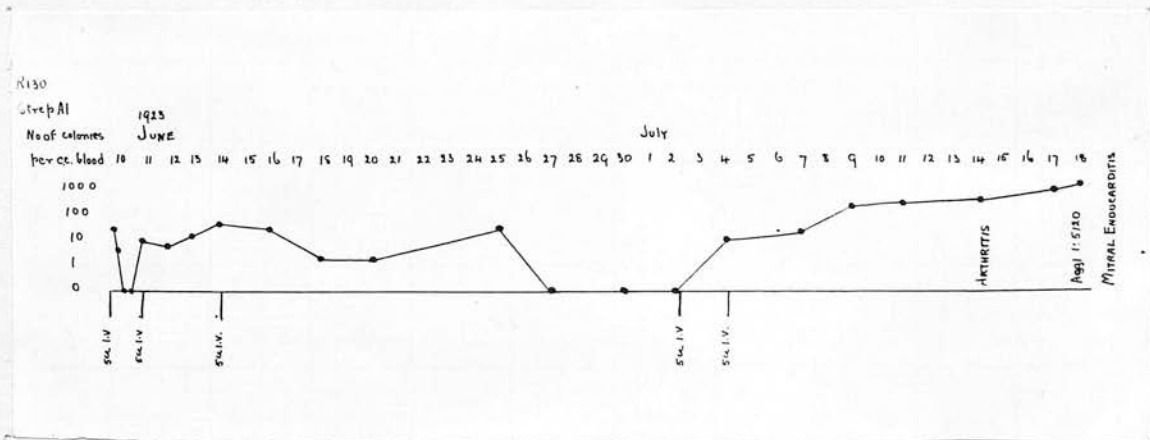


Chart VII.

Representing the course of the bacteriemia in the same rabbit.

the mitral valve. Smaller vegetations were found also on the ventricular endocardium on the posterior wall of the ventricle exactly opposite the mitral valve cusp. The serum collected after death of the animal agglutinated the streptococcus used for the inoculations in a dilution of 1 in 5000. Here we have apparently clearer evidence of a certain amount of damage done by the preliminary injections. It is difficult to understand the survival of the organisms in the blood of the rabbit for so long a period as in this case unless there had been some local damage done wherein the organism could multiply and from which it could be discharged into the blood. We have again the paradox of a progressive and fatal septicaemia with a high agglutinating activity manifested by the serum even after death of the animal. It was on this animal that the observations on delay in growth in blood culture and destructive action of the blood upon the streptococci within it were made. The condition of the organism and its destructibility by the animal's blood appeared to be exactly similar to the same phenomena in the human patient.

In these three cases we have certain resemblances to the process as we see it in man. The method of producing the disease by repeated injections at intervals resembles the intermittent discharge of organisms from a focus of infection into the blood stream. For a long time it goes on without producing any recognizable disease focus. It may even produce a certain degree of general immunity. But finally comes the combination of circumstances favouring

localization at a given site and the development of an infective endocarditis. Usually there has been a certain amount of effect on general health, not enough in itself to constitute a disease process but possibly of importance. It is these determining factors which for the moment lack definition.

The first point relating to them is that the occurrence of endocarditis in a series of animals treated as nearly as possible in identical fashion is quite unpredictable. Rabbits Nos. 60 and 61 illustrate this point. It is also true in the case of rabbit No. 130 which was one of four receiving almost identical treatment. Anaerobic cultures were no more certain to produce the disease than were aerobic. Injections every two days had no better results than injections every week. There seemed to be some factor connected with the particular rabbit which determined what should happen. The impression received is that it is more likely to be due to a traumatic factor than such a thing as hypersensitiveness, which might be expected to be produced more regularly.

Bacterial anaphylaxis is a subject upon which definite conclusions have been difficult to reach. In one case of this series, rabbit No. 80, weekly injections of streptococci from case Wi were made intravenously as follows:-

20/1/24	1 c.c. broth culture.
27/1/24	2.5 c.c. do.
3/2/24	5 c.c. do.

Up to this point the procedure was uneventful and no signs followed the inoculations.

10/2/24	5 c.c. broth culture.	The injection was
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interrupted after about 4 c.c. had been given. After two or three minutes' delay the remaining 1 c.c. was introduced. Almost before the injection had been completed the animal began to struggle. Breathing became slow and very laboured, the nostrils dilating and moving with every inspiration. The animal uttered a short sharp cry. When placed on the floor it lay on its side and almost stopped breathing. Gradually the respirations increased in depth and frequency and recovery became complete. The whole group of phenomena had occurred in less than ten minutes.

17/2/24 5 c.c. broth culture. No immediate symptoms. Animal found dead next day. At the post mortem were found chronic inflammatory lesions of the appendix, mesenteric glands and spleen and from spleen and glands was isolated an organism of the pasteurilla group.

The symptoms following the fourth injection were extraordinarily suggestive of an anaphylactic process but it cannot in any definite fashion be asserted that this was the cause.

Whatever be the localizing factor in these cases, despite the fact that there are certain resemblances between the rabbit disease and that in the human being yet it must be remembered that the process in the rabbit is usually of short duration (7 to 17 days) and in the lesion there is no evidence of organization of the vegetation. It is not really comparable with the subacute infective process in man so much as with the acute form of the disease.

Experiments with Pneumococci.

In view of Mair's extraordinarily high percentage of cases of endocarditis in his animals it seemed likely that this might provide a certain means of producing the disease at will and so provide material for the study of the disease in its early stages. (MAIR 1923). Accordingly the following series of experiments was undertaken.

In the first place there was a large number of animals which at various times each received a single injection of broth culture of pneumococci for the purpose of testing the virulence of strains isolated. All the organisms used were bile soluble and were pathogenic to mice - some to a very extraordinary degree. Yet it was observed on many occasions that a culture which killed mice in very low dilutions on inoculation intraperitoneally might be entirely without effect when inoculated intraperitoneally or even intravenously into rabbits. Passage through mice sometimes resulted in steady increaseⁱⁿ virulence but this was rather the exception than the rule. Similarly passage through rabbits was not by any means invariably followed by increase in virulence. The strains were all isolated from cases of disease in man and only one out of 14 strains was at all regularly lethal for rabbits in reasonably small doses. COTONI, TRUCHE & RAPHAEL (1922) have commented on similar findings in their studies of the pneumococcus. Moreover the individuality of the experimental animal used has proved to play a very important part in the results of virulence tests. This is of course much more marked where the dose of organisms

inoculated has been something approximating to the minimal lethal amount. One marked instance of this is recorded.

Pneumococcus strain X. was isolated from the pus from an empyema following pneumonia. It was isolated upon blood agar and grown thereafter in blood broth. (This medium was described by GRIFFITH (1923) and consists of equal parts of glucose free broth and rabbit's blood. It has been found to maintain the virulence of the pneumococcus if subcultured each week for at any rate seven months without animal passage). For inoculation a few drops of blood broth culture were transferred to broth and incubated for 18 hours. On 6/12/22 1 c.c. of such a culture was inoculated into rabbit No. 58 (1950 grammes).

On 11/12/23 the animal died with well marked endocarditis of the aortic valve and a small lesion on the pulmonary valve. A pure culture of pneumococci was obtained from the heart blood, and on 13/12/23 1 c.c. of a 24 hour broth culture inoculated intravenously into rabbit No. 64 (1510 grammes) without producing any effect.

Recourse was also had to the stock culture which was subcultured upon broth and after twenty four hours' growth used for inoculating a series of six rabbits as follows:-

TABLE XXXII.

Date 14/12/23. Intravenous injections Pneumo X.

Rabbit No.	Weight.	Dose.	Result.
65	1490	1 c.c.	Nil.
66	2640	1 c.c.	Nil.
67	1270	1 c.c.	Nil.
68	1670	0.5 c.c.	Nil.
69	2550	0.5 c.c.	Septicaemia.
70	2260	0.5 c.c.	Nil.

Only one rabbit out of six succumbed to the inoculation and that a heavy rabbit and to the smaller dose.

The strain isolated from this rabbit was used to inoculate still another one - No. 71 (2070 grammes) but without effect.

The experiments with inoculations of one dose only of pneumococci were carried out with a great variety of strains, varying dosage and different methods of inoculation, subcutaneous, intramuscular, intraperitoneal and intravenous. It would serve no useful purpose to record the details in each case. Suffice it to say that the results obtained were as indicated in Table XXXIII.

TABLE XXXIII.

Total number of animals receiving one dose of pneumococci	= 62
Number which developed endocarditis	= 3
" " " arthritis	= 0
" " " peritonitis	= 7
" " " septicaemia (acute)	= 14
" " " no pneumococcal lesion	= 34
" " " local abscesses	= 4

The protocols of the animals which developed endocarditis are as follows:-

Rabbit No. 1. Received an intraperitoneal inoculation of 5 c.c. of a 24 hour broth culture of pneumococcus strain I. on 5/9/23. On 10/9/24 the animal was killed as it appeared ill and there was found a well marked mitral endocarditis. The pneumococcus was recovered from the heart blood.

Rabbit No. 58. Weight 1950 grammes.

On 6/12/23 was inoculated intravenously with 1 c.c. of strain X. On 10/12/23 showed marked spasms of body and limbs with slight tendency for these to be unilateral. On 11/12/23 the spasms had disappeared but the animal died in the evening. There was found endocarditis of the mitral (marked) and pulmonary (slight) valves only with a small abscess in the septum between the ventricles. There were also slight pericarditis and marked pulmonary oedema. There were multiple small abscesses in the liver but the kidneys were unaffected.

Rabbit No. 143. Weight 2350 grammes.

On 13/6/24 was inoculated intravenously with 1 c.c. of a broth culture (24 hours) of strain XIV. On 16/6/24 the animal suffered from severe diarrhoea and died on the night of 18/6/24. Observations upon the blood drawn from the ear showed that there was on each day a number of pneumococci present which varied between 5000 and 9000 per c.c. of blood.

There were found at autopsy severe peritonitis, pleural and pericardial effusions, small abscesses in the liver and a slight degree of mitral endocarditis.

There was also a small abscess in the

septum between the ventricles near the attachment of the tricuspid valve.

The lungs contained areas of haemorrhage.

These three cases show that endocarditis may be produced in this way but the findings suggest that the lesion is, as it were, an incident in the course of a pneumococcal septicaemia.

Most of the deaths from septicaemia, that is bacteraemia with no definite macroscopic local focus, and from peritonitis (which was in all cases accompanied by a bacteraemia) occurred in animals inoculated with strain XIV. Eight animals inoculated with this organism died of acute septicaemia and five with peritonitis. This strain was by far the most virulent used. A number of animals died in the course of 24 to 48 hours with general septicaemia. If however the rabbits survived from four to six days peritonitis was a very common finding. The few who survived longer, animals which had received smaller doses, showed in three cases severe ophthalmitis, in five others no lesion at all. It would appear then that pneumococcal peritonitis may be induced by intravenous injections in rabbits if the balance between virulence or dose of organisms and the animal's resistance is hit off with exactness. There has been in all these cases a septicaemia concurrently, but of a minor degree. The organisms were not visible in films but were present to the extent of 8000 to 10,000 per c.c., a number of the order which as will be seen later is found in cases of well established endocarditis. It is suggested

that the bacteriaemia in these cases may be derived in part from the lesion in the peritoneum. Where the septicaemia is, if the term may be permitted, primary, the organisms are frequently visible in films and may number hundreds of thousands per cubic centimetre of blood.

The next group of experiments refers to animals which received a series of injections of living pneumococci the doses being carefully graded and spread out over a long period.

The strains used were II. and III., the former having been isolated from an empyema, the latter from a case of mastoiditis and meningitis. Strain III. grew in the manner usually associated with cultures of pneumococcus mucosus. Both strains were soluble in bile and were actively pathogenic for mice.

The inoculum used was obtained from cultures in broth, the strain being subcultured every day. This method of cultivation led to marked loss of virulence but it was thought that such a change might help in production of local lesions without permitting the development of an ordinary septicaemia.

The preliminary doses were small intraperitoneal inoculations, usually 0.5 c.c. of 24 hours broth culture. This was followed in most cases by a sharp drop in the weight of the animal. When this had been rectified a further dose of 1 c.c. was given intraperitoneally. The

next week 0.5 c.c. of 24 hour broth culture was injected intravenously. In the following week doses of this amount were injected intravenously on each of three successive days. This dosage was continued for three weeks and then a week allowed to elapse before a further inoculation was made. Inoculation was then recommenced, 1 c.c. being injected intravenously on each of three successive days for a further period of three weeks. Then the dose was increased to 1.6 c.c., 3 c.c. and 5 c.c. in the succeeding three weeks. The weight of the animals was recorded weekly on a chart. If the animal showed very marked and progressive loss of weight the inoculations were stopped as this sign has been found to be a very good indication of the development of some focus of infection, though frequently due to the commencement of some natural disease. Slight variations occurred in the matter of the preliminary doses but these were unimportant. The chart of rabbit No. 29 is given as indicating the course of injections and weight curve in an animal which died at the end of the period from infection with an organism of the pasteurella group. (Chart VIII.)

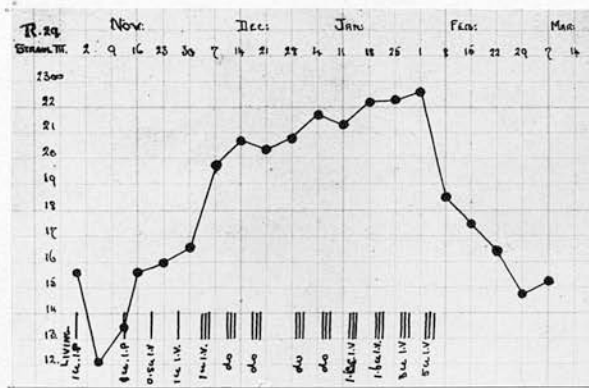


Chart VIII.

Curve of weight and details of injections of pneumococci in the case of rabbit No. 29.

Nine animals altogether were treated in this way, five with strain III. and four with strain II. The salient points in the case of each animal are recorded in Table XXXIV.

TABLE XXXIV.

Rabbit No.	Strain	1st injection.	last injection	No. of injections.	Result.
21	II.	29--9-23	16--1-24	21	Nil - stopped owing to loss of weight.
23	II.	17-10-23	16--1-24	20	Nil. do.
27	II.	30-10-23	6-2-24	30	Nil.
28	II.	30-10-23	6-2-24	28	Nil.
20	III.	26--9-23	6--2-24	28	Nil.
22	III.	17-10-23	5--2-24	27	Died within five minutes of receiving second injection of last series.
29	III.	30;10;23	6--2-24	30	Killed 10/3/24 Marked chronic pasteurella infection.
30	III.	30-10-23	6--2-24	30	Nil.
31	III.	30-10-23	6--2-24	30	Nil.

This experiment then ended in complete failure, none of the animals developing endocarditis.

The very sudden death of rabbit No. 22 was noteworthy. There was sudden collapse and difficulty of breath with death within five minutes. It was suggestive of anaphylactic death. The lungs were not distended. There were multiple small abscesses in the liver and the spleen was slightly enlarged. The serum of the animal, collected after death, produced precipitation of autolysed pneumococci in a dilution of 1 in 10 and agglutination of a fresh culture in a dilution of 1 in 5. It cannot definitely be determined whether true hypersusceptibility played any part in producing the effect.

The survivors of this experiment were kept for use in subsequent experiments described below.

The next group of animals were first immunized with killed cultures and later submitted to inoculation with living organisms.

The inoculum used was similar to that described by MAIR (1923). Broth cultures of pneumococci were grown for 24 hours at 37°C. These were then left in the incubator for a further 24 hours by which time the culture had become almost clear. They were then placed in the water bath at 45°C. for a further 24 hours by which time the autolysis was complete and the fluid sterile.

Each rabbit used was inoculated intravenously with 1 c.c. of this autolysate and a similar amount one week later. Thereafter in each week the animals received three injections on each of three consecutive days, the dose at first being 1 c.c. but later it was increased to 2 c.c. and at last to 5 c.c. Weight charts were prepared and the attempt made to graduate the dose so that loss of weight was avoided. The earlier doses frequently had a very marked effect on the weight of the animal.

The chart of rabbit No. 40 which died at the end of the period of immunization serves to indicate the dosage employed. (Chart IX.)

weight charts showed that the inoculations were not without ill effect upon the animal inoculated. Nos. 54, 55 and 40 seem to have died as a result of the inoculations, no other case of death being found. In the case of rabbit No. 55 the dosage was too great.

The response to the inoculations with regard to antibody production was strikingly small, particularly the animals inoculated with strain II., only two of which gave evidence of even a moderate amount of antibodies in the serum. The animals inoculated with strain III. were more active in this respect.

At this stage we therefore had available seven rabbits immunized against strain II. and four immunized against strain III. Of the former group four animals, Nos. 43, 44, 47 and 49, and of the latter, two, Nos. 33 and 34, were selected for the next stage of the experiment.

A period of three weeks was allowed to elapse from the date of the last injection. Each rabbit then received an intravenous injection of 1 c.c. of a living broth culture of the appropriate strain.

This was followed, one week later, by a series of three similar injections on three successive days. The following week three doses of 2 c.c. each were given; then in the next week the dose was 3 c.c. and in some cases a further series of injections of 5 c.c. was made one week later.

The chart of rabbit No. 34 illustrates the dosage employed (Chart X.). In the case of this animal injections were discontinued on 6/3/24. A specimen of blood from the

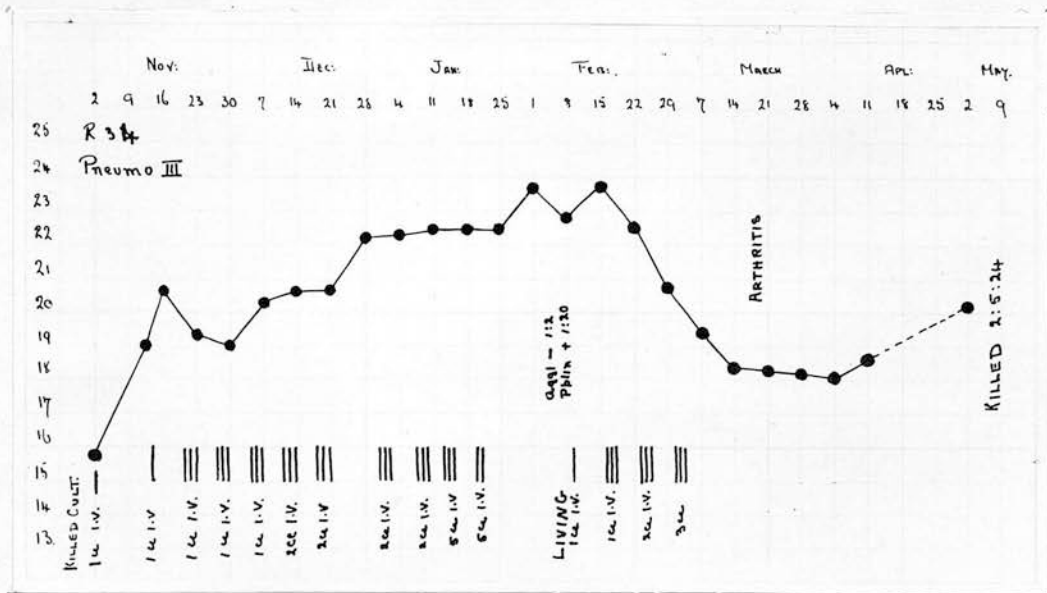


Chart X.

Weight curve (in hundreds of grammes) and course of injections of rabbit No. 34.

ear vein on 10/3/24 was found to contain pneumococci. The blood was frequently examined thereafter with the following results:-

10-3-24	Blood culture -
11-3-24	2 colonies per c.c.
13-3-24	Blood culture -
15-3-24	do. negative.

On 17-3-24 it was noticed that the left knee joint was swollen.

27-3-24	Blood	21 colonies per c.c.
28-3-24	do.	4 do.
31-3-24	do.	9 do.
1-4-24	do.	4 do.
3-4-24	do.	7 do.
5-4-24	do.	4 do.
7-4-24	do.	19 do.
9-4-24	do.	22 do.
11-4-24	do.	12 do.

16-4; 29-4; 1-5 Blood sterile.

The persistence of the septicaemia had at first suggested that there was probably endocarditis but its subsequent disappearance negatived this view. At the autopsy on 2/5/24 the only lesion found was in the knee joint which contained a slight excess of sterile clear fluid. Around the joint there was marked thickening of the tissues with apparently overgrowth of bone. The joint was not disorganised. There was no endocarditis. A septicaemia of this type is in my experience rare in rabbits suffering from arthritis only. The organism isolated was virulent for mice.

Rabbit No. 33 showed slight decrease in weight after the 3 c.c. injections. The inoculations were then stopped and the animal slowly recovered. The blood was sterile on 10/3/24 and 27/3/24, the last injection having been made on 6/3/24.

Rabbit No. 49 showed a very marked loss of weight after the series of injections of 3 c.c. ending on 6/3/24. He subsequently recovered completely.

None of the others were adversely affected in any way and the experiment was stopped on 14/3/24.

As a result of this procedure of preliminary immunization with autolysed cultures and, after a rest of three weeks, subsequent inoculation with graded doses no animal developed endocarditis and only one developed arthritis.

Some observations upon the rate of disappearance of pneumococci from the blood of rabbits following intravenous inoculation had shown that in an animal which had just eliminated one injection its power to deal with a second one was much enhanced while there were no antibodies demonstrable in the serum. If however it was allowed to remain uninoculated for a long period the power to eliminate inoculated organisms became less marked. These experiments have not yet been completed but they led to the experiment now recorded. A series of five animals was taken.

Rabbit No. 82 was a fresh animal, having received no previous inoculation of any organism.

Rabbit No. 98 had been inoculated as follows:-

13-3-24	5 c.c. of strain II. intravenously.
20-3-24	5 c.c. strain II. intravenously.

Rabbit No. 23 received between 29-9-23 and 16-1-24 twenty injections of strain II. intravenously.

Rabbit No. 45 received between 2-11-23 and 22-1-24 twenty eight injections of killed culture of strain II. intravenously. On 20-3-24 it received 5 c.c. of living culture intravenously without effect .

Rabbit No. 48 had been treated with killed cultures in the same way as rabbit No. 45, but had received no subsequent inoculation of living organisms.

Each animal received an intravenous injection of 5 c.c. of 24 hour broth culture of strain II. on each of the days 24th, 25th and 26th of March 1924.

The result is given in brief in Table XXXVI.

TABLE XXXVI.

Rabbit No.	Previous inoculation	Time since last inoculation	Agglutinin	Result.
82	Nil	-	-	Died in 24 hrs. after 2nd dose.
98	2 living	4 days	+ 1 in 32	Nil.
23	20 living	66 days	Nil 1 in 2	Endocarditis.
45	28 killed	6 days	+ 1 in 2	Endocarditis.
48	1 living 28 killed	72 days	- 1 in 2	Arthritis.

Of this series of five animals one, the fresh animal, died of acute septicaemia; one, recently immunized with living cultures and highly immune, developed no lesion, the other three possessing little evidence of immunity from the point of view of antibodies developed local lesions, one in the joints and two in the heart valves.

Agglutination is of course only an indirect method of estimating the capacity of an animal to deal with an organism. Accordingly specimens of blood were drawn off from the ear vein immediately before the second and third injections were made, ^{and} the number of organisms present in each cubic centimetre estimated by plating. This procedure was repeated at frequent intervals thereafter.

Rabbit No. 82 on the morning after the first inoculation had a septicaemia of 20,000 per c.c. The next morning it was dead.

Rabbit No. 98 showed no organisms in its blood at any time.

The figures observed in the case of the other three animals are recorded on the accompanying chart. (Chart XI)

The details of inoculations received by rabbits No. 23 and 45 are also given on a separate chart. (Chart XII.)

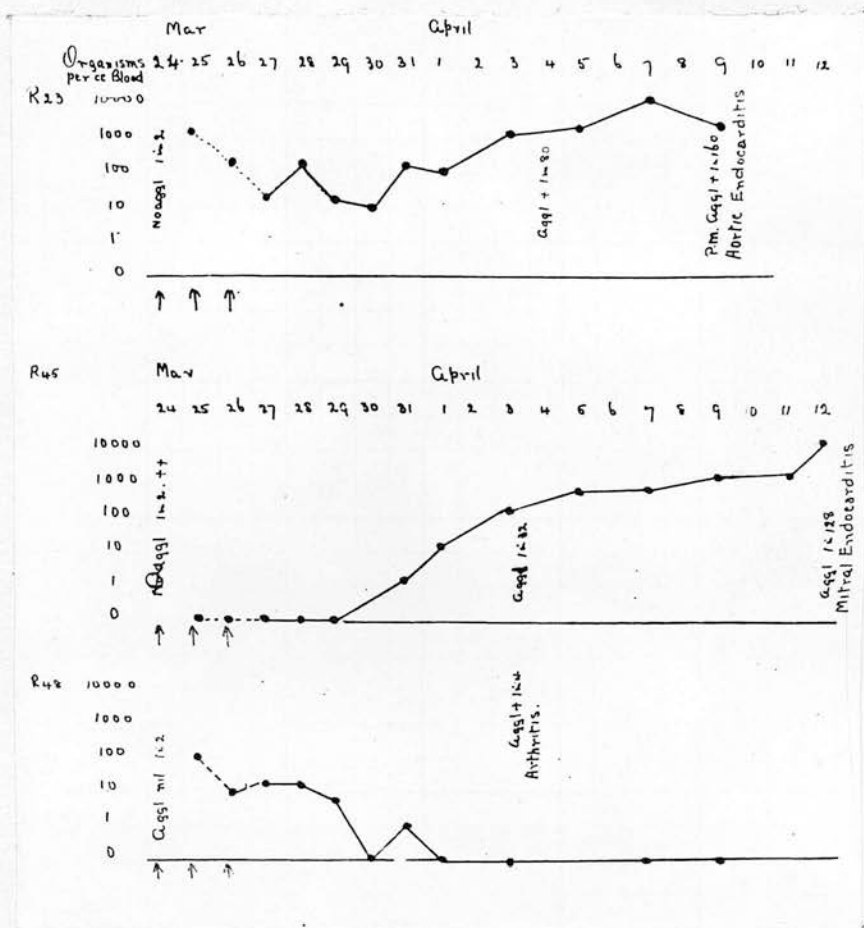


Chart XI.

Showing course of bacteriaemia in rabbits Nos. 23 (upper), 45 (middle) and 48 (lower).

The arrows indicate the points at which injections of 5 c.c. of pneumococcus culture were made into the ear veins. Cultures were made on these days before the injection was made.

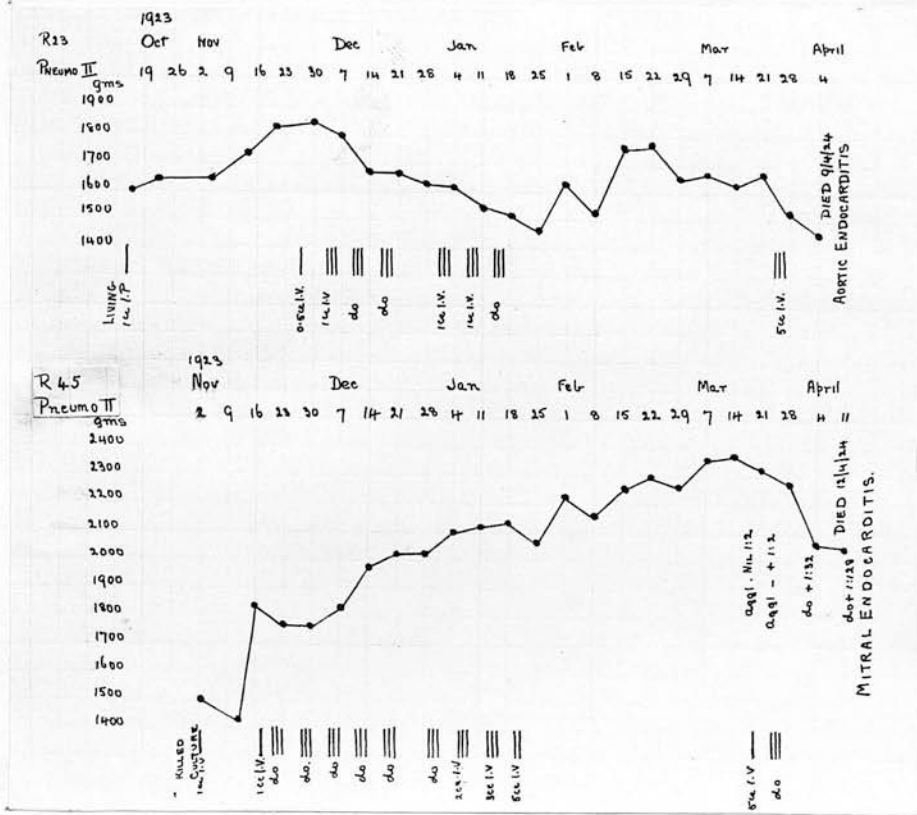


Chart XII.

Weight curves and course of injections of rabbits Nos. 23 (upper) and 45 (lower).

From chart XI. it will be seen that the animals differed in their capacity to deal with the injections. Rabbit No. 45 whose serum showed slight agglutinating action upon the pneumococcus had no organisms in its blood during the first five days. Rabbit No. 48 became free of bacteriaemia on the sixth day. Rabbit No. 23 had a septicaemia throughout the period of observation.

The two animals which developed endocarditis both showed a preliminary diminution in the bacteria in the blood, in one case complete disappearance and then a slow and gradual increase up till the time of death. In both cases there was a marked increase in the agglutinin titre in the blood serum. Even in the serum collected after death this was high.

In both cases there was marked endocarditis with definite evidence of heart failure - engorged spleen, fatty and congested liver and slight ascites. There were no inflammatory lesions in other organs.

The observation indicates that capacity to remove organisms from the blood stream does not necessarily mean capacity to deal with and destroy organisms thus removed.

Further it would seem to suggest that the secondary rise in the bacteriaemia was of the nature of a gradually increasing overflow from the focus of inflammation in the valve.

It also appears to suggest that the production of endocarditis in a previously undamaged valve depends on the attainment of a very delicate balance between the

virulence and dose of the organism on the one hand and the resistance of the animal on the other.

As a result of this experiment it was decided to use all the surviving animals from previous experiments and inoculate them with doses of the same size as those used in this case. Eleven animals in all were used in varying stages of immunization. The result however was completely negative, apparently partly due to the fact that the culture had lost a certain amount of its virulence.

A fresh attempt was made to reproduce Mair's results with ten fresh rabbits. In this case gradually increasing doses of autolysed culture were given on three successive days in every alternate week for a period of 3 months. No further injections were given for 2 months when doses of living culture were inoculated intravenously starting with a single injection of a serum broth culture freshly grown from the virulent blood broth culture. Then in the next week three doses of 1 c.c. were given on each of three days. In the next weeks the doses were increased until in the fourth week the inoculum consisted of 5 c.c. The last - fifth - week the animals received on each day 5 c.c. of a six hour culture in serum broth. Care was taken throughout to see that the culture fully maintained its virulence.

The strain used was pneumococcus XIV.

Only one rabbit of the ten developed endocarditis. The chart of this rabbit will serve to indicate the details of the inoculations made. (Chart XIII.)

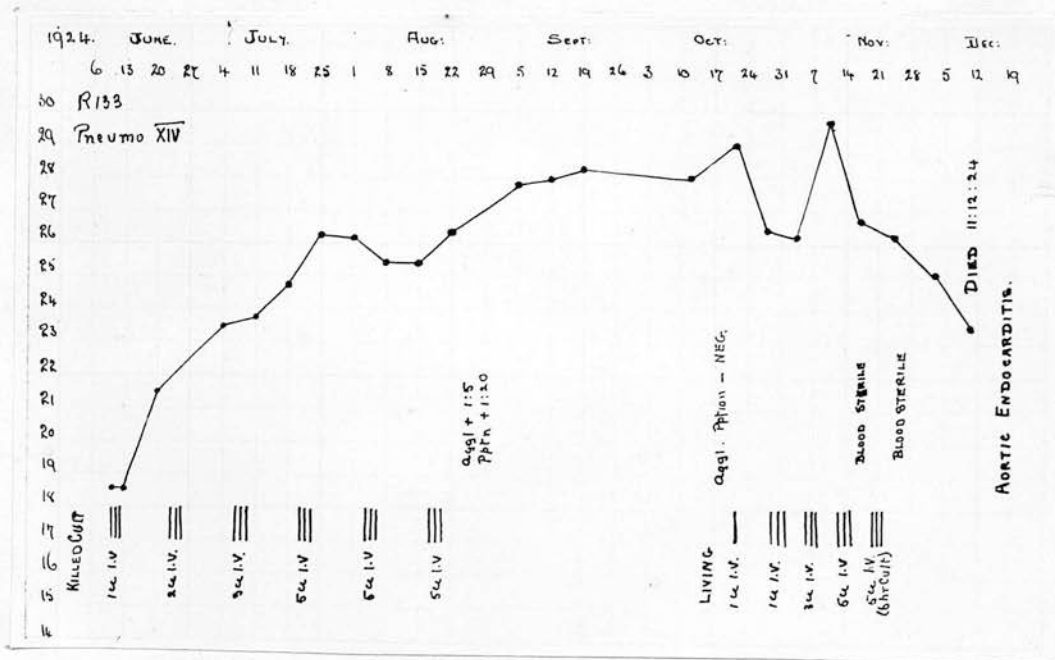


CHART XIII.

Showing weight curve (in hundreds of grammes) and inoculations of rabbit No. 133.

The strain used was rather more virulent than those employed in the previous experiments. Even after eight months of subculture in blood broth without any passage the lethal dose for a mouse was one ten thousandth of a cubic centimetre of a 24 hour serum broth culture while 1 c.c. was fatal to a rabbit when inoculated intravenously.

On the other hand the response to the immunizing process appeared to be rather more marked. Particulars of the individual rabbits are given in Table XXXVII.

TABLE XXXVII.

Rabbit No.	Antibody Titre.		Result.
	After Immunization	After rest.	
133	Pption + 1 in 20 Aggl. + 1 in 5	Nil 1 in 5	Endocarditis.
134	Pption + 1 in 80 Aggl. + 1 in 5 ?1 in 10	do.	Died intercurrent disease 2 mos. after course of living organisms.
135	Pption + 1 in 80 Aggl. + 1 in 5	do.	Nil.
136	Pption + 1 in 10 Aggl. + 1 in 10	do.	Nil.
137	Pption + 1 in 5 Aggl. + 1 in 5	do.	Nil.
138	Nil 1 in 2	do.	Nil.
139	Pption + 1 in 20 Aggl. + 1 in 2	Pption + 1 in 5 Aggl. -	Nil.
140			Died during immunization.
141	Pption + 1 in 40 Aggl. + 1 in 5	Nil.	Died after course of living organisms. Cause unknown.
142	Nil 1 in 2	Nil.	Nil.

These experimental results have not yielded the information which it had been hoped would result from them and the frequency of incidence of endocarditis is too low to permit of any conclusive deductions. They do however afford some illumination of the problems of endocarditis in the following respects.

(1) Non-haemolytic streptococci may sometimes produce endocarditis in rabbits as a result of a single injection.

(2) Injections of streptococci which have been innocuous may, if repeated over a long period at intervals, give rise to endocarditis.

(3) The endocarditis is accompanied by bacteraemia

as well as on the laboratory culture and in the case of pneumococci the organism isolated has been possessed of at any rate some virulence for mice.

NAME	AGE	HISTORY												BLOOD CULTURES				BACTERIA IN SECTIONS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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AND:	14	+	+	-	10m 12h	-	+	-	+	+	+	10 ² C.C.	Q	21-8-24	STREPS		29-8-24	TOXAEMIA	P.A.T.	+	+																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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WHE:	20	+	?	-	2h 5h?	+	+	+	+	+	+	10 ³ A.R.	1	16-12-22	STREPS		23-1-23	H.F.	M.A.	+	+																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
WIL:	29	+	-	-	8h 2h?	-	+	+	+	+	+	10 ² M.S.	2	15-2-24	STREPS	STREPS (CONTIN)	11-2-24	H.F. AND TOXAEMIA	M.A.	+	+																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
GROUP II																							AIN:	30	-	+	-	9h 1h?	-	+	+	+	+	+	99 ¹ M.S.	1	28-8-23	PNEUMO		23-9-23	PNEUMONIA	A.M.	PNEUMONIA AND INDEFINITE MASSES	PNEUMONIA - RED MASSES - STREPTOCOCCI	BRO:	42	-	+	+	1h ?	+	-	?	+	+	+	99 ¹ A.R.	1	21-4-22	PNEUMO		26-4-22	MENTINGITIS	A.	+	+	FOX:	28	-	-	-	5h 1h?	+	+	?	+	+	+	10 ² M.S.	1	20-10-22	PNEUMO		20-10-22	H.F. AND PNEUMONIA	M.A.	+	+	LAT:	27	+	+	-	2h 2h?	+	+	?	+	+	+	10 ¹ A.R.	5	9-11-22	STREPS	S. SHARON	12-11-22	SEPTICAEMIA	M.A.	+	+	GROUP III																							ASK:	38	+	?	-	2h ?	+	+	+	+	+	+	10 ¹ A.R.	1	18-6-20	NEG.		29-8-20	H.F. AND TOXAEMIA	A.M.	COCCI AND INDEFINITE MASSES		COO:	23	+	-	-	6h 3h?	+	+	+	+	+	+	10 ³ A.R.	1	16-6-20	NEG.		1-8-20	H.F.	M.A.	INDEFINITE	DEFINITE	CRO:	44	-	-	-	3h 1h?	+	+	+	+	+	+	10 ¹ M.S.	1	10-2-22	NEG.		23-2-22	CEREBRAL EMBOLUS	M.A.	INDEFINITE	COCCI FRUIT.	CRUD:	29	-	-	-	11h 1h?	+	+	+	+	+	+	10 ⁴ A.R.	6	22-8-23	NEG.		2-9-23	CEREBRAL HEM.	A.M.	INDEFINITE	COCCI FRUIT.	CUT:	24	-	-	-	2h 1h?	+	-	-	+	+	+	10 ¹ A.R.	1	20-12-22	NEG.		16-2-23	H.F.	A.M.	INDEFINITE	DEFINITE	HAY:	50	-	+	+	1h ?	-	+	?	+	+	+	10 ¹ A.R.	1	26-9-22	NEG.		8-9-22	H.F.	M.A.	INDEFINITE	DEFINITE	JUL:	45	-	+	+	1h ?	-	-	?	+	+	+	10 ² A.R.	2	2-12-22	NEG.		4-12-24	NOT FOUND	A.	NEG.	NEG.	MAR:	32	-	+	-	6h 1h?	+	+	+	+	+	+	10 ² A.R.	1	1-12-19	NEG.		8-1-20	H.F.	A.M.	NEG.	NEG.	MON:	43	-	+	-	2h ?	+	+	+	+	+	+	10 ² M.S.	3	9-10-22	NEG.	B. COLI S. SHARON	14-10-22	H.F. AND PERITONITIS	M.A.	COCCI AND INDEFINITE MASSES	DEFINITE	NEA:	27	+	-	-	2h ?	+	+	-	+	+	+	10 ¹ A.R.	1	1-12-19	NEG.		18-1-20	CEREBRAL EMBOLUS	A.M.	INDEFINITE	DEFINITE IN PLEURA	SKI:	38	EMPH	-	-	1h 2h?	-	-	-	+	+	+	10 ¹ M.	1	21-11-22	NEG.		5-12-22	H.F. AND PERITONITIS	M.	DEFINITE IN BLOOD INDEFINITE - NEG.	DEFINITE	VAL:	28	-	+	+	2h 10h?	+	+	+	+	+	+	10 ² M.S.	1	17-7-20	NEG.		10-9-20	H.F. AND PNEUMONIA	M.	INDEFINITE	RED MASSAGE COCCI INDEFINITE	GROUP IV																							BAY:	29	-	+	-	4h 1h?	-	+	+	+	+	+	10 ¹ M.S.					2-12-22	H.F. AND INFARCT OF LUNG	M.A.	VERY INDEFINITE	DEFINITE	BUR:	30	-	-	-	10h 1h?	+	-	-	+	+	+	10 ² M.S.					26-9-22	PNEUMONIA	A.M.	INDEFINITE	DEFINITE	DLB:	40	-	+	+	1h ?	+	+	+	+	+	+	10 ⁴ M.S.					21-5-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	HEW:	28	-	+	+	3h 2h?	+	+	+	+	+	+	10 ³ M.S.					7-6-20	H.F. AND INFARCT OF LUNG	A.	INDEFINITE	RED MASSES COCCI INDEFINITE	HUN:	42	-	-	-	5h 3h?	+	?	-	+	+	+	10 ² A.R.					22-3-22	H.F.	A.M.	INDEFINITE	DEFINITE	PARK:	47	-	-	-	7h 10h?	+	-	-	+	+	+	10 ² M.S.					27-6-22	H.F.	M.A.	INDEFINITE	DEFINITE	PER:	39																						POT:	52	+			10h 1h?							10 ²						4-9-22	? H.F.	P.	INDEFINITE	DEFINITE	RAB:	39	?			2h ?	-	+	+	+	+	+	A.R.					2-9-22	H.F.	A.M.	+	+	REE B:	30	+	+	-	2h 2h?	+	+	+	+	+	+	99 ¹ A.R.					17-7-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	USH:																																																																																																																																																																																																																																																																																																
AIN:	30	-	+	-	9h 1h?	-	+	+	+	+	+	99 ¹ M.S.	1	28-8-23	PNEUMO		23-9-23	PNEUMONIA	A.M.	PNEUMONIA AND INDEFINITE MASSES	PNEUMONIA - RED MASSES - STREPTOCOCCI																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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GROUP III																							ASK:	38	+	?	-	2h ?	+	+	+	+	+	+	10 ¹ A.R.	1	18-6-20	NEG.		29-8-20	H.F. AND TOXAEMIA	A.M.	COCCI AND INDEFINITE MASSES		COO:	23	+	-	-	6h 3h?	+	+	+	+	+	+	10 ³ A.R.	1	16-6-20	NEG.		1-8-20	H.F.	M.A.	INDEFINITE	DEFINITE	CRO:	44	-	-	-	3h 1h?	+	+	+	+	+	+	10 ¹ M.S.	1	10-2-22	NEG.		23-2-22	CEREBRAL EMBOLUS	M.A.	INDEFINITE	COCCI FRUIT.	CRUD:	29	-	-	-	11h 1h?	+	+	+	+	+	+	10 ⁴ A.R.	6	22-8-23	NEG.		2-9-23	CEREBRAL HEM.	A.M.	INDEFINITE	COCCI FRUIT.	CUT:	24	-	-	-	2h 1h?	+	-	-	+	+	+	10 ¹ A.R.	1	20-12-22	NEG.		16-2-23	H.F.	A.M.	INDEFINITE	DEFINITE	HAY:	50	-	+	+	1h ?	-	+	?	+	+	+	10 ¹ A.R.	1	26-9-22	NEG.		8-9-22	H.F.	M.A.	INDEFINITE	DEFINITE	JUL:	45	-	+	+	1h ?	-	-	?	+	+	+	10 ² A.R.	2	2-12-22	NEG.		4-12-24	NOT FOUND	A.	NEG.	NEG.	MAR:	32	-	+	-	6h 1h?	+	+	+	+	+	+	10 ² A.R.	1	1-12-19	NEG.		8-1-20	H.F.	A.M.	NEG.	NEG.	MON:	43	-	+	-	2h ?	+	+	+	+	+	+	10 ² M.S.	3	9-10-22	NEG.	B. COLI S. SHARON	14-10-22	H.F. AND PERITONITIS	M.A.	COCCI AND INDEFINITE MASSES	DEFINITE	NEA:	27	+	-	-	2h ?	+	+	-	+	+	+	10 ¹ A.R.	1	1-12-19	NEG.		18-1-20	CEREBRAL EMBOLUS	A.M.	INDEFINITE	DEFINITE IN PLEURA	SKI:	38	EMPH	-	-	1h 2h?	-	-	-	+	+	+	10 ¹ M.	1	21-11-22	NEG.		5-12-22	H.F. AND PERITONITIS	M.	DEFINITE IN BLOOD INDEFINITE - NEG.	DEFINITE	VAL:	28	-	+	+	2h 10h?	+	+	+	+	+	+	10 ² M.S.	1	17-7-20	NEG.		10-9-20	H.F. AND PNEUMONIA	M.	INDEFINITE	RED MASSAGE COCCI INDEFINITE	GROUP IV																							BAY:	29	-	+	-	4h 1h?	-	+	+	+	+	+	10 ¹ M.S.					2-12-22	H.F. AND INFARCT OF LUNG	M.A.	VERY INDEFINITE	DEFINITE	BUR:	30	-	-	-	10h 1h?	+	-	-	+	+	+	10 ² M.S.					26-9-22	PNEUMONIA	A.M.	INDEFINITE	DEFINITE	DLB:	40	-	+	+	1h ?	+	+	+	+	+	+	10 ⁴ M.S.					21-5-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	HEW:	28	-	+	+	3h 2h?	+	+	+	+	+	+	10 ³ M.S.					7-6-20	H.F. AND INFARCT OF LUNG	A.	INDEFINITE	RED MASSES COCCI INDEFINITE	HUN:	42	-	-	-	5h 3h?	+	?	-	+	+	+	10 ² A.R.					22-3-22	H.F.	A.M.	INDEFINITE	DEFINITE	PARK:	47	-	-	-	7h 10h?	+	-	-	+	+	+	10 ² M.S.					27-6-22	H.F.	M.A.	INDEFINITE	DEFINITE	PER:	39																						POT:	52	+			10h 1h?							10 ²						4-9-22	? H.F.	P.	INDEFINITE	DEFINITE	RAB:	39	?			2h ?	-	+	+	+	+	+	A.R.					2-9-22	H.F.	A.M.	+	+	REE B:	30	+	+	-	2h 2h?	+	+	+	+	+	+	99 ¹ A.R.					17-7-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	USH:																																																																																																																																																																																																																																																																																																																																																																																																															
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CRO:	44	-	-	-	3h 1h?	+	+	+	+	+	+	10 ¹ M.S.	1	10-2-22	NEG.		23-2-22	CEREBRAL EMBOLUS	M.A.	INDEFINITE	COCCI FRUIT.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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HAY:	50	-	+	+	1h ?	-	+	?	+	+	+	10 ¹ A.R.	1	26-9-22	NEG.		8-9-22	H.F.	M.A.	INDEFINITE	DEFINITE																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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MAR:	32	-	+	-	6h 1h?	+	+	+	+	+	+	10 ² A.R.	1	1-12-19	NEG.		8-1-20	H.F.	A.M.	NEG.	NEG.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
MON:	43	-	+	-	2h ?	+	+	+	+	+	+	10 ² M.S.	3	9-10-22	NEG.	B. COLI S. SHARON	14-10-22	H.F. AND PERITONITIS	M.A.	COCCI AND INDEFINITE MASSES	DEFINITE																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
NEA:	27	+	-	-	2h ?	+	+	-	+	+	+	10 ¹ A.R.	1	1-12-19	NEG.		18-1-20	CEREBRAL EMBOLUS	A.M.	INDEFINITE	DEFINITE IN PLEURA																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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VAL:	28	-	+	+	2h 10h?	+	+	+	+	+	+	10 ² M.S.	1	17-7-20	NEG.		10-9-20	H.F. AND PNEUMONIA	M.	INDEFINITE	RED MASSAGE COCCI INDEFINITE																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
GROUP IV																							BAY:	29	-	+	-	4h 1h?	-	+	+	+	+	+	10 ¹ M.S.					2-12-22	H.F. AND INFARCT OF LUNG	M.A.	VERY INDEFINITE	DEFINITE	BUR:	30	-	-	-	10h 1h?	+	-	-	+	+	+	10 ² M.S.					26-9-22	PNEUMONIA	A.M.	INDEFINITE	DEFINITE	DLB:	40	-	+	+	1h ?	+	+	+	+	+	+	10 ⁴ M.S.					21-5-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	HEW:	28	-	+	+	3h 2h?	+	+	+	+	+	+	10 ³ M.S.					7-6-20	H.F. AND INFARCT OF LUNG	A.	INDEFINITE	RED MASSES COCCI INDEFINITE	HUN:	42	-	-	-	5h 3h?	+	?	-	+	+	+	10 ² A.R.					22-3-22	H.F.	A.M.	INDEFINITE	DEFINITE	PARK:	47	-	-	-	7h 10h?	+	-	-	+	+	+	10 ² M.S.					27-6-22	H.F.	M.A.	INDEFINITE	DEFINITE	PER:	39																						POT:	52	+			10h 1h?							10 ²						4-9-22	? H.F.	P.	INDEFINITE	DEFINITE	RAB:	39	?			2h ?	-	+	+	+	+	+	A.R.					2-9-22	H.F.	A.M.	+	+	REE B:	30	+	+	-	2h 2h?	+	+	+	+	+	+	99 ¹ A.R.					17-7-20	H.F.	A.M.	INDEFINITE	RED MASSES COCCI INDEFINITE	USH:																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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TABLE XXXVIII.

- * = blood cultures made by writer.
- ? = information not conclusive.
- + = congenital heart lesion.
- D = days.
- W = weeks.
- M = months.
- 5M? (under "Duration of disease") = known to have lasted 5 months, actual date of onset not determinable.
- Strep. = non-haemolytic streptococcus.
- AR = Aortic regurgitation. MS = Mitral stenosis.
- MR = Mitral do.
- HF = Heart failure.
- Am = large aortic and small mitral lesion.
- P. = Pulmonary.
- T. = Tricuspid.

Microscopic examination of the
valves from cases of subacute
infective endocarditis.

The experiments described upon the technique of blood culture have seemed to make it clear that failure to isolate an organism from the blood stream during life has not been due to defective technique. It can with justice be claimed to indicate that the organisms were not present in the specimen of blood examined and where the examination has been frequently repeated there is a strong presumption that they were not circulating in the blood at all. Sir Thomas Lewis has kindly given me an opportunity of examining the valves from a large number of cases in his collection with a view to determining how far the microscopic examination of these agrees with or refutes the findings made on examining the blood during life.

HARBITZ (1899) in the course of an examination of the valves in 26 cases of "chronic infective endocarditis" found bacteria present in 16 of these. In the other 10 he was unable to detect any bacteria at all.

LIBMAN (1913 I.) reports details of 18 cases in what he terms the bacteria free stage". He states that he has observed cases in which bacteria could not be isolated from the blood but masses of them were observed on the valves after death. The possibility of the occurrence of terminal invasion in some of these cases does not seem to have been excluded. In the remainder however no bacteria could be demonstrated in the valves. Libman draws attention to the

The vegetation on the valve in subacute infective endocarditis may be regarded as having a base which consists of the tissue of the cusp proper or an extension or modification thereof, upon which is deposited a mass of material of greater or less extent, composed of modified fibrin. This latter material is practically devoid of, or at any rate, very poor in cellular elements. It does not stain with Weigert Gram fibrin stain except at the extreme periphery where, as a rule, but to a varying degree in different cases, can be seen a narrow zone of blood elements, the cells and the fibrin net work being normal in appearance. In the main mass of the vegetation there is no evidence of the presence of a network such as is usually to be seen in sections of fresh fibrin.

In this study two points only fall to be considered in relation to the histology of the lesions. They are

- (a) The distribution of the bacteria.
- (b) The zone of reaction at the junction between the vegetation proper and the tissues of the cusp.

Distribution of bacteria in the vegetation.

In the cases of Group I. of this series, with one exception, there was no difficulty whatever in determining the presence of bacteria in the vegetations. The staining was done by Gram's method, decolourization being effected by the use of clove oil and counterstaining by weak carbol fuchsine.

By this method the cocci could be readily seen and easily defined. They were as a rule seen in pairs or as individual organisms, the detection of chain formation being only rarely possible. The most striking feature about the sections stained in this way was the very evident tendency for the organisms to be found at the periphery of the vegetation. With a low power objective large masses of stained material could be seen in this situation. Many of these were apparently directly in contact with the blood stream but a great many were separated from the surface by layers of fibrin of greater or less thickness.

Between these peripheral masses of bacteria and the tissues of the cusp there could also be seen masses of bacteria of varying size. A common appearance was that of a mass which was frequently of similar size to that of the large cells to be described later as being present in the zone of reaction where the fibrin joined the tissues of the cusp.

Closer examination usually revealed a considerable number of isolated individual organisms in the fibrin in its deeper parts. It was very uncommon to find organisms in the valve tissue.

The organisms were on the whole difficult to decolourize but in the deeper parts they seemed to hold Gram's stain slightly less firmly than at the periphery.

A variety of other staining methods was also tried but none seemed to present any advantage over Gram's method with the exception of that described by TWORT (1924). This method has proved of the very greatest assistance. The

stain is a double dye resulting from the combination of light green and neutral red the resulting precipitate being dissolved in propyl alcohol. In sections stained by this method the red acts selectively upon the nuclei of cells and upon bacteria, the remaining constituents being stained varying shades of green. The contrast is very striking and the colours such that examination of a large number of sections is accomplished without undue fatigue. For ordinary purposes staining for five minutes in a mixture of two parts of stain and one of distilled water gave excellent results. Later it was found useful to stain for fifteen minutes in order to bring out clearly certain points to be described later. Using this method it was very easy to detect even isolated bacteria against the green background of the fibrinous material. Examination in detail showed that bacteria at the peripheral part of the mass frequently were more deeply stained than those in the interior and that in some of the masses a large number of cocci might be difficult to resolve microscopically while others alongside them stood out quite clearly. There were in fact to be observed marked differences in staining reaction in these masses which were undoubtedly streptococci.

Further it could be very clearly seen that many large mononuclear cells which had made their way from the valve cusp into the fibrin were packed with cocci, sometimes to such an extent as to obscure the elements of the cell altogether.

Now the use of Gram's method was found to give rise in some sections to certain difficulties of interpretation.

In a given section a small mass of material might be found to react positively under such conditions as led one to consider that decolourization had not been perfect. Further decolourization frequently removed a considerable amount of the stain but not all and the resolution of the stained material was very difficult. One was left with the uncertainty as to whether the material was bacterial in origin or was a mass of fibrin particularly resistant to decolourization. Moreover bacteria which had been decolourized took on the counterstain and were of the same colour as the background and consequently somewhat difficult to see. It was precisely under these conditions that Twort's stain proved of great value. A fairly extensive trial of the stain, involving some hundreds of sections of valves with deposits of fibrin upon them has not shown any case where the fibrin stained other than green. Nor has any material yet been observed excepting bacteria which gives rise to the characteristic red colourization. Moreover all bacteria, whether Gram positive or Gram negative are coloured red and, further, the personal factor involved in the decolourization in Gram's method is eliminated.

In one of the cases of Group I. (Ree, J.) there were no definite bacteria to be seen in the sections stained by Gram's method. There was however a fringe seen in the periphery of the fibrin which resisted decolourization more than the material in its neighbourhood. The difficulty arose as to what this material was. Its position suggested strongly that it was a mass of bacteria. But it was impossible to say definitely if this were true as the granular material of which it

consisted could not be definitely said to be cocci. It seemed likely to be one of the following three things:-

- (1) Calcium particles.
- (2) Fibrin.
- (3) Bacteria.

A section stained by the silver nitrate method showed that the granules were not calcium. The application of Twort's stain showed that the mass stained definitely red and could be clearly distinguished from the matrix of green fibrin. It was not possible however to define exactly the individual organisms in the mass.

Now in this case the blood culture was positive, revealing streptococci. And yet in the vegetation it was difficult to be quite certain of the presence of organisms. There seems no doubt that the masses described were of the nature of bacteria. What is not clear is whether the modification had occurred during the life of the patient or after death as a result of faulty fixation. The specimen was an old one and there were many crystalline bodies present in the sections such as might result from destruction of red blood corpuscles and liberation of haemoglobin through faulty fixation. The specimen had been sent from some distance and it was some time before it was fixed in the routine way employed in the Cardiology Department.

It is however important to note that the difficulty met with here in a case of undoubted streptococcal endocarditis is just such as arises in the cases in Groups III. & IV. and will be further discussed.

The four cases in Group II. call for little comment.

In all three pneumococcal cases the bacteria were easily recognisable.

In the case of Ain the well formed clearly staining pneumococci were chiefly on the surface of the vegetation. In addition there could be seen in the periphery of the fibrin masses of material staining with Gram's stain but not quite so resistant to decolourization as the pneumococci. Twort's stain showed this material to stain the same colour as bacteria, and in some places there could be seen rounded cocci quite different in appearance from the pneumococci at the surface. The patient had pneumonia and it is suggested that the masses in the vegetation were probably different organisms from the pneumococci which were a terminal invasion.

In the case of Brow there was considerable evidence of chronic inflammation and, in addition, in the cusp an area of infiltration with polymorphs almost amounting to abscess formation, in which cocci could be seen in large numbers. This latter picture is not seen in the streptococcal infections. The patient died of meningitis. Here also the occurrence of a superadded infection cannot be excluded.

In the case of Fox who died of pneumonia similar considerations are to be taken into account.

In the case of Lat the actual occurrence of the superadded invasion was observed and histologically there is found in this case actual abscess formation in the heart muscle.

From the point of view of the present discussion case

Ain provides us with evidence that the appearance of the masses in the periphery of the fibrin, which in this case are undoubtedly bacterial, is not due to bad fixation of the tissues because alongside them are to be seen perfectly stained pneumococci as though from a culture of the organism. It is important to note this because many of the specimens were old and formalin fixation had been employed and it seemed feasible to attribute the appearances to faulty technique. That this was not so is proved also by another case Crudg, where at post mortem, a portion of a vegetation was fixed in alcohol as soon as it was removed from the body and the results were not affected in any way.

The findings here in the cases of Ain, Fox and Lat strongly suggest that the chronic endocarditis was not due to the pneumococcus but to some other organism and the death was brought about by a pneumococcal infection, which was additional.

Group III. This group of cases gave rise to very great difficulty. In only three cases was it possible by Gram's method to say with definiteness that bacteria were present. In Ask and Ski cocci could be seen in considerable numbers in the blood upon the surface of the vegetation. In the former case the cocci were very large and the arrangement strongly suggested that the organisms were staphylococci. In the latter there were numbers of cocci and a few Gram positive bacilli. Now this patient had died with signs of peritonitis and it would seem probable that the organisms seen were derived from that focus of infection. Similarly in case

Mont who died with peritonitis following a septic infarct of the spleen, from which haemolytic streptococci and B. coli were isolated at post mortem the cocci seen on the surface of the sections might have a similar origin.

In cases Jul and Mar no bacteria of any kind could be demonstrated.

In all the remaining cases and in the three cases first mentioned (Ask, Ski and Mont) there were to be seen in the periphery of the vegetation, separated from the surface by a narrow interval, masses of material staining with Gram's stain but tending to be decolourized more readily than the well formed organisms. In all cases there was doubt as to what constituted the masses. In all but two of these cases Twort's stain showed that the masses undoubtedly consisted of cocci. In the case Nea it was possible to define cocci only in one or two small areas although large masses of ill defined material stained quite clearly red with the stain. Finally in case Val, in which there was much fibrosis there were found small masses of red staining material but the individual elements were not sufficiently defined.

Of these twelve cases, therefore, which showed no evidence of bacteria during life, only two have failed to show any evidence of bacteria in the valves. In nine cases it has been possible to demonstrate cocci. In six of these there is no room for doubt that the bodies are actually cocci. In two others the staining is well marked but the number of definitely coccal bodies is small. (One of these two cases is Crudg, from whose blood six cultures during life failed

to reveal any organisms). The remaining case shows material in just the position in which bacteria might be expected to be found and staining in the same way as a mass of bacteria but not showing any really definite organisms except in one or two small areas. In the tenth case the stained material was found but no clear cocci.

The impression one obtains as a result of this study is that all these masses are really masses of bacteria. The difficulty of defining the organisms would apparently be due to the fact that they are somewhat degenerated. As in the definite cases in which bacteria are certainly present there is variation in the definiteness of staining of the individual cocci, so also from case to case there is the same sort of variation. The series would seem to represent phases in the process of disintegration of the bacterial masses seen so clearly in the cases with bacteriaemia.

Two difficulties in accepting this view are the apparent absence of bacteria from the blood stream and the persistence of the organism for such lengthy periods in the vegetation. The reason for the failure of blood culture to reveal organisms would appear to be in part anatomical. Most of the organisms are shut off from the blood stream by the superficial fibrin. Possibly also a number, if not all, of the bacteria are dead and would not be revealed by cultural methods even if they did escape from the fibrin through dislodgement of the covering layer.

And the fibrin which prevents them from entering the blood stream may also protect the organisms from the attacks of the cells, chiefly apparently wandering tissue cells, so

that complete removal of them would await the slow process of autolysis and natural death of all the bacteria in the mass. Further if this view be correct we have a graded series of cases from fully fledged septicaemias to cases with fibrosed valves containing only a few bacteria. This view accords with clinical findings. There are slight differences between the various cases but there is an essential resemblance between them. The main point of difference appears to be in regard to temperature. Some cases are febrile throughout, some show only very occasional rises of temperature, but all show fever at some time or other. It is difficult to explain the fever in the absence of bacteria. If these enclosed foci are present the rise of temperature might easily be due to escape of bacterial products.

Finally it may be stated that examination of a series of cases of rheumatic and terminal endocarditis has revealed in the fibrin no evidence whatever of any material staining as these masses do in the vegetations of the cases of infective endocarditis.

In the cases in Group IV. there was only one in which undoubted bacteria could be demonstrated by both Gram's and Twort's methods. In all the other cases the same fringe of material could be seen in sections stained by the former method, well marked if only slight decolourization was used, much less so if the decolourization was carried out in the ordinary way.

By Twort's method all these masses stained quite distinctly red. In six cases the cocci could be demonstrated

quite clearly. In the remaining four such demonstration was difficult and only possible in limited areas of the red stained material. It is to be noted that in three of these four cases there was well marked calcification of the vegetation. In two other cases showing calcification the cocci were quite distinct.

From the sum of these results it seems justifiable to conclude that where the blood culture has been positive bacteria have been readily demonstrated in the valve. Where it has been negative bacteria present in sections have apparently come from a terminal infection or present appearances suggesting that they are in a condition somewhat different from that of the organisms in the valves of bacteriaemic cases.

Histology of the zone of reaction.

In examining the series of sections one has been impressed by the peculiar character of the reaction at the junction between the fibrin of the vegetation and the tissues of the valve cusp. Writers have referred (LIBMAN(1912) CLAWSON (1924)) to the mononuclear character of the cells. There is indeed in many cases, though not in all, a marked lack of leucocytes. In many of the bacteriaemic cases however there is a considerable number of polymorph leucocytes to be seen. But in almost all there is a very definite reaction on the part of the fixed tissue cells. There appears to be a gradual enlargement and swelling of the

protoplasm of the cells, apparently connective tissue cells, which become oval, rounded or irregular in shape. Further there is frequent evidence of multinucleation and in marked cases actual formation of giant cells. These large cells tend to migrate out into the fibrin and approach the bacterial masses where they ingest large numbers of bacteria and may be seen in various stages of disintegration. Now the interesting point about the various stages in development of these cells is that they closely approximate to the changes seen in the rheumatic nodule (submiliary nodule of ASCHOFF (1906)) which is regarded as being specific to rheumatic fever. The study of these changes is not yet complete but it would seem necessary to accept with caution such a view of any lesion of the valves or endocardium. POYNTON & PAINE (1913) it is true regard infective endocarditis as rheumatic in nature but the view is not generally accepted. One would be inclined to attribute the cellular peculiarities of the reaction to the chronicity of the infection. Dr. Grant and I have found in sections of hearts from pigs suffering from a subacute endocarditis due to the bacillus of swine erysipelas changes identical with those found in this condition.

In view of the alleged relationship of acute rheumatism to subacute infective endocarditis it has seemed advisable to study the heart muscle in these cases. Sections of the muscle from the interventricular septum have been examined in each case. In no case has there been found any collection of cells resembling even remotely the submiliary nodule of rheumatic myocarditis. This point is being investigated further and more extensively for it is well known that the distribution of the nodules is frequently very sparse in acute rheumatism and therefore large numbers of sections need to be examined before a negative result becomes of much value.

General Discussion.

The results of this investigation would appear to lead to certain conclusions upon the nature of this disease.

In the first place it would seem to be unnecessary in the majority of cases to look beyond the group of non-haemolytic streptococci for the aetiological agent. This organism is much more frequently isolated from these cases than any other. But there appears good evidence that the bacillus of Pfeiffer may occasionally produce the same type of disease. And there is no a priori reason why other organisms of low virulence should not produce similar effects. On the other hand it is doubtful if pneumococci staphylococci or haemolytic streptococci have ever really been found in these cases except as accidental invaders of an already infected patient.

The septicaemia is to be regarded not as a primary factor in the established disease but as something of the nature of an accident, resulting from the overflow of bacteria from the focus of infection into the circulating blood. The term "bacteria free stage" is one which might lead to confusion. There are undoubtedly many cases in which the bacteriaemia is absent and possibly others in which it is intermittent. Of this last type of case there has been no example in this series. But even these cases without bacteriaemia are not all "bacteria free". The great majority of them have bacteria present in the lesion on the valve. They should be referred to therefore as "non-bacteriaemic" or "non septicaemic". Whether the further small residue would show any bacteria in other parts of the valves than those examined can be settled only by

examining serial sections of the whole of the lesions, which has not yet been done. It is possible however that a small number of cases are really free of bacteria.

There is no evidence available that the bacteria present in the circulating blood actually multiply there nor that they are more resistant to the bactericidal action of the blood than the ordinary strains. What evidence has been collected suggests that they are less capable of growth on laboratory media than the strains kept growing on such media.

It suggests in fact that some at least of the organisms are like those in an old culture rather than those in a young one.

Further the patient's blood in vitro appears to be able to deal with and destroy the organisms it contains. It is also well equipped with antibodies and these seem to increase as the disease progresses. The maintenance of the septicaemia seems therefore only explicable on the basis of constant renewal from the focus. Failure to deal with the bacteria in the focus is not explained. It is suggested that the anatomical position of the cocci in the vegetation may help to account for this. As to the part the leucocytes play in removing the streptococci no evidence is available. It is however a very striking fact that invasion of the vegetation by these cells is not very marked and may be absent. On the other hand invasion by mononuclear cells of the tissues is seen very constantly and also ingestion of the bacteria by these cells. It is certain that in the older lesions the part of the vegetation

near the cusp tissues is devoid of bacteria, which are to be found only at the periphery. Even at the periphery the leucocytes seem to show little evidence of a tendency to attack the bacteria.

The "immunity" of the patient suggests that specific therapy is not a very hopeful line of treatment. What appears to be necessary is something to stimulate the natural phagocytosis or some drug to penetrate the vegetation and destroy the bacteria.

These various facts would also tend to explain in part the absence of suppuration in the tissues despite the septicaemia. As to the mode of production of the lesions it seems clear that production of endocarditis in previously undamaged valves is difficult in the experimental animal. Even where it has been apparently effected it cannot be asserted that preliminary traumatization sufficient to permit of localization of organisms had not been produced. Specific sensitization of the tissues has not been proved to occur and in one case was certainly not generally present. So far as it was tested the source of the organism had no effect on the results of the experiments.

Lewis & Grant have clearly shown that aortic valves which are the site of a congenital malformation are peculiarly liable to infection. It has often been noted that infection of the valves on the right side of the heart occurs most frequently in patients with congenital abnormalities. But one of these cases (Pot) was an infection of the pulmonary valve alone without any congenital abnormality.

To obtain clear evidence of previous damage to cardiac

valves is extremely difficult. In the first place it is not often possible to record in any given case whether the patient had signs of valvular disease before the onset of the infection either because there is no record of examination at a sufficiently early date or because it is quite impossible to say when the infective process began. Further the examination of the valves does not allow of such determination because it is impossible to say how much of the chronic inflammation and fibrosis is due to a preexistent condition and how much the result of the infective process. Many authors have stated that the disease always occurs in a damaged valve. This may be true but the evidence lacks conclusiveness. It is usually stated also that rheumatic fever is a very common occurrence in the history of patients with infective endocarditis. Figures relating to this point are also very liable to error because of the lack of precision in the various definitions of rheumatic fever. In this series of 37 cases there is a clear history of rheumatic fever or chorea in 11; in three no information is available.

Despite this lack of precise information it must be admitted that it is likely that a damaged valve would be a locus minoris resistentiae and play a large part in determining the incidence of infection, whether the damage were due to previous disease or to congenital defect or to mere bombardment by particles such as bacteria. And the frequency of disease on the left side is to be associated in all probability with the greater strain on the tissues of these

chambers, and consequent liability to damage, rather than to any hypothetical effect of the oxygen or carbon dioxide tension of the blood on the organism.

Summary.

1. Organisms can be isolated from the blood of many cases of subacute infective endocarditis but not from all.
2. The organism most commonly isolated is a streptococcus of non-haemolytic type.
3. Cultures made from the blood during the last few days of life may reveal organisms which have entered the blood stream as part of a terminal invasion.
4. Pneumococci and other pyogenic organisms isolated from these cases are most frequently terminal invaders.
5. Cultures made after death from the blood are unreliable as a means of studying diseases due to streptococci unless the observations are adequately controlled, as these organisms occur very commonly as terminal or agonal invaders of the blood stream.
6. Failure to isolate streptococci from the blood in cases of this disease has not been due to defective technique.
7. The technique of blood culture has been studied experimentally. Trypsinized media appear to be the best but satisfactory results can be obtained with media containing sodium citrate in a concentration of 0.2 per cent.
8. The course of the septicaemia in this disease has been studied quantitatively. The number of organisms is usually small and the increase in numbers is slow and irregular.
9. The serum of the patient contains agglutinins for the organism producing the disease.

10. The blood of a patient with infective endocarditis can sterilize itself in vitro.
11. The bacteria isolated from cases of this disease do not form a homogeneous group.
12. There is evidence that the bacteria isolated are not in the same condition as those in a laboratory culture, but that they are less capable of active growth.
13. Delay in growth in blood cultures is chiefly dependent upon the peculiarities of the organism present in the blood.
14. Experimental production of endocarditis in rabbits is irregular and uncertain.
15. Details of the progress of the septicaemia and production of antibodies in the experimental animal resemble closely those in the human disease.
16. No evidence has been obtained of the existence of any property of elective localization in the organisms isolated from the human or the experimental disease.
17. Experiments are recorded on the production of endocarditis by the inoculation of pneumococci into immunized rabbits.
18. In most of the cases of infective endocarditis with a negative blood culture bacteria can be demonstrated in the vegetation but they appear to be in a degenerate condition.
19. Attention is drawn to the resemblance between the lesions in the valves in this disease and that in the submiliary nodule in rheumatic fever.
20. Submiliary nodules have not been found in the heart

muscle of cases of subacute infective endocarditis.

21. Attempts at specific therapy by means of a specially prepared serum and by means of transfusion of blood from an immunized donor have not produced any beneficial effect in the cases in which they have been tried.

22. The significance of these findings in relation to certain features of the disease is discussed.

I desire to express my indebtedness to Sir Thomas Lewis, F.R.S., for facilities afforded me in investigating cases in his wards and for the opportunity of studying the specimens in his collection; to Professor T. R. Elliott, F.R.S., Dr. Chas. Bolton, F.R.S., Dr. F. J. Poynton and Dr. Batty Shaw for opportunities afforded me of investigating their cases. Dr. W. Mair was good enough to let me see the protocols of his experiments before I attempted to repeat them. Professor A. E. Boycott, F.R.S., has given me much helpful advice in the course of the investigation.

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Finally I would express my appreciation of the technical assistance afforded me by my laboratory assistant, Mr. E. P. Murrell.

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PLATE I.

Heart of WIL

Mitral lesions.
Streptococci isolated from blood during life.

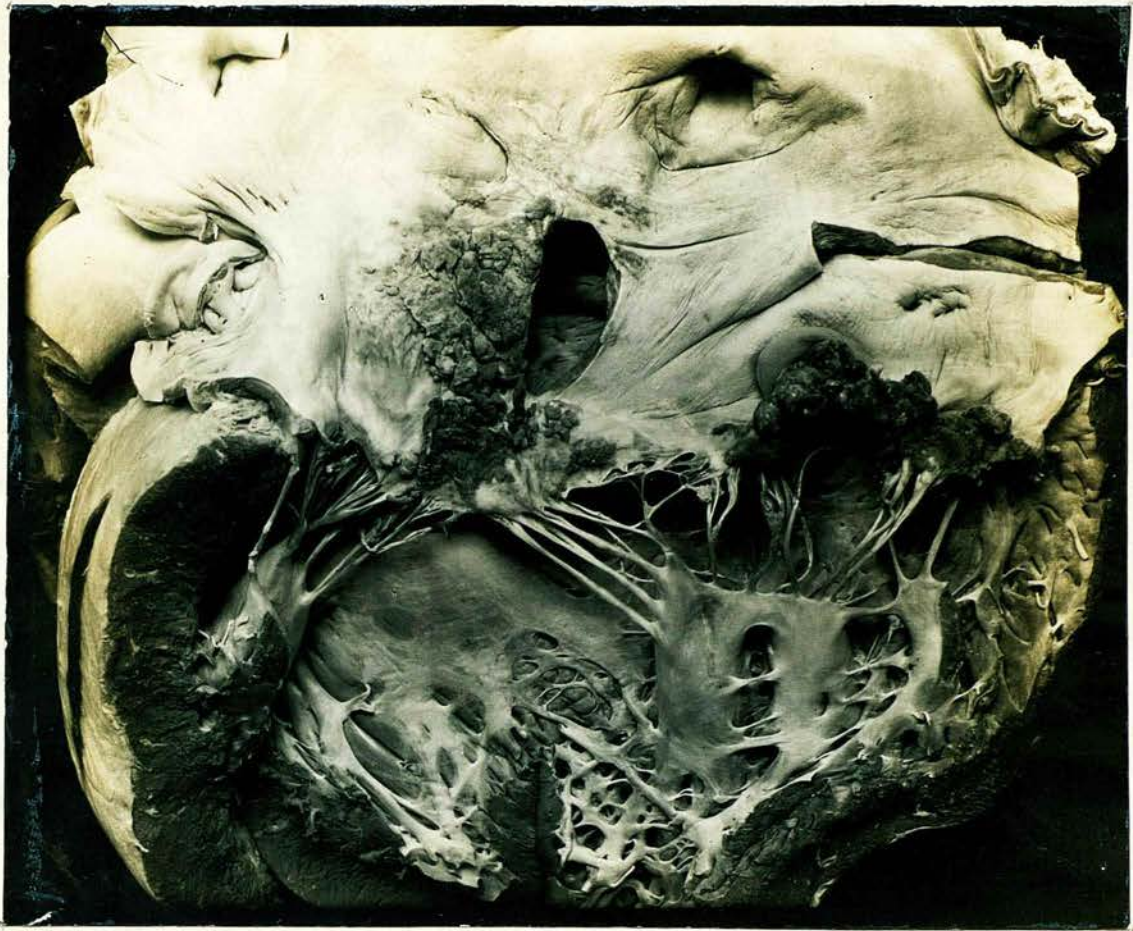


PLATE 2.

Heart of DUD

Mitral lesion spreading to auricle
Streptococci isolated from blood during life
The hole in auricular wall is an area from which
a portion was removed for sectioning.

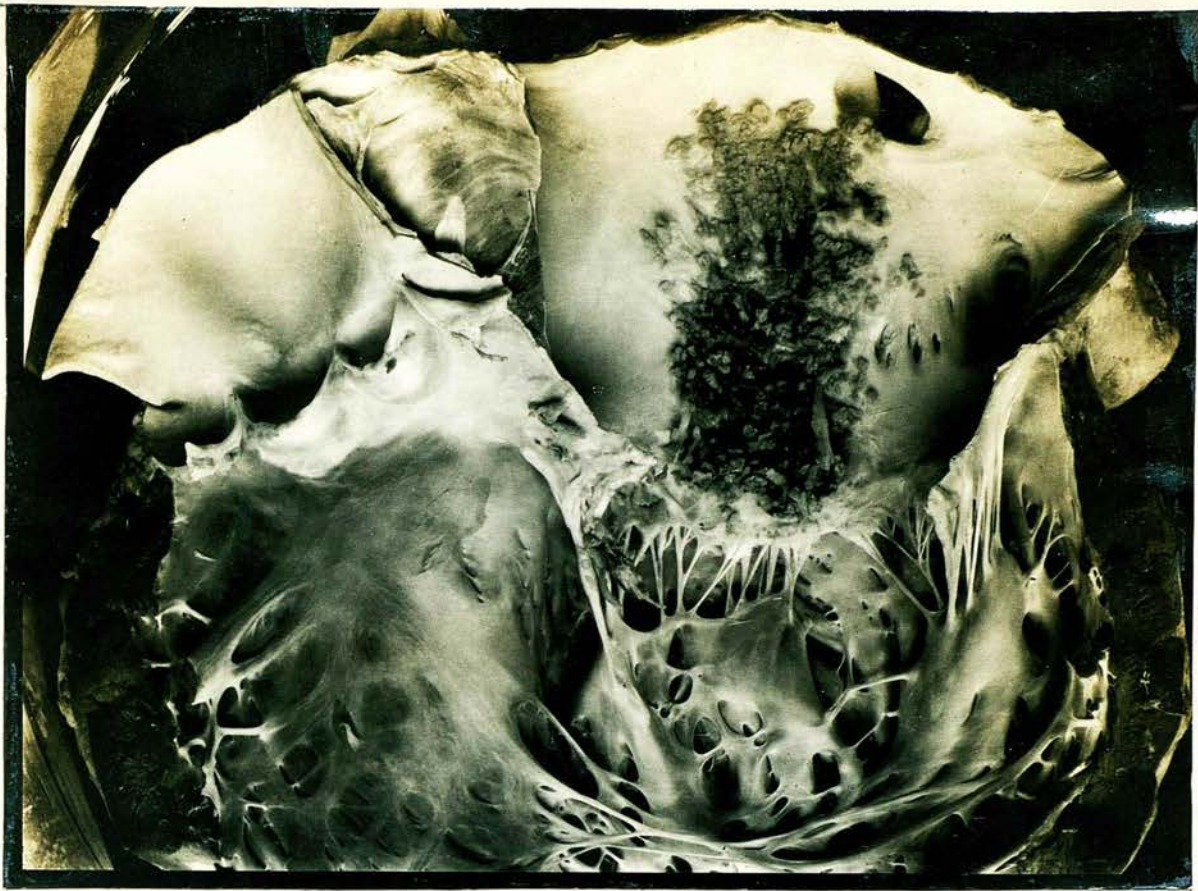


Plate 3.

Heart of FOX

Mitral lesion spreading to auricle.
Pneumococcus isolated from blood 2 days before
death.



Plate 4.

Heart of CRUDG

Mitral and aortic lesions
No bacteria in blood during life.



PLATE 5.

Heart of rabbit No. 133.

Aortic endocarditis produced by injections
of pneumococci into a partially immunized
animal.

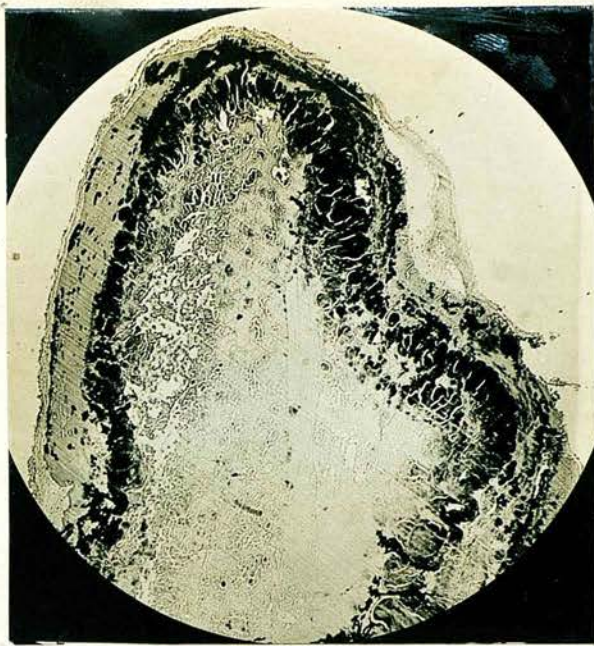


PLATE 6.

Section of vegetation of case Pett - stain Gram.
Zeiss ocular 4. objective aa.
Showing peripheral position of bacteria - black
in photograph
Blood cultures positive during life - streptococci.



PLATE 7.

Similar section - stain Twort.
The fringe of bacteria stains red (black in photograph.)
Note that fringe, though peripheral, is separated from
the edge of the section by a band of fibrin.



PLATE 8.

Section of vegetation of case Bow. Stain Twort. Low power. Bacterial mass stained red (dark in photograph). Streptococci in blood culture during life.



PLATE 9.

Section of vegetation of case AM. Stain Twort. Low power. Showing peripheral red (dark) masses of streptococci with other less deeply stained masses in the substance of the vegetation. Streptococci repeatedly cultivated from the blood.



PLATE 10.

Section of vegetation. Case Crudg. Stain Gram.
Zeiss ocular 4. Eyepiece 22.
Showing a fringe of blue staining (dark in photograph). If the section was properly decolourized very little of this remained.
Blood culture negative during life.

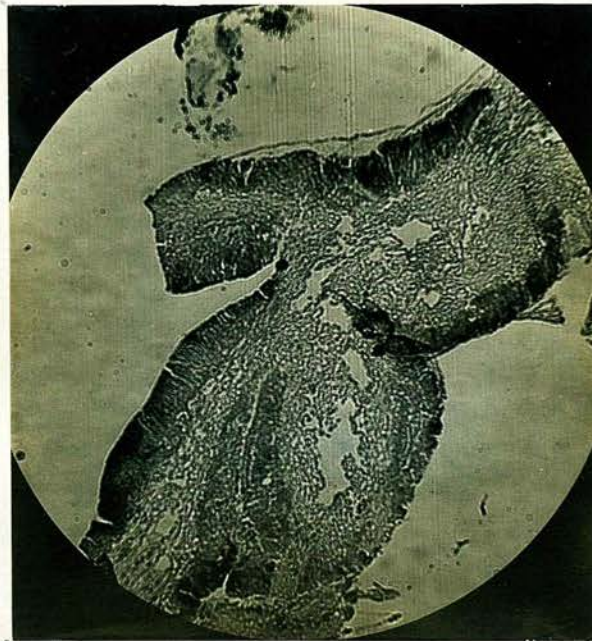


PLATE 11.

Similar section stained with Twort's stain. The fringe is stained red (dark in photograph) and appears more definite than in Plate 10.

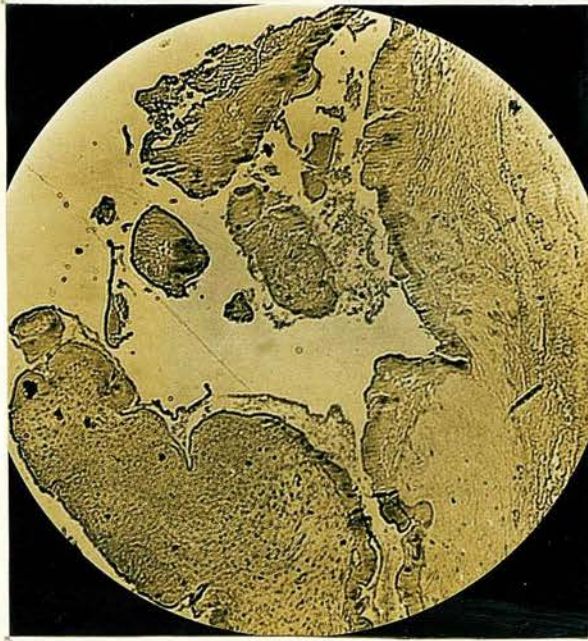


PLATE 12.

Section of vegetation of case Bur. Stain Gram. Low power.
Very little faintly stained material in peripheral
part of vegetation.

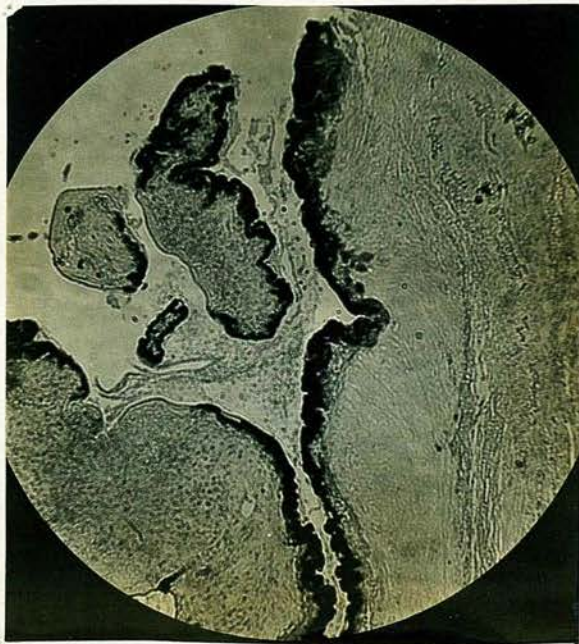


Plate 13.

Similar section. Stain Twort.

The peripheral red stained material is very
clearly seen.

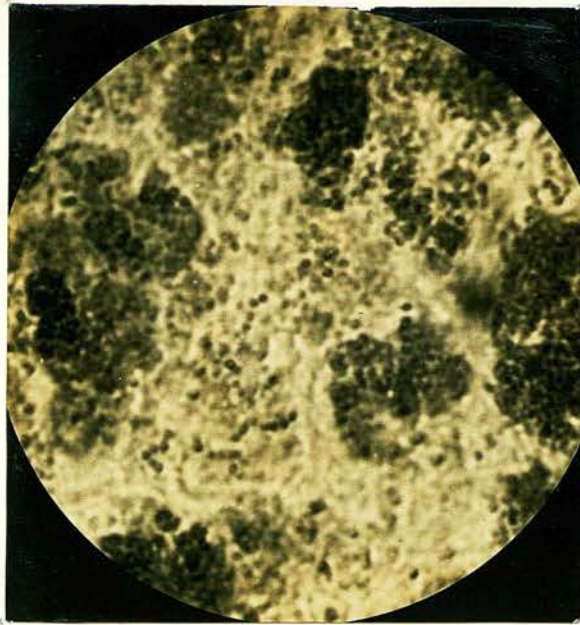


PLATE 14.

Section of vegetation of case Am. Stain Gram.
Zeiss ocular 8, objective 1/12th.
Septicaemia present during life.

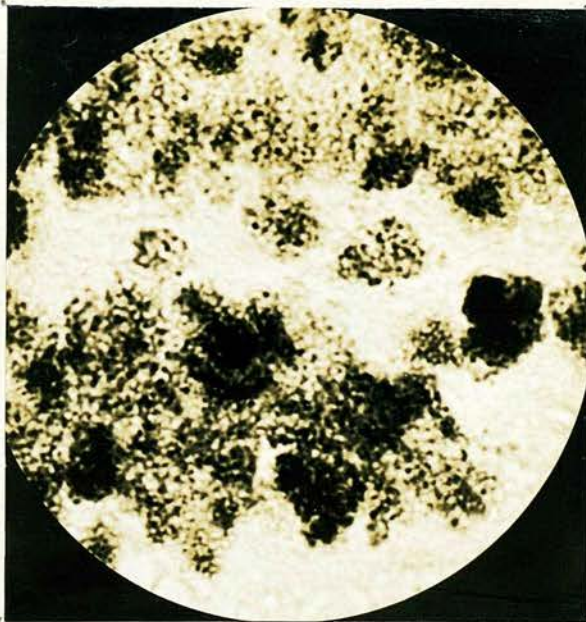


PLATE 15.

Similar section. Stain Twort. Same magnification.
Cocci stained red (dark in photograph).

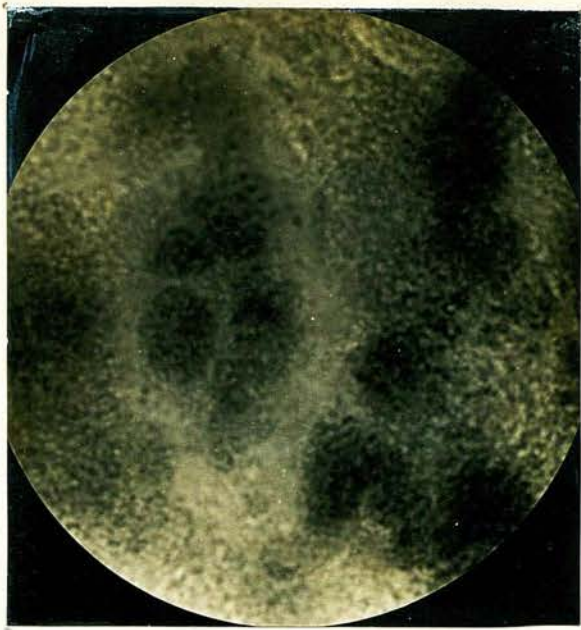


PLATE 16.

Section of vegetation of case Coo. Stain Gram.
Zeiss ocular 8, objective 1/12th.
Portion of bluish fringe. Consists of granular
(coccoid) material some of which is definitely
blue (dark) and the rest less definitely so, shad-
ing off into red. Blood culture negative.

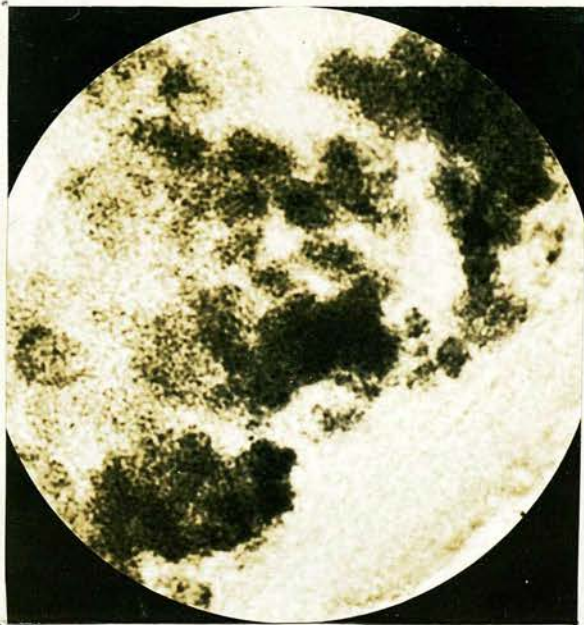


PLATE 17.

Similar section. Stain Twort. Showing the red cocci
(dark) contrasted with the green (light) fibrin of
the vegetation.

They are not so large as in case Am.

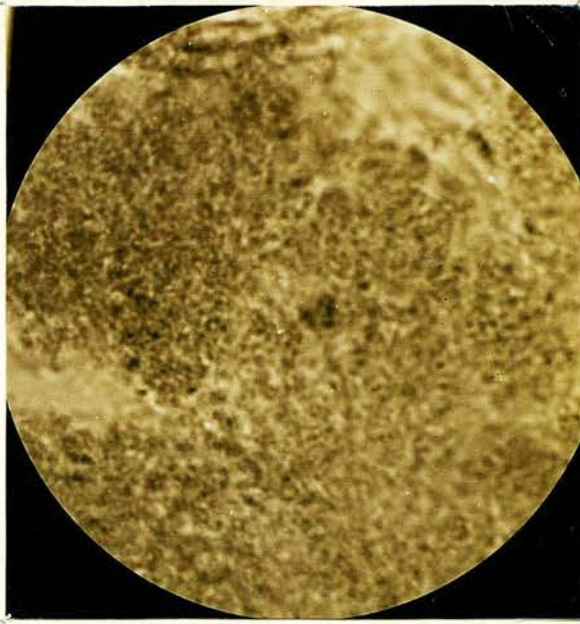


PLATE 18.

Section of vegetation of case Crudg. Stain Gram.
Zeiss ocular 8, objective 1/12th.
Showing the indefinitely staining granular
material in the periphery of the vegetation.
Repeated negative blood cultures.

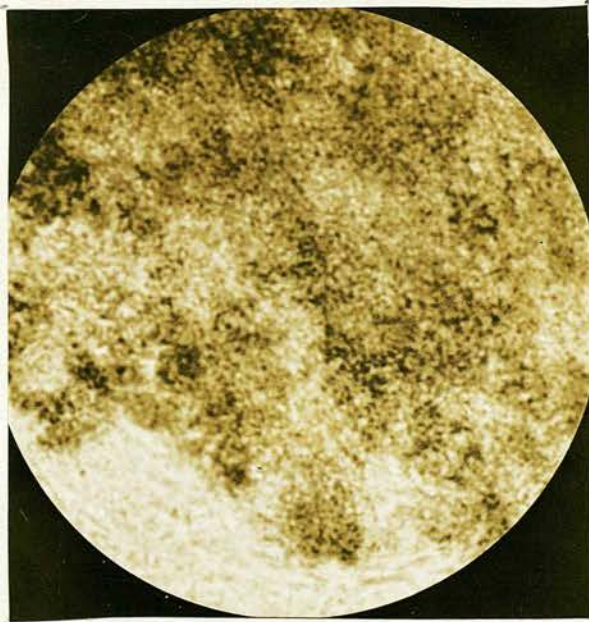


PLATE 19.

Similar section. Stain Twort.

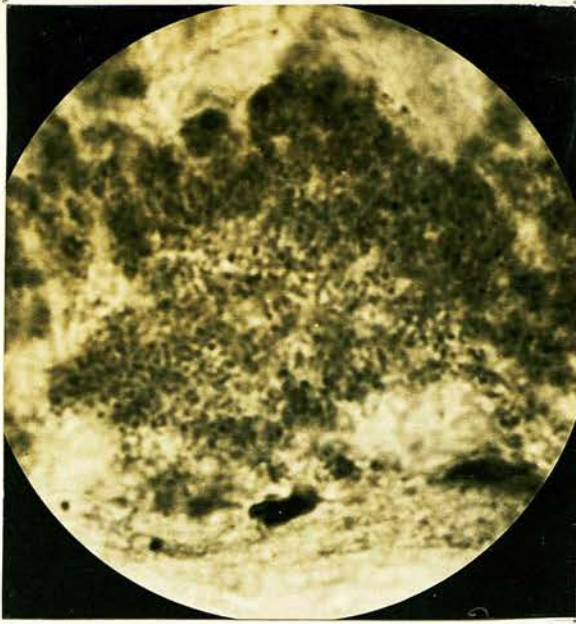


PLATE 20.

Another portion of vegetation of Crudg. Stain Twort.
The granular bodies are larger and more nearly resemble cocci.

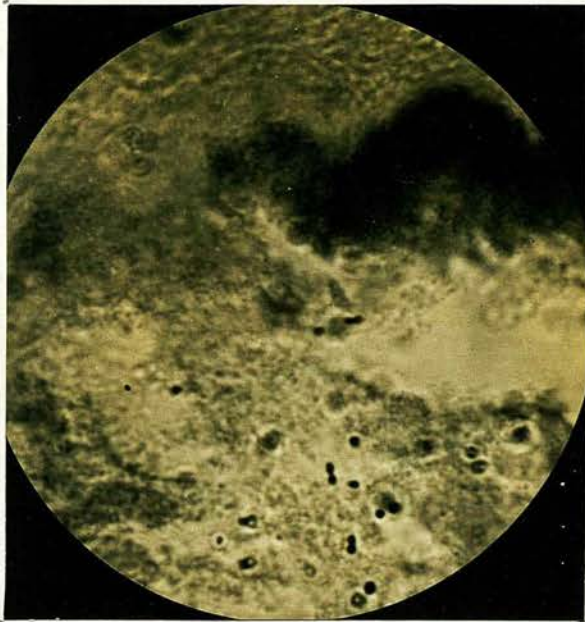


PLATE 21.

Section of vegetation of case Ain. Stain Twort.
Zeiss ocular 8. objective 1/12th.
To contrast size of pneumococci (below) with granules of red mass (dark) above.

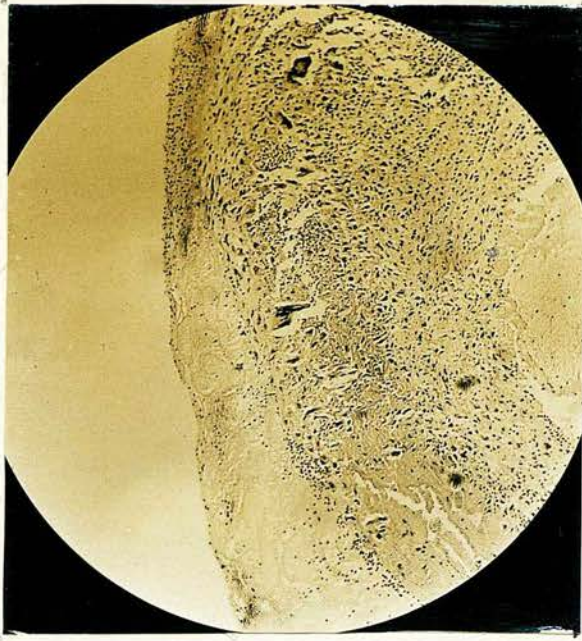


PLATE 22.

Section of vegetation, case Jul. Stain Haematoxylin and eosin.

Zeiss ocular 4, objective A.A.

Showing giant cell at junction of fibrin (left) and valve tissue (right).

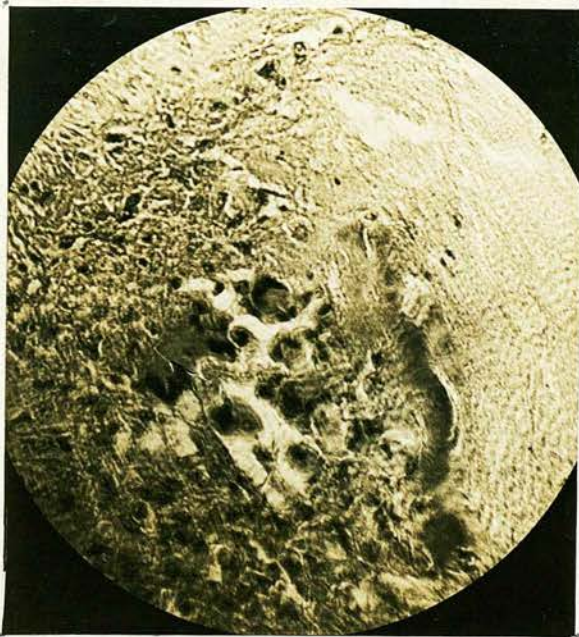


PLATE 23.

Section of vegetation, case Mon. Stain Haematoxylin and eosin.

Zeiss ocular 4, objective DD.

Showing group of multinucleate cells in similar position, the limitations of the cells being somewhat ill defined.

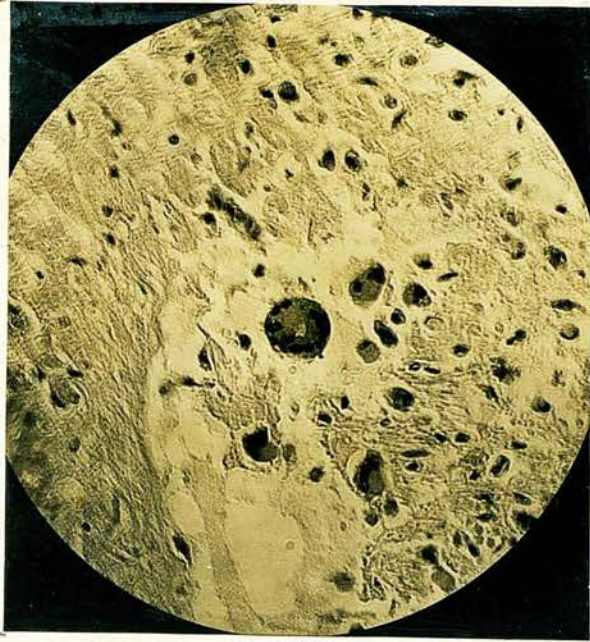


PLATE 24.

Section of vegetation of case Crudg. Stain Haematoxylin and eosin.

Zeiss ocular 4, objective DD.

Showing giant cell, several smaller multinucleate cells and some large swollen mononuclear tissue cells.

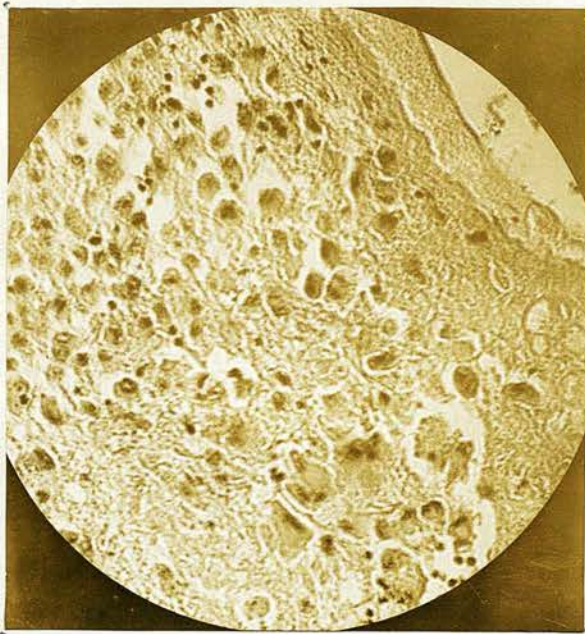


PLATE 25.

Section of vegetation of case Park. Stain Haematoxylin and eosin.

Zeiss ocular 4, objective DD.

Showing stages of transition from mononuclear tissue cells (left) to giant cells (right).

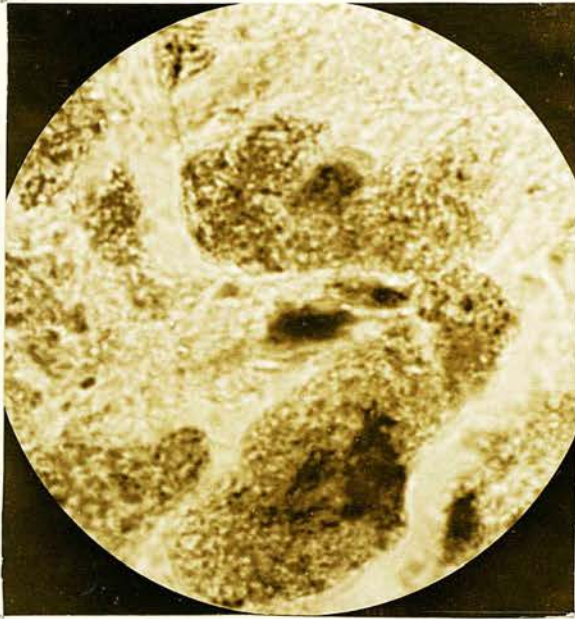


Plate 26.

Section of vegetation of case GUN.
Stain Twort.
Zeis ocular 8. objective 1/12.
Showing large mononuclear cells containing
ingested bacteria.