AN INVESTIGATION INTO THE CAUSE OF RHEUMATIC FEVER IN CHILDREN.

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(With 5 Charts.)

In 1900 Poynton and Paine published the results of their researches into acute rheumatism and described the *Diplococcus rheumaticus*. From that time bacteriological interest has chiefly centred round the streptococci as the causal agents of acute rheumatic fever. The great variety of types, however, believed by various authors to be the aetiological organism leaves it open to doubt whether the true cause has been discovered. A great difficulty has always been the inability to isolate organisms in every case of the disease from the blood or joints; success usually being obtained in a large number of cases only when moribund or *post-mortem* cases have been examined. Loeb (1908), out of 45 patients examined, obtained positive cultures in 10 cases from the blood and in one from the knee-joint, and concluded that the streptococcus isolated was unlikely to be the causal agent as it failed to show any feature which differentiated it from other members of the group. Harrison (1913) failed to isolate a streptococcus from the blood in 26 cases, isolated streptococci in two joint fluids out of 27 but found no evidence that they were identical with *S. rheumaticus*; he failed to isolate that organism from the throats of patients. Rosenow (1913) obtained the high percentage of 7 positive cultures out of 8, and stated that the organism closely resembled *S. rheumaticus*. Herry (1914) isolated a diplococcus with remarkable constancy in all manifestations of the disease. Swift and Kinsella (1917) found the exudates from the joints in acute rheumatic arthritis uniformly sterile; 85 blood cultures from 58 patients yielded only 7 positive cultures of non-haemolytic streptococci; similar streptococci were isolated *post mortem* from active endocardial lesions in less than 50 per cent. of cases. Clawson (1925) after examining the organisms isolated from 20 cases concluded that they were not a specific group. Lynch (1927) failed to demonstrate organisms in the blood from fifteen cases of rheumatic fever. Coombs and Poynton (1926) in a review of the work done since 1906 conclude that there is no rival to the streptococcal theory of acute rheumatism, but admit that while many observers have found this organism others have failed to do so, and that when found the organism is very difficult to classify. Small (1927) and Birkhaug (1927) independently isolated a streptococcus giving similar sugar reactions: this organism will be referred to later.

A further difficulty in assigning the rôle of causative organism to any

particular type of streptococcus is the fact that the characteristic non-suppurative arthritis can be produced in rabbits by many members of the group. Thus, Topley and Weir (1921) produced lesions in the joints and hearts of rabbits with streptococci isolated from rheumatic fever, meningitis, ulcerative endocarditis, and peritonitis.

The present paper is a preliminary report on an investigation carried out on forty-six cases of acute and sub-acute rheumatism in children aged from four to fifteen years. Thirteen were boys and thirty-three were girls. At the outset it was hoped to investigate cases during the first few days of the first attack, but this was only possible in a small number.

Material Examined and Method of Isolation of Organisms.

Cultures were made from the tonsils or tonsillar region in cases where the tonsils had been removed, as it is very generally agreed that sore throats, tonsillitis, and enlarged tonsils are associated with acute rheumatism. Post-nasal swabs were also taken, and in thirty-three cases blood cultures were made as well. For the blood cultures approximately 20 c.c. of blood was withdrawn from a vein and in some cases transferred direct into 100 c.c. of glucose broth, and in others allowed to clot and the clot broken up in 100 c.c. of glucose broth after the manner of Clawson (1925), who, however, used a larger quantity of broth. The cultures were incubated at 37° C. and examined and plated at intervals of a week for one month: all the cultures remained sterile with the exception of the growth of *Staphylococcus albus* in four and *B. subtilis* in one.

The tonsillar and post-nasal swabs were cultured in glucose broth for 24 hours, then plated out on broth agar; single colonies were sub-cultured into broth and the process repeated until it appeared under the low power of the microscope that a pure culture had been obtained.

Nature of Organisms Isolated.

It is interesting that from 90 of the primary cultures streptococci were the only organisms grown; in the remaining two *M. pharyngis siccus* and a pneumococcus were grown together with a streptococcus. In all, 146 strains of streptococci were isolated and inoculated into the carbohydrates lactose, saccharose, inulin, mannite, raffinose and salicin. The broth used for the reactions was the low buffer medium previously worked out by myself in conjunction with Prof. McIntosh (Lazarus-Barlow, 1928). The reactions obtained as regards the last four carbohydrates are recorded in Table I.

Table I. *Sugar reactions.*

Inulin	Mannite	Raffinose	Salicin	No. of strains	Group
O.	O.	A.	A.	101	I
O.	O.	A.	O.	30	II
A.	O.	A.	A.	11	III
O.	O.	O.	A.	2	IV
O.	O.	O.	O.	2	V

A. = acid and no gas. O. = no change.

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It is seen that these strains fall into five distinct groups arbitrarily numbered I to V. When first tested 19 cultures fermented inulin, raffinose, and salicin, and it was thought that they were probably mixed cultures of a pneumococcus and a streptococcus. Accordingly they were treated as follows: to a broth culture, sterile ox-bile was added up to 10 per cent., the culture shaken and placed in the incubator at 37° C. for a quarter of an hour; the culture was then centrifuged and the organisms re-inoculated into the carbohydrates. By this means eight strains which were originally placed in group III were placed in group I. Eleven strains, however, still fermented inulin, and these were further tested with a batch of medium containing inulin specially re-purified by Mr Lawson of the Middlesex Hospital. In all 11 cases an acid reaction was again produced.

A similar organism was isolated from the throat of one non-rheumatic child out of 10 cultured.

Owing to the unusual carbohydrate reactions these 12 strains were studied more closely. These organisms were isolated from ten patients; in 5 from the tonsillar region, in 4 from the post-nasal space and in 1 from both sites. Four of these cases, so far as could be ascertained, were suffering from their first attack; their sex and ages were two girls aged 9 years and 11 years, and two boys aged 12 years. The organism is a short chained streptococcus which often appears as a diplococcus; it is about 1·0μ in diameter, has no capsule, is a facultative anaerobe, is bile-insoluble and does not liquefy gelatin. On broth agar it forms small discrete colonies about 1·0 mm. in diameter with a finely granular appearance and regular outline under the low power. In broth there is usually a uniform turbidity in young cultures, the growth tending to fall to the bottom of the tube after a few days: only one strain formed a rather granular growth in broth from the first. The addition of 1·0 per cent. glucose produces a much more profuse growth. On blood agar the organism grows well and causes no change in the medium. Half an hour at 56° C. killed every strain except one. The organism is non-pathogenic to rabbits, guinea-pigs, and mice.

While these researches were proceeding Small (1927) described an organism isolated from the blood of a case of acute rheumatic fever in the first instance, and later from other rheumatic and arthritic conditions. He named this organism *Streptococcus cardioarthritidis*. Shortly afterwards Birkhaug (1927) described a non-methaemoglobin-forming, bile-insoluble, toxin-producing streptococcus which he originally isolated on more than one occasion from a girl aged 5 years suffering from acute rheumatic fever, and later from other cases. The toxin from this organism produced a skin reaction in a larger proportion of cases with a history of rheumatic fever compared with normal persons. In both of these series the organisms fermented inulin, raffinose and salicin with great regularity.

Dr. Small kindly sent me a strain of his organism and this has been compared with strains isolated as described above. Later three strains were received

from Dr. Birkhaug and these too were included in agglutination and other tests carried out for purposes of comparison. Two strains from this hospital sent to Dr. Small were examined by him and in a private communication he stated that he has no doubt that they were strains of *S. cardioarthritidis*.

Agglutinations.

Agglutination tests were carried out using both high and low titre antisera prepared against two strains of *S. cardioarthritidis*. No clean-cut results were obtained, quite marked agglutination taking place with some strains of *S. mitis* and *S. salivarius* which were used for comparison. The results are summarised in Table II.

Table II. *Summary of agglutination reactions.*

	S. cardioarthritidis				*S. mitis*				*S. salivarius*			
		Agglutination				Agglutination				Agglutination		
	No. tested	Good	Fair	Nil	No. tested	Good	Fair	Nil	No. tested	Good	Fair	Nil
Small's antiserum (high titre)	16	15	1	—	33	15	7	11	6	3	—	3
L–B 9 antiserum (high titre)	16	15	1	—	33	20	9	4	6	3	—	3
Small's antiserum (low titre)	16	16	—	—	33	18	9	6	6	1	4	1
L–B 9 antiserum (low titre)	16	16	—	—	33	19	9	5	6	1	4	1

Further agglutination tests were carried out using the serum from rheumatic cases taken on admission to hospital and at monthly intervals for six months. Small's strain, Birkhaug's strain R.F. 1 *b*, and strain L–B 9 of the present series were the organisms tested. Little or no evidence of the presence of agglutinins was found in any of the 69 sera tested from 12 patients. Those agglutinins present were most active against strain L–B 9.

Absorption of Agglutinins.

Attempts were made to differentiate the groups of organisms more sharply by means of absorption of agglutinin tests. The same three strains of *S. cardio-*

Table III. *Absorption of agglutinins.*

	Small's antiserum							R.F. 1 *b* antiserum							L–B 9 antiserum						
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Homologous organism	4	4	4	4	4	3	2	4	4	4	4	4	4	3	4	4	4	4	4	3	1
After absorption with:																					
Strain Small	.	.	.	.	.	.	.	.	3	3	2	2	1	1	.	4	4	3	3	2	.
L–B 9	.	3	3	3	.	.	.	.	3	3	2	.	.	.	.	.	.	.	.	.	.
R.F. 1 *b*	.	3	3	3	2	.	.	.	.	.	.	.	.	.	.	4	3	3	3	1	.
L–B 19 (mitis)	.	3	2	3	2	.	.	.	3	2	3	2	2	.	.	4	3	3	2	2	1
L–B 27 (mitis)	.	4	4	3	3	2	.	.	3	3	3	2	2	.	.	4	4	4	3	3	2
L–B 80 (saliv.)	.	4	4	3	2	3	.	.	3	3	3	2	2	2	.	4	4	4	2	1	.
L–B 81 (saliv.)	.	4	3	3	3	3	.	.	4	3	3	2	2	2	.	4	4	4	3	2	.
L–B 100 (saliv.)	.	4	4	4	3	2	.	.	3	4	3	2	2	2	.	3	3	3	2	2	.

(4 = complete agglutination; 3 and 2 = incomplete agglutination; 1 = trace of agglutination)

arthritidis were used together with two strains of *S. mitis* and three strains of *S. salivarius*. Antisera were prepared against the three strains of *S. cardio-arthritidis* and absorption carried out with a heavy emulsion of organisms at 56° C. for 2 hours: the diluted sera were then put up against the homologous organism. The results which were not clearly defined are shown in Table III.

ATTEMPT TO SHOW THE PRESENCE OF AN EXOTOXIN.

Owing to the infrequency with which organisms have been isolated from the blood in cases of acute rheumatic fever experiments were made to determine if an exotoxin was produced by *S. cardioarthritidis*. These tests were carried out before Birkhaug's strains had been received. Two strains of *S. cardio-arthritidis*, together with strains of *S. mitis* and *S. salivarius* were tested. The technique used for the production of the exotoxin was that described by Parker (1924), the cultures being finally filtered through Pasteur-Chamberland candles L 2. Rabbits were inoculated intravenously with various amounts of filtrate up to 10 c.c. and four-hourly readings of their temperatures recorded. In no case could any definite effect be seen which might be ascribed to a toxin, though occasionally a definite sharp but unsustained rise in temperature was recorded. Possibly readings at more frequent intervals would have been of value (see below).

THE PRODUCTION OF AN ENDOTOXIN OR LYSATE.

A lysate of the organism was prepared by the method described by Duval and Hibbard (1927), and various dilutions inoculated intradermally into the author's forearm. A reaction occurred with some of the stronger solutions and completely disappeared in 48 hours: a week later, however, the erythema in these same areas appeared again, and also in the control area with heated lysate. The reappearance could only be accounted for by the fact that rabbit had been eaten 36 hours previously. Owing to this it was thought best to exclude all foreign protein except the organisms themselves if possible, and the method adopted was the following. The organisms were grown either in broth or on agar, washed in saline, and an emulsion made containing about 10,000 million organisms per c.c. as estimated by an opacity method. This emulsion was placed, with two gross of sterile $\frac{1}{4}$ in. diameter steel balls, into a sterile 9 in. nickel-steel tube of $1\frac{3}{4}$ in. internal diameter and $\frac{1}{8}$ in. walls. The ends of the tubes were plugged with rubber bungs and the whole vigorously shaken for 5 to 6 hours in a special shaker working at at least 500 oscillations per minute. Owing to the friction of the steel balls the temperature rose to about 40° C. and the tubes were allowed to remain in the shaker until quite cold again before being removed. After filtration through Chardin paper to remove dirt, etc. the emulsion was finally filtered through a Pasteur-Chamberland candle L 2.

Intracutaneous tests on rheumatic and non-rheumatic persons using the lysate were inconclusive and need repeating. Intracutaneous tests on rabbits

in dilutions of 1 in 5000 up to the undiluted lysate were negative. A rabbit was then inoculated intravenously with increasing quantities of lysate from strain L–B 9, and on four occasions the temperature was recorded at hourly intervals (see Chart I).

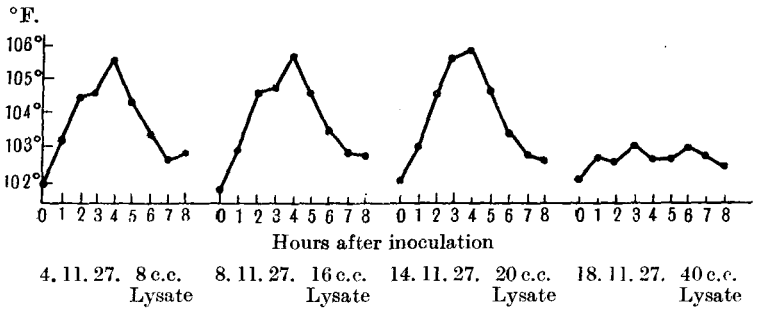

Chart I.

From the charts it is apparent that a definite reaction is caused by the intravenous inoculation of the lysate.

The Production of an Antilysate.

Six days after the last inoculation, the above animal was bled and the serum tested for the presence of an antilysate: three rabbits were inoculated intravenously as follows: (*a*) 5 c.c. unheated lysate, (*b*) 5 c.c. unheated lysate + 5 c.c. of antiserum, (*c*) 5 c.c. lysate heated to 100° C. for one hour. The temperatures were recorded at hourly intervals and are seen in the following chart (Chart II).

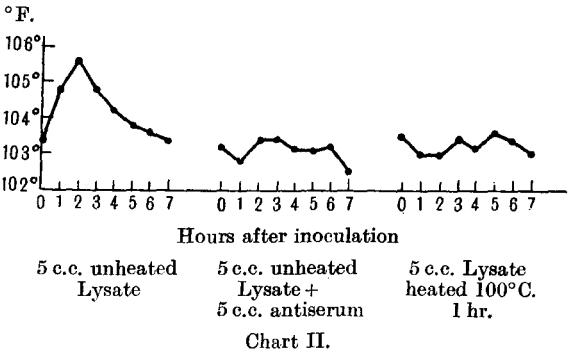

Chart II.

This single experiment seemed to indicate that the inoculation of lysate into a rabbit will give rise to an antilysate and that heating the lysate for an hour at 100° C. destroys its toxicity.

A similar experiment carried out at the same time using the lysate from Small's strain was valueless, as the lysate failed to cause any appreciable rise in temperature. This difficulty is constantly arising because, owing to the great variation in susceptibility of the rabbits, it is impossible to state whether

lysates of equal potency have been obtained on two separate occasions using the same organism and the same technique.

During the time that these experiments were being carried out Dr R. G. White, Director of the Metropolitan Asylums Board's Antitoxin Establishment, was inoculating two horses to see whether an antitoxic serum could be produced. Owing to the difficulty of producing large quantities of lysate at frequent enough intervals, filtrates from broth cultures, and, later, the cultures themselves, were used for inoculation purposes. In the experiments about to be described the unconcentrated antiserum thus produced from strain L–B 9 was used. Further experiments with this serum after concentration, and with the concentrated antiserum to Small's strain are being carried out at the present time.

Lysates were prepared from Small's strain, Birkhaug's strain R.F. 1 *b* and strain L–B 9. Control inoculations were carried out using 5 c.c. of normal horse serum, the antiserum, and pooled serum from rheumatic patients. Chart III shows the mean temperature curves for six rabbits in each case; from these it is evident that the sera alone have no effect on the temperatures.

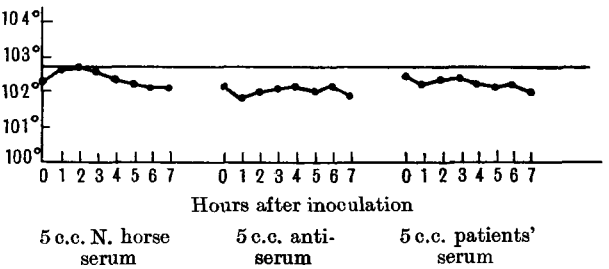

Chart III.

In the first experiment four pairs of animals were tested with each strain as follows: (1) 5 c.c. of lysate + 5 c.c. of saline, (2) 5 c.c. of lysate + 5 c.c. of normal horse serum, (3) 5 c.c. of lysate + 5 c.c. of antiserum, (4) 5 c.c. of lysate + 5 c.c. of pooled serum from rheumatic patients. The various mixtures of lysate and serum were incubated for half an hour at 37° C. before being inoculated. As a lethal effect was doubtful, the effect of the lysates was tested by observations on the temperatures. It has been shown (Lazarus-Barlow, 1928, 2) that the normal temperature of a rabbit is 102·6° F. but that individual animals may normally run a steady temperature either above or below this; and also that mathematically a variation of 1·5° F. from the mean was probably a real variation, and that 2·0° F. was certainly so. During the experiment no food was given, as this causes a rise in the temperature curve. The temperatures were taken immediately before inoculation, and at hourly intervals for one minute by the clock, by inserting a half-minute clinical thermometer into the rectum for $2\frac{3}{4}$ in. They were as recorded in Table IV.

In Chart IV the mean temperature of each pair, and the means for the complete series are seen.

Table IV.

	Weight in grm.	Temperatures ° F. Before inoculation	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
Small's strain									
Lysate + saline	1460	102·8	105·3	104·5	104·4	103·1	102·4	102·4	102·2
+ horse serum	1250	102·2	103·0	103·8	103·8	103·2	102·9	102·6	102·3
+ antiserum	1530	102·3	102·7	103·3	102·2	101·9	101·9	101·8	101·8
+ rheumatic serum	1450	102·0	Died immediately						
Birkhaug's R.F. 1 *b*									
Lysate + saline	1520	101·6	102·3	102·7	103·4	103·6	102·6	102·3	102·0
+ horse serum	1270	101·4	102·1	103·3	103·4	102·8	102·3	101·8	101·8
+ antiserum	1660	102·0	103·1	103·0	102·6	102·0	102·0	101·8	101·6
+ rheumatic serum	1420	102·4	101·9	102·6	104·0	103·2	102·7	102·1	102·2
L–B 9									
Lysate + saline	2250	103·3	104·8	105·6	104·8	104·2	103·8	103·6	103·4
+ horse serum	1210	101·6	102·4	102·6	102·8	102·2	101·8	101·8	101·9
+ antiserum	1850	103·2	102·8	103·4	103·4	103·2	103·1	103·2	102·5
+ rheumatic serum	1780	102·1	102·1	102·4	102·4	102·3	102·0	101·9	101·9
Small's strain									
Lysate + saline	1150	102·6	103·6	103·8	104·8	104·0	103·4	102·8	102·4
+ horse serum	1240	102·7	105·0	104·2	105·8	105·4	103·6	102·7	102·5
+ antiserum	1320	101·6	102·4	102·7	103·9	103·8	103·5	102·7	102·2
+ rheumatic serum	1360	101·8	103·0	103·4	104·6	104·2	102·9	102·5	101·9
Birkhaug's R.F. 1 *b*									
Lysate + saline	1100	102·7	105·1	104·7	104·9	105·2	104·5	104·3	104·5
+ horse serum	1100	102·5	102·9	101·8	102·8	104·0	104·4	103·7	103·1
+ antiserum	1110	102·3	104·1	103·8	106·4	105·7	104·1	102·6	102·2
+ rheumatic serum	1130	102·7	104·3	103·2	104·7	105·2	105·0	103·8	102·7
L–B 9									
Lysate + saline	1020	102·3	103·3	104·6	104·0	102·8	102·6	102·1	101·9
+ horse serum	950	102·0	102·3	103·4	102·8	102·2	102·1	102·1	101·8
+ antiserum	1010	102·3	102·5	104·2	102·8	101·8	102·0	102·2	102·0
+ rheumatic serum	990	101·9	Died during inoculation						
Means of above									
Lysate + saline		102·5	104·1	104·3	104·4	103·8	103·2	102·9	102·7
+ horse serum		102·6	103·0	103·2	103·6	103·3	102·8	102·5	102·3
+ antiserum		102·3	102·9	103·4	103·7	103·1	102·8	102·4	102·1
+ rheumatic serum		102·3	102·8	102·9	103·9	103·7	103·3	102·6	102·2

In the case of Small's strain 5 c.c. of lysate cause a sharp rise of temperature reaching its maximum in three hours and gradually falling to the normal again. Much the same rise occurs when 5 c.c. of either normal horse serum or rheumatic serum is added, whereas 5 c.c. of lysate + 5 c.c. of antiserum produce little effect. With Birkhaug's strain a similar sharp rise is seen with the lysate alone, and is maintained for a longer period. Normal horse serum has checked the effect of the lysate very considerably, whereas with the antiserum and with rheumatic serum a definite rise is seen, but in both cases the drop is sharper than with the lysate alone. With L–B 9 there is also a sharp rise with the lysate alone and a hardly appreciable rise in the case of lysate + antiserum; here, again, normal horse serum has had some neutralising effect on the lysate. Taking the mean temperatures for the series it appears that 5 c.c. of lysate will cause a sharp rise reaching its maximum of about 2° F. in 3 hours, and gradually falling to the normal again; 5 c.c. of normal horse serum or antiserum check the rise somewhat, the latter tending to bring the

temperature back to normal earlier. Serum from rheumatic patients has little or no effect.

In a second experiment 10 c.c. of lysate were inoculated, the saline and

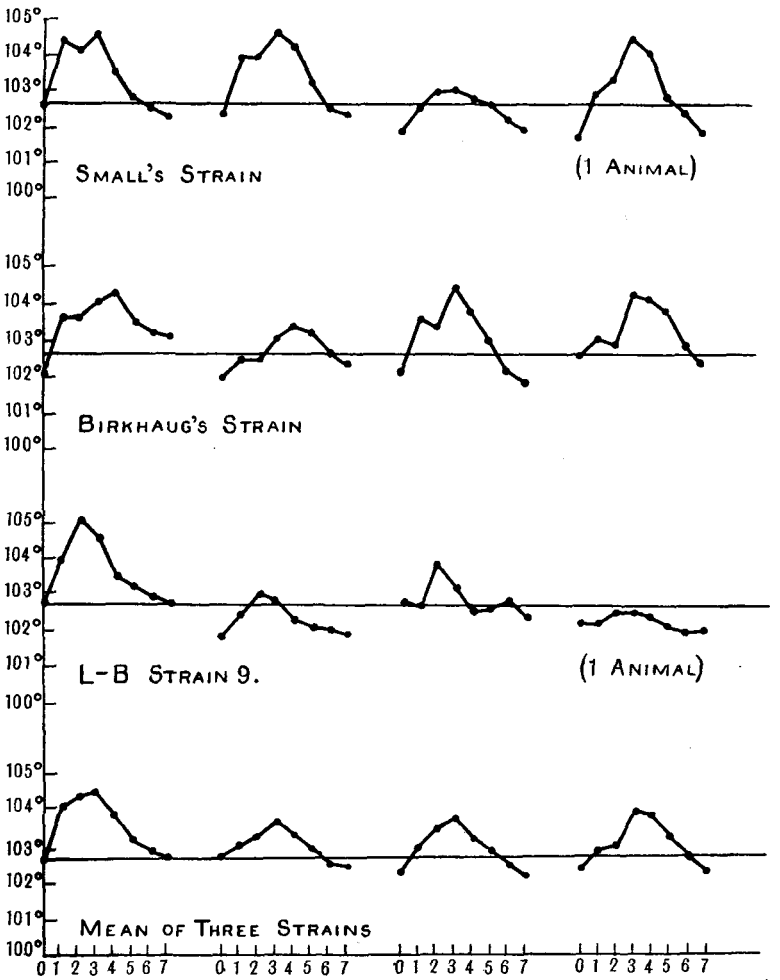

Chart IV.

sera being again 5 c.c. The temperatures were recorded in the same manner as in Exp. 1 (see Table V) and are recorded in Chart V.

In the case of Small's strain 10 c.c. of lysate caused a profound effect in one rabbit, the temperature falling from 102·8° F. to 95·9° F. in 2½ hours when death occurred. Nothing could be seen *post mortem* except a small amount of blood-stained peritoneal exudate, and extensive congestion of the

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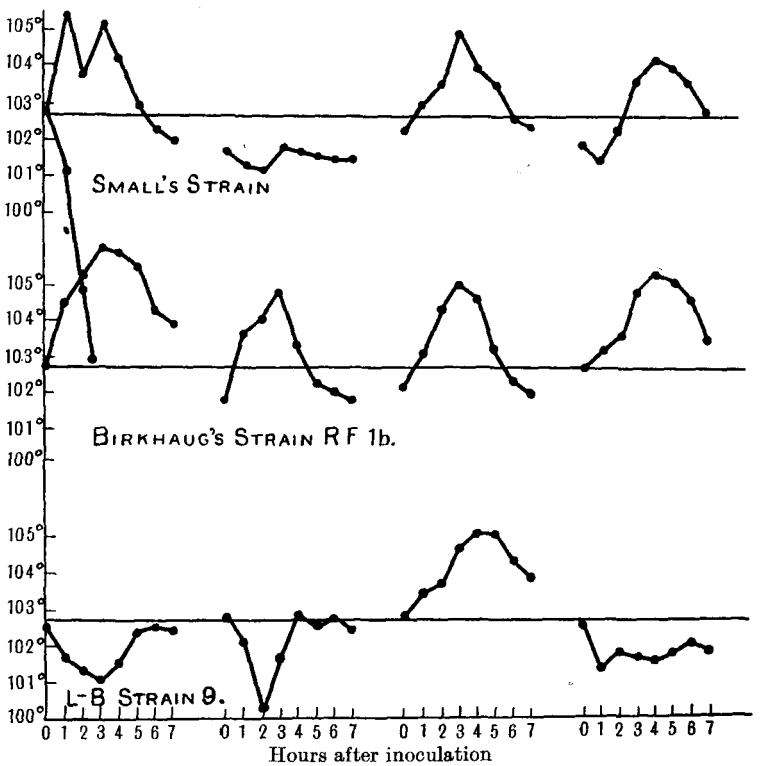
Table V.

	Weight in grm.	Temperatures ° F. Before inoculation	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
Small's strain									
Lysate + saline	1110	102·8	101·1	97·9	95·9	Died in 2½ hours			
+ saline	850	102·6	105·5	103·8	105·2	104·3	103·0	102·3	102·0
+ horse serum	1150	101·7	101·4	101·2	101·9	101·7	101·6	101·5	101·5*
+ antiserum	1190	102·3	103·0	103·6	105·0	104·0	103·5	102·6	102·4†
+ rheumatic serum	1100	101·9	101·4	102·3	103·6	104·3	104·0	103·6	102·3
Birkhaug's R.F. 1 *b*									
Lysate + saline	1100	102·7	104·5	105·1	106·0	105·9	105·5	104·3	103·9‡
+ horse serum	1340	101·7	103·6	104·0	104·8	103·3	102·2	102·0	101·8
+ antiserum	1200	102·1	103·0	104·3	105·0	104·5	103·1	102·3	102·0
+ rheumatic serum	1330	102·6	103·1	103·5	104·7	105·3	105·1	104·6	103·4
L–B 9									
Lysate + saline	1000	102·5	101·6	101·3	101·0	101·4	102·3	102·5	102·4§
+ horse serum	1100	102·7	102·0	100·2	101·6	102·8	102·5	102·6	102·4
+ antiserum	1280	102·8	103·4	103·6	104·6	105·0	105·0	104·3	103·8‖
+ rheumatic serum	1240	102·5	101·3	101·7	101·6	101·5	101·7	102·0	101·8

* Very ill, died in 3 days. † Died in 19 days. ‡ Died in 10 days.
§ Died in 17 days. ‖ Died in 11 days.



10 c.c. Lysate | 10 c.c. Lysate + 5 c.c. N. horse serum | 10 c.c. Lysate + 5 c.c. anti-serum | 10 c.c. Lysate + 5 c.c. patients' serum

Chart V.

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viscera. When an attempt was made to repeat this result, using a smaller animal, only an accentuation of the temperature curve obtained by the inoculation of 5 c.c. was obtained. This shows clearly the great difference in susceptibility of individual animals. When 10 c.c. of lysate + 5 c.c. of horse serum was given the animal appeared to be ill and the temperature remained low, but no lethal effect was obtained until three days later: 5 c.c. of antiserum with the lysate resulted in a sharp rise in temperature which reached its maximum in 3 hours, whereas the lysate alone caused a rise reaching its maximum in 1 hour. This animal died in 19 days. The antiserum seems either to prevent an immediate lethal effect or to minimise the immediate reaction to some extent, even if the animal die later. Rheumatic serum had no appreciable effect. With Birkhaug's strain the results were similar to those obtained when 5 c.c. of lysate were inoculated, only accentuated. The animal inoculated with lysate alone died in 10 days; the antiserum delayed and minimised the rise of temperature and brought it down to the normal again within 7 hours; the animal was alive 21 days later. In the case of L–B 9 the lysate produced an appreciable fall in temperature; the animal was decidedly ill and died in 17 days: horse serum combined with lysate produced much the same temperature curve. The curve resulting from 10 c.c. of lysate + 5 c.c. of antiserum is similar to the curve obtained with 5 c.c. of lysate alone; the animal, however, died in 11 days.

Owing to the great difference in the reaction of the animals in this experiment it is useless to take the mean readings as was done in Exp. 1, but taking the data as a whole, and making allowances for the different potencies of the lysates, it would seem that 10 c.c. of lysate will produce a marked effect on the temperature and usually kill the animal sooner or later, and that the addition of 5 c.c. of antiserum either minimises the immediate effect or prolongs the life of the animal. Only those animals inoculated with lysate and lysate + antiserum were kept for more than a week owing to lack of accommodation, so that the ultimate result of the inoculation of lysate + horse serum and lysate + rheumatic serum has not been investigated.

Of the original batch of lysates made for these experiments there was only enough left in the case of L–B 9 to inoculate 15 c.c. and 15 c.c. + 5 c.c. of antiserum. Of these two animals the one receiving 15 c.c. of lysate died in 70 minutes and the one receiving 15 c.c. of lysate + 5 c.c. of antiserum was alive 20 days later. Nothing could be seen *post mortem* except congestion of all the viscera. In the animal which died three days after receiving 10 c.c. of lysate of Small's strain + 5 c.c. of horse serum, emphysema and congestion of the lungs and congestion of the liver were seen *post mortem*, but degenerative changes were so advanced that histological examinations were not made. In the other four animals the *post-mortem* findings and histological appearances were as follows:

1. 10 *c.c. lysate of Small's strain* + 5 *c.c. antiserum: died in* 19 *days.*

Emphysema and congestion of lungs. Congestion of coronary vessels. ? Some thickening of the mitral valve. Visceral congestion.

Heart muscle and mitral valve. Congestion of the capillaries in the heart muscle and of the coronary vessels. Definite separation of the muscle bundles. The valve appeared healthy.

Liver. Congestion of the main vessels throughout and of the capillary spaces in parts. Capillary spaces distended throughout. Some cloudy swelling.

Kidney. Marked congestion especially amongst the straight tubules. Some glomerular congestion. Degeneration of the convoluted tubules, the cytoplasm being granular and broken up; in places the nuclei have disappeared. Many tubules filled with granular debris. The cytoplasm of the straight tubules shows some degeneration, the nuclei in many places lying in a clear space within the cell wall.

2. 10 *c.c. lysate of* R.F. 1 *b: died in* 10 *days.*

Emphysema and congestion of lungs which contained localised small purplish areas. General congestion. Some peritoneal exudate.

Heart muscle. Some separation of the muscle bundles and poor striation in parts.

Lung. Marked congestion and emphysema. In places there are small areas of complete collapse.

Liver. Marked congestion throughout: the cells appear healthy.

Kidney. Congested throughout. Some swelling and degeneration of the cells of the convoluted tubules in places, but on the whole the organ appears to be in good condition.

3. 10 *c.c. lysate of* L–B 9*: died in* 17 *days.*

Emphysema of lungs. Marked congestion of coronary vessels. General congestion.

Heart muscle and mitral valve. Congestion of the muscle; the individual fibres are much separated in places, and for the most part the striation is poor or absent. The mitral valve appeared healthy.

Liver. Congested. In places there is some granularity of the liver cells, and in isolated areas the cells have been destroyed and replaced by an infiltration of fine fibrous tissue.

Kidney. Congested, especially in the region between the cortex and medulla. There is some degeneration of the convoluted tubules, marked in places.

4. 10 *c.c. lysate of* L–B 9 + 5 *c.c. antiserum: died in* 11 *days.*

Emphysema and congestion of lungs, with isolated purplish patches at the edges. Congestion of the coronary vessels and ? some swellings on the mitral valve. General congestion.

Heart muscle and mitral valve. The muscle is congested. Some separation of the muscle bundles and of the muscle fibres. In the mitral valve there are numerous large and several small areas of haemorrhage. The haemorrhages are chiefly on the auriculo-ventricular aspect of the valve, and here the endothelium appears swollen. In one place the sub-endothelial haemorrhage has burst through the endothelium. There is a large vessel engorged and containing a large number of polymorphonuclear leucocytes.

Lung. Very congested. There are large areas of marked emphysema throughout the section, and in places areas of complete collapse.

From these histological specimens it is seen that the lysates of the three strains used have some definite effect on the heart muscle and possibly also on the mitral valve. The liver and kidneys also are affected, and in those cases examined emphysema and areas of collapse in the lungs were marked features.

Further animal experiments using concentrated sera are being carried out, and the sera are being used therapeutically as suitable cases occur.

SUMMARY OF RESULTS.

1. Bacteriological examination from the tonsils and post-nasal space in forty-six cases of acute and sub-acute rheumatism resulted in streptococci alone being isolated.

2. The streptococci isolated fell into three groups, *S. mitis*, *S. salivarius*, and eleven strains of *S. cardioarthritidis*.

3. Comparison of the strains of *S. cardioarthritidis* with strains isolated in America show them to be closely related.

4. No evidence was found of an exotoxin.

5. Evidence was found of the presence of an endotoxin or lysate which produces an antitoxin.

6. Intravenous inoculation of the lysate into rabbits appears definitely to affect the heart muscle and lungs, and to some extent the liver and kidneys.

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