A CONTRIBUTION TO THE BACTERIOLOGY OF RHEUMATIC FEVER.

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PLATE VI.

Since Wasserman in 1899, experimenting with a diplococcus which he had isolated from a case of chorea, produced what he regarded as typical rheumatic fever in a series of eighty rabbits, much has been written for any against his view.

I do not propose in this paper to, in any way, review the position or to criticize the work of other observers. The bacterial origin of rheumatic fever seems to need no defence. The clinical features, the epidemic nature and the irregular periodicity all bring it in line with well-known bacterial diseases. Recently I have described amyloid degeneration in four cases of long standing subacute rheumatism. These cases were very typical, pure cases of rheumatism and neither in the clinical history nor in the histological examination of the organs after death was there anything to suggest any of the ordinary causes of amyloid degeneration.

Though it is true that the causes of amyloid degeneration are not clearly understood, yet it is generally recognized that the condition is secondary to some infective agent, and that, most generally, bacterial. All the experimental evidence of Krakow, Maximow, Davidsohn and others goes to support the bacterial cause.

When, however, we come to deal with the specific bacterium of rheumatism we enter much more controversial fields. It seems to me that we can dismiss from our consideration all the organisms which have been described at various times as causal except the diplococcus of Wasserman, and that which has been isolated and described in England by Poynton and Paine,[1, 2, 3] Walker,[4] Saw,[5] and myself;[6] this bacterium is probably the same as that

described by Wasserman. By these observers this organism has been isolated from definite cases of acute rheumatism, has been cultivated outside the body, has produced in rabbits and monkeys polyarthritis, endocarditis and other manifestations of rheumatic fever; and has again been isolated from the lesions in these organs. The case at first sight seems very strong, but various objections have been lodged, and it is with these that I propose dealing here.

The organism isolated, it has been thought, is not causal, the infection being simply a terminal one. Had this objection not been raised in authoritative quarters it would have been hardly worth dealing with. Terminal bacterial infections are not by any means common; besides the organism has been isolated from patients during life and when they have not been specially ill. Again, if we get a terminal bacterial infection, we expect it to be general and would expect to find the causal organism in the blood. In the three cases which I have examined after death (two of them only a few hours after), culture tubes inoculated from the blood remained sterile, and cultures of the organism were got from isolated areas only in the synovial membranes.

Acute rheumatism, it is claimed, is simply an attenuated pyæmia, and the organism isolated is an ordinary Streptococcus pyogenes. This objection has been supported by many eminent bacteriologists and must therefore be examined in detail. Rheumatism in many respects does resemble pyæmia. Both exhibit themselves in arthritis, endocarditis, etc., but there are definite distinguishing features, and save perhaps in the early stages clinicians do not mistake the one condition for the other.

Considering the very severe symptoms, and often the rapidly fatal issue in cases of acute rheumatism it seems to me a travesty of terms to speak of it as an "attenuated pyæmia." Again in pyæmia, and especially where the organism is not very active, pus formation is the common result; with acute rheumatism pus formation is the exception. As will be seen from the subjoined experiments pus was quite common in arthritis following injections of various forms of streptococci, whereas in the arthritis following inoculation with *Micrococcus rheumaticus* pus was not got in a single case.

For purposes of comparison I have examined and carried out inoculation experiments with twelve strains of streptococci and three of the special rheumatic organism:

Streptococcus 1 was isolated from a case of cellulitis; *2*, from the pus in the knee joint in a case of pyæmia; *3*, from pus in the mastoid cells in a case of middle ear disease; *4*, from a similar case; *5*, from a pyæmic abscess in the region of the appendix following an operation on a septic knee joint; *6*, from the throat in a case of measles; *7*, from the throat of a patient with scarlet fever; *8* and *9*, from separate cases of diphtheria; *10*, from a case of measles; *11*, from an acute tonsillitis; and *12*, from a case of diphtheria.

Micrococcus rheumaticus was isolated from three cases of definite acute rheumatism. These organisms were all identical in their cultural characters, but as the majority of the inoculations were carried out with one strain only, it is taken as the standard throughout the paper.

MICROCOCCUS RHEUMATICUS.

Micrococcus rheumaticus was isolated from a case of acute rheumatism on December 9, 1904, and since that time it had been subcultured frequently, often, however, intervals of two months elapsing between the times of subcultivation. The history of the case and the results of the inoculation experiments have already been published[7] and need not be further referred to here.

Morphological Characters and Staining Reaction.—It may be stated as a general rule that the coccus we have isolated, and which for convenience we call *Micrococcus rheumaticus* or *Streptococcus rheumaticus* is in its morphological characters and staining reactions indistinguishable from strains of *Streptococcus pyogenes.*

Cultural Characters.—Micrococcus rheumaticus grows quite readily at the room temperature. The growth on gelatine at the room temperature is very definite in twenty-four hours, and much more copious than the growth of any of the varieties of streptococci which were used. The acid production is extremely marked in any of the ordinary media, but some of the strains of streptococci used gave quite as marked an acid reaction, and as will be seen in the tables below the acid production and the reactions in the various sugars gave no help in distinguishing the one class of organism from the other.

The only definite and very distinctive reaction was the production of acid and precipitation of the bile salts by *Micrococcus*

rheumaticus in McConkey's bile salt lactose broth. No reaction was got in this medium with any of the strains of streptococci used. This difference was so marked that the observations were repeated with exactly similar results. The vitality of the organism outside the body I have referred to in a previous publication, but this was most strikingly illustrated during these investigations. Constant subculture was necessary to keep the streptococci alive, and three of the strains were lost during the course of the investigations. With *Micrococcus rheumaticus* there was not the slightest difficulty; several months could elapse and quite active subcultures could be obtained both in blood agar and in ordinary agar.

	Raffinose.			Inulin.			Saccharose.			Salacin.			Mannite.		
	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3
Mic. rh.	o	x	x	o	o	o	o	(x)	x	x	x	x	o	o	o
Strepto. I.	o	o	o	o	o	o	o	o	o	o	o	(x)	o	o	o
" II.	o	o	o	o	o	o	o	x	x	o	o	o	o	o	o
" III.	o	o	o	o	o	o	o	o	o	o	o	x	o	o	o
" IV.	o	o	o	o	o	o	o	x	x	o	o	o	o	o	o
" V.	o	o	o	o	o	o	o	x	x	o	o	o	o	o	(x)
" VI.	o	o	o	o	o	x	o	o	o	o	o	o	o	o	o
" VII.	o	x	x	o	o	x	o	(x)	x	o	o	o	o	o	o
" VIII.	o	x	x	o	o	o	o	o	x	o	x	x	o	o	o
" IX.	o	x	x	o	o	(x)	o	x	x	o	x	(x)	o	o	o
" X.	x	x	x	o	o	(x)	o	x	x	o	o	x	o	o	o
" XI.	o	o	o	o	o	o	o	o	(x)	o	o	o	o	o	o
" XII.	o	x	x	o	o	o	o	x	x	o	x	x	o	o	o

x indicates acid change; o indicates a negative result. Brackets ( ) indicate feeble development of the change.

	Milk.			Litmus Milk.			Taurocholate Broth.			Neutral Red.		
	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys. 2	Dys. 3
Mic. rh.	o	xc	xc	x	xc	xc	x	x	x	o	o	x
Strepto. I.	o	o	o	o	o	(x)	o	o	o	x	x	x
" II.	o	o	o	o	(x)	(x)	o	o	o	o	o	o
" III.	o	o	o	o	x	o	o	o	o	o	o	o
" IV.	o	o	o	o	o	o	o	o	o	o	o	o
" V.	o	o	o	(x)	(x)	(x)	o	o	o	(x)	(x)	(x)
" VI.	o	(xc)	(xc)	x	x	x	o	o	o	o	o	o
" VII.	o	xc	xc	x	xc	xc	o	o	o	o	o	x
" VIII.	o	o	o	x	x	x	o	o	o	o	x	x
" IX.	o	xc	xc	x	xc	xc	o	o	o	o	o	x
" X.	o	xc	xc	xc	xc	xc	o	o	o	o	o	x
" XI.	o	o	o	x	x	x	o	o	o	o	o	x
" XII.	o	o	o	x	x	xc	o	o	o	o	o	o

Brackets ( ) indicate feeble development of the respective change. c indicates coagulation.

The following tables show the cultural reactions in sugars, etc., of the strains of streptococci examined and of *Micrococcus rheumaticus.*

	Glucose Broth.			Lactose Broth.			Maltose Broth.		
	Hrs. 24	Dys. 2	Dys. 3	Hrs. 24	Dys 2	Dys. 3	Hrs 24	Dys. 2	Dys. 3
Mic. rh.	x	x	x	x	x	x	x	x	x
Strepto. I.	x	x	x	(x)	x	x	x	x	x
" II.	x	x	x	x	x	x	x	x	x
" III.	No growth.			No growth.			No growth.		
" IV.	x	x	x	(x)	x	x	x	x	x
" V.	x	x	x	x	x	x	x	x	x
" VI.	x	x	x	x	x	x	x	x	x
" VII.	x	x	x	x	x	x	x	x	x
" VIII.	x	x	x	x	x	x	x	x	x
" IX.	x	x	x	(x)	x	x	o	x	x
" X.	x	x	x	x	x	x	x	x	x
" XI.	x	x	x	x	x	x	(x)	x	x
" XII.	x	x	x	x	x	x	x	x	x

INOCULATION WITH STREPTOCOCCUS.

Cultures for these experiments were made on ordinary sloped agar tubes and an emulsion was made in 0.85 per cent. saline solution immediately before inoculation. It has not been deemed necessary to deal in detail with the microscopic examination of the various organs, though this has been carried out in a considerable number of cases.

The tables appended give the main results necessary for comparative purposes.

In this table lesions such as are common to any septic infection, *e. g.*, cloudy swelling, etc., will not be referred to.

Organism.	Rabbit.	Date and Site of Injection	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 1.	1	17 Jan., 1906, intravenous.	Half agar tube.	Killed, 5 Feb.	Swelling left wrist, 22 Jan.; fluctuation in left and right wrists with redness of skin, 30 Jan.	Purulent; in right and left wrists.	Nil.	Nil.
	2	17 Jan., 1906, right knee joint.	Half agar tube.	Killed, 5 Feb.	Tenderness, swelling, fluctuation in right knee.	Purulent; in right knee with infiltration with pus of tissues round.	Nil.	Nil.
	3	26 Feb. to 2 Apr., intraperitoneally.	Two loops to one agar tube.	Killed, 2 Apr.	Nil.	Nil.	Nil.	Nil.
	4	4 June, intravenous.	One agar tube.	Killed, 11 July.	Ill for two days.	Nil.	Nil.	Nil.
	5	4 June, intravenous.	Two agar tubes.	Killed, 11 July.	"	Nil.	Nil.	Nil.
Streptococcus 2.	1	24 Jan., intravenous.	Half tube.	Died, 4 Feb.	31 Jan., very ill, no evidence of joint affection.	Nil.	Nil.	Septicaemia; cultures from heart blood.
	2	24 Jan., intravenous.	Half tube.	Died, 16 Feb.		Nil.	Nil.	Heart very much dilated; septicaemia.
	3	26 Feb. to 2 Apr. 7 injections intraperitoneally.	Two loops to one tube.	Killed, 2 Apr.	Nil.	Nil.	Nil.	Nil.
	4	5 June, intravenous.	Half tube.	Died, 10 June.	—	—	—	Septicaemia; (organisms in blood).

Organism.	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 3.	1	17 Jan., subcutaneous.	One tube.	Killed, 2 Apr.	Pus formed at site of injection.	Nil.	Nil.	Nil.
	2	17 Jan., knee joint.	Half agar tube.	Killed, 2 Apr.	Swelling, tenderness and fluctuations in injected joint.	At death joint dislocated and fixed.	Nil.	Nil.
	Same rabbit (2)	3 Mar., intraperitoneally.	One agar tube.	—	Joint not altered by this injection.	—	—	—
	3	24 Jan., intravenously.	Half agar tube.	Killed, 2 Feb.	Lameness, swelling in right wrist, 30 Jan.; 31 Jan., fluctuation.	Purulent; right wrist.	Nil.	Nil.
	4	7 injections, 26 Feb. to 22 Mar. intraperitoneally.	Two loops to one tube.	Died, 23 Mar.	—	—	—	Septicaemia; streptococci in general circulation.
	5	4 June, intravenously.	One tube.	Killed, 27 June.	Nil.	Nil.	Nil.	Nil.
	6	4 June, intravenously.	One tube.	Killed, 27 June.	Nil.	Nil.	Nil.	Nil.
Streptococcus 4.	1	17 Jan., subcutaneous.	One tube.	Killed, 2 Apr.	Nil.	Nil.	Nil.	Nil.
	2	17 Jan., knee joint.	Half tube.	Killed, 15 Feb.	Swelling and fluctuation in injected knee.	Purulent; knee; erosion of bone; infiltration of tissues round knee.	Nil.	Nil.
	3	24 Jan., intravenously.	One tube.	Killed, 2 Apr.	Nil.	Nil.	Nil.	Nil.

Organism.	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 4.	4	26 Feb. to 2 Apr., 7 injections intraperitoneally.	Two loops to one tube.	Killed, 2 Apr.	Nil.	Nil.	Nil.	Nil.
	5	30 May, intravenously.	One tube.	Killed, 16 June.	6 June, lameness left fore limb.	Purulent; left wrist.	Nil.	Nil.
	6	30 May, intravenously.	Two tubes.	Killed, 16 June.	6 June, tenderness and swelling left wrist; 12 June, fluctuation.	Purulent; left wrist.	Nil.	Nil.
Streptococcus 5.	1	16 Feb., intravenously.	Fourth tube.	Killed, 22 Feb.	17 Feb., swelling and pain in right wrist; 22 Feb., fluctuation, pain and swelling in left wrist.	Purulent; right wrist; synovial membranes in left wrist congested; no pus.	Nil.	Nil.
	2	26 Feb. to 2 Apr., 5 injections intraperitoneally.	Two loops to one tube.	Killed, 5 Apr.	Nil.	Nil.	Nil.	Nil.
	3	5 June, intravenous.	One and one-half tubes.	Died, 7 June.	Nil.	Nil.	Nil.	Septicaemia; organisms in blood.
	4	5 June, intravenous.	One and one-half tubes.	Killed, 16 June.	10 June, lame left fore limb; 12 June, fluctuation in left wrist.	Purulent; left wrist.	Nil.	Nil.
Streptococcus 6.	1	8 May, subcutaneous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.

Organism	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 6.	2	16 May intravenous.	One tube.	Killed, 4 June.	Paralysis of both hind limbs, 22 May.	Nil.	Nil.	An abscess in lumbar region of the spinal canal pressing on the spinal cord.
		This organism died before further experiments could be made with it.						
Streptococcus 7.	1	16 May, intravenous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
	2	27 June, intravenous.	One tube.	Killed, 11 July.	Nil.	Nil.	Nil.	Nil.
	3	27 June, intravenous.	Two tubes.	Killed, 11 July.	Nil.	Nil.	Nil.	Nil.
	4	15 June to 10 July, subcutaneous and intraperitoneal; 5 injections.	Half to one tube.	Killed, 24 July.	Nil.	Nil.	Nil.	Pus at the site of subcutaneous injection.
Streptococcus 8.	1	8 May, intravenous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
	2	16 May, intravenous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
	3	29 May, intravenous.	Two tubes.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
		The organism died before further experiments could be made.						
Streptococcus 9.	1	16 May, intravenous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.

Organism.	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 9.	2	12 July, intravenous.	Three tubes.	Killed, 17 July.	Nil.	Nil.	Nil.	Nil.
	3	15 June to 10 July, subcutaneous and intravenously 5 injections.	Half to one tube.	Killed, 24 July.	Nil.	Nil.	Nil.	Pus at the site of subcutaneous inoculation.
Streptococcus 10.	1	16 May, intravenous.	One tube.	—	—	—	—	—
	2	25 May, intravenous.	Two tubes.	Died, 5 June.	Paralysis of both hind limbs.	Nil.	Aortic endocarditis; vegetations large; ulceration of valves (ulcerative endocarditis).	Abscess in spinal column pressing on lower part of spinal cord; pyaemic abscesses in kidney.
	3	18 June, intravenous.	One tube.	Killed, 12 July.	Nil.	Nil.	Nil.	Nil.
	4	26 June, intravenous.	One tube.	Killed, 11 July.	Nil.	Nil.	Nil.	Nil.
	5	15 June to 10 July, subcutaneous and intraperitoneally; 5 injections.	Half to one tube.	Killed, 24 July.	Nil.	Nil.	Nil.	Pus at the site of subcutaneous inoculation.

Organism.	Rabbit	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Streptococcus 11.	1	16 May, intravenous; and 15 May to 10 July, subcutaneously and intraperitoneally.	One tube. Half to one tube.	Killed, 17 July.	15 June, paralysis of both hind limbs, from which it completely recovered; abscess at site of subcutaneous injection.	Nil.	Nil.	Nil.
	2	25 May, intravenous.	Two tubes.	Died, 1 June.	27 May, swelling and tenderness left wrist.	Purulent; left wrist.	Nil.	Nil.
	3	18 June, intravenous.	One tube.	Killed, 25 June.	21 June, lameness left fore limb; swelling of left wrist with considerable oedema.	Purulent; left wrist.	Nil.	Nil.
Streptococcus 12.	1	16 May, intravenous.	One tube.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
	2	20 May, intravenous.	Two tubes.	Killed, 3 July.	Nil.	Nil.	Nil.	Nil.
	3	28 June, intravenous.	One tube.	Killed, 12 July.	Nil.	Nil.	Nil.	Nil.
	4	15 June to 10 July, subcutaneous and intraperitoneal, 5 injections.	Half to one tube.	Killed, 24 July.	Nil.	Nil.	Nil.	Abscess at site of subcutaneous inoculation.

Summary of Inoculations with Streptococcus.

Intravenous	34
Intraperitoneal	7
Subcutaneous	3
Both intraperitoneal and subcutaneous	4
Total	48
Number of deaths	7 or 14.5%
Number of animals with arthritis	9 or 18.7%
Number of animals with endocarditis	1 or 2.0%

In all cases the arthritis was definitely purulent. In animals injected subcutaneously pus formed at the site of injection.

In the single experiment with endocarditis, there was distinct ulceration of the valves and the organisms were invading the adjacent muscle of the heart. There were pyæmic abscesses in the kidney, and an abscess in the lower dorsal vertebra. This lesion was evidently pyæmic endocarditis, a condition which everyone admits may occur in the course of a septic infection whatever be the organism present.

INOCULATION WITH MICROCOCCUS RHEUMATICUS.

Rabbit 1.—Inoculated in the right knee on November 8, 1905; was killed on March 26, 1906. There was stiffness about the right knee, but otherwise the animal appeared to be in perfect health. On March 8 it received an intraperitoneal injection of one half tube of culture. The following day the knee was swollen and painful, but in four days the acute symptoms had passed off. It was reinoculated in the peritoneal cavity on March 15 and 22, and on each occasion the acute symptoms reappeared and subsided again. At the post-mortem examination the joint contained a very small quantity of viscid exudation, which on examination was mainly composed of large mononucleated cells. Polymorphonuclear leucocytes were present but were very few in numbers. There was considerable destruction of cartilage with numerous small superficial erosions of the bone. No organisms of any kind were found.

There was a small recent vegetation on one of the aortic cusps. No cultivations were obtained from it. The other organs showed nothing apart from the usual toxic changes.

Rabbit 2.—Inoculated on March 29 with two tubes intravenously. April 2 the animal was lame in the right fore limb. No swelling could be made out. The lameness became less, but the animal was killed on April 6. There was well-marked aortic endocarditis, the vegetations being about a sixteenth of an inch in diameter, and pure cultures of the organism were obtained from these. With the exception of very slight injection in the synovial membranes of the left elbow nothing abnormal could be made out in any of the joints.

Rabbit 3.—Inoculated intravenously with one tube of culture on March 29,

1906; showed lameness in the left fore limb on April 5, and was killed the following day. At the post-mortem examination there was very evident congestion in the synovial membranes in the left elbow. There was also a slight amount of congestion in the left hip joint. Tubes were inoculated from portions of the synovial membranes in the congested joints but no growth was obtained. There was no endocarditis.

Rabbit 4.—Inoculated intravenously with one tube of culture on May 24, 1906; showed no symptoms of any kind during life. It was killed on June 12, but nothing pathological was made out. There was no arthritis and no endocarditis.

Rabbit 5.—Inoculated intravenously with two tubes of cultures on May 24; showed no symptoms during life. It was killed on June 12. The mitral and tricuspid segments were distinctly thickened and though no distinct vegetations could be made out there was slight nodular thickening along the free edge of the tricuspid segments. There was a firm fibrous nodule about an eighth of an inch in diameter in the septum between the ventricles. On microscopic examination this showed well-marked interstitial myocarditis. In the kidneys were some small pin-point whitish areas seen under the capsule. On section these areas extended in a wedge into the kidney substance, some of them passing almost to the hilus. On microscopic examination they were seen to be composed of masses of small lymphoid cells, with very slight development of fibrous tissue.

These interstitial charges in the heart and kidney may have been accidental and independent of the injection, but in none of the other animals examined during this investigation was the same heart condition seen. The kidney lesion was observed in three animals and these had been inoculated with Micrococcus rheumaticus. In some recent experiments by Dr. Henry Wade of Edinburgh a number of dogs were inoculated with the well-known infective granuloma of the dog. In the great majority of these experiments the kidneys showed interstitial nephritis, and in the early stages the appearance was identical with that seen in these rabbits. The interstitial changes in the kidney of the dog are undoubtedly toxic in origin, and I am inclined to attribute the changes I have described also to toxines.

Rabbit 6.—Inoculated intravenously on May 30 with one tube of culture. No symptoms developed. The rabbit was killed on June 22. There was no evidence of arthritis. On the tricuspid segments there were some very recent, fairly firm, but rather doubtful vegetations. No organisms were found. There was marked œdema of the aortic segments, but no trace of endocarditis. On microscopic examination it was difficult to determine whether the deposit on the tricuspid segments was not merely blood clot.

Rabbit 7.—Inoculated intravenously on May 30 with two tubes of culture. No symptoms developed. The rabbit was killed on June 22. There was no evidence of arthritis or of endocarditis. In both kidneys there were numerous small dark wedge shaped areas depressed slightly below the surface. The naked eye appearance suggested infarctions. On microscopic examination the area examined was found to be composed of masses of small lymphoid-like cells, and was identical with the areas seen in Rabbit 5. No bacteria could be found in these areas.

Rabbit 8.—Inoculated intravenously on June 5 with three agar tubes of the culture. No symptoms developed, and the rabbit was killed on June 22. There was no arthritis, but on the anterior mitral valve segment there was a small recent vegetation. No culture tubes were inoculated from this vegetation, but on microscopic examination a few organisms, practically unaltered in appearance, were found just at the junction of the vegetation with the tissue of the valve.

Rabbit 9.—A young rabbit of 600 grams weight was inoculated intravenously on June 13 with the culture from four agar tubes. On June 17 the rabbit looked very ill, and could not be got to move about. It was impossible to decide as to the presence or absence of arthritis. The animal died on June 18 and at the post mortem examination a slight amount of serous fluid was found in both elbows, but there was no congestion of the synovial membranes. On microscopic examination the fluid contained some mononucleated cells, but hardly any polymorphonuclear leucocytes. No bacteria were detected. Cultures were negative. The synovial membranes of both knees were very markedly congested, and there was very evident dilatation of the vessels along the inner side of the condyles of both femora. There was a slight amount of clear fluid in the joint cavities. On microscopic examination there were a fair number of polymorphonuclear cells, but the large proportion of the cells were mononucleated. These were markedly vacuolated and showed abundant phagocytosis of polymorphonuclear cells.

Cultures in milk broth, made by inoculation with small pieces of the membranes, gave pure cultures of the organism, whereas those inoculated with the fluid in the joint remained sterile.

No vegetations were made out. Microscopic examinations of the heart blood showed no organisms, and cultures from it remained sterile. Microscopic examination of the synovial membranes showed irregular scattered areas of polymorphonuclear leucocytes and in these areas only were the organisms found (Fig. 1). This experiment is of extreme interest, for it seems to me to be in line with many cases of rheumatism. The organism is localized and produces its main effect by a toxin; it also proves that failure to obtain the organism from the blood or even from the joint exudation is no proof that the organism is not present.

Rabbit 10.—Inoculated intravenously with four tubes of culture on June 25; showed very definite lameness in the right fore limb on June 28, and on June 29 in the right hind limb. The rabbit recovered completely and was killed on July 18. Nothing abnormal could be made out on naked eye examination. Microscopic examination is not yet completed.

Rabbit 11.—Inoculated intravenously with four tubes of culture on June 25; showed very slight lameness in the right fore limb on June 28. No tenderness or swelling could be made out. The animal was quite well the following day. It was killed on July 18. Microscopic examination is not yet complete. No organisms were obtained in culture from the joints.

Rabbit 12.—Inoculated intravenously on June 26 with the growth on five culture tubes. On June 29 there was very definite lameness in the right hind limb. On July 1 there was very distinct improvement in the condition. On July 2 the animal was dragging the left hind limb also, and on July 18 when

Organism.	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis	Other Lesions.
Micrococcus rheumaticus.	1	8 Nov., 1905 right knee joint.	A few loopsful.	26 Mar., 1906, killed.	Swelling and tenderness later, stiffness in right knee.	Non purulent with some destructive changes.	Very recent aortic.	
	2	29 Mar., intravenously.	Two tubes.	Killed, 8 Apr.	Lameness right fore limb.	Slight injection in synovial membrane of right elbow.	Well marked aortic.	
	3	29 Mar., intravenously.	One tube.	Killed, 6 Apr.	Lameness left fore limb.	Congestion of synovial membrane in left elbow.	Nil.	
	4	24 May, intravenously.	One tube.	Killed, 12 June.	Nil.	Nil.	Nil.	Nil.
	5	24 May, intravenously.	Two tubes.	Killed, 12 June.	Nil.	Nil.	Thickening of mitral and tricuspid segments.	Interstitial myocarditis; acute interstitial nephritis.
	6	30 May, intravenously.	One tube.	Killed, 22 June.	Nil.	Nil.	Doubtful vegetations on tricuspid and aortic segments.	Nil.
	7	30 May, intravenously.	Two tubes.	Killed, 22 June.	Nil.	Nil.	Nil.	Acute interstitial nephritis.
	8	5 June, intravenously.	Three tubes.	Killed, 22 June.	Nil	Nil.	Recent vegetation on mitral segment.	Nil.
	9	13 June, intravenously.	Four tubes.	Died, 18 June.	Indefinite, but animal acutely ill.	Acute in both knees.	Nil.	Nil.

Organism.	Rabbit.	Date and Site of Injection.	Quantity of Injection.	Date of Death.	Symptoms Noted During Life.	Post Mortem.		
						Arthritis.	Endocarditis.	Other Lesions.
Micrococcus rheumaticus.	10	25 June, intravenously.	Four tubes.	Killed, 18 July.	Lameness in right fore and right hind limb (recovered).	Nil.	Nil.	Examination not completed.
	11	25 June, intravenously.	Four tubes.	Killed, 18 July.	Lameness in right fore limb (recovered).	Nil.	Nil.	Examination not completed.
	12	26 June, intravenously.	Five tubes.	Killed, 18 July.	Lameness right hind limb; partial recovery.	Congestion of synovial membranes on both hips.	Nil.	Acute interstitial nephritis.
	13	10 July, intravenously.	Four tubes.	Killed, 13 July.	Indefinite; animal acutely ill.	Right knee.	Nil.	Septicaemia.
	14	10 July, intravenously.	Two tubes.	Died, 13 July.	Indefinite, but acutely ill.	Both knee joints.	Minute vegetations in mitral segments.	Septicaemia.
	15	10 July, subcutaneously.	Five c.c. broth culture.	Killed, 24 July.	Nil.	Nil.	Nil.	Nil.

it was killed, both hind limbs were weak and they appeared to be paralyzed. When the animal was allowed to run about it could move its limbs fairly well.

At the post mortem examination there was definite congestion of the ligamentum teres and synovial membranes generally in both hips. Nothing abnormal was made out in any of the other joints. There was no endocarditis. In the kidney there were several small wedge-shaped areas resembling infarctions. These were dark red and were depressed slightly below the surface of the organ. Microscopically these areas corresponded with those described in Rabbits 5 and 7.

Rabbit 13.—Inoculated intravenously on July 10 with the growth from four culture tubes. The following day the animal was evidently ill, but no arthritis could be made out.

On July 13 it was killed. There was slight injection of the synovial membrane in the right knee, and microscopic examination showed the presence of polymorphonuclear and mononucleated cells and also bacteria similar to Micrococcus rheumaticus.

Cultures from the heart blood gave a pure growth of the micrococcus. There were no vegetations.

Rabbit 14.—Inoculated intravenously on July 10 with the growth from two agar tubes. On July 11 it was very ill, and was found dead in its cage on July 13. There was congestion in the synovial membranes of both knee joints, and from the right pure cultures of the micrococcus were obtained. A pure culture was also obtained from the blood in the heart. There were numerous minute vegetations along the free margin of the mitral valve segments. On microscopic examination the vegetations were very definite and there was infiltration of the tissue of the valve with polymorphonucleated and mononucleated cells.

Rabbit 15.—Inoculated subcutaneously with 5 c.c. of a broth culture of Micrococcus rheumaticus on July 10, 1906. No pus developed at the site of inoculation. The rabbit was killed on July 24. No pathological lesions were detected.

Summary of Inoculations with Micrococcus Rheumaticus:

Intravenous	13
Into knee joint	1
Subcutaneous	1
Total	15
Number of deaths	2 or 13.3%
Number of animals with arthritis	9 or 60.0%
Number of animals with endocarditis (including one doubtful case)	5 or 33.3%

In all the experiments the arthritis was non-purulent, and the knee joints were frequently affected. Recovery or improvement took place if the animal was allowed to live. I would also call special attention to the presence of acute interstitial nephritis and myocarditis in the rheumatic cases. These were not seen in any

of the streptococcal cases. Their significance I am not yet in a position to state.

AGGLUTINATION.

By this means also differentiation is made out between the various forms of streptococci and *Micrococcus rheumaticus*. These reactions were all done the same day with similar solutions, and the results were checked by the independent observations of Dr. J. W. Dawson, to whom I am indebted for very much help during the course of these investigations.

The Agglutination was tested in sedimentation tubes. Dilution was 1 in 30.
Reaction xx = Definite Clumping.

I. (*Control*) *Emulsions alone:* In peptone broth of the separate organisms gave the following reactions:

Micrococcus rheumaticus	A few clumps.
Streptococcus I	No clumps or very few.
Streptococcus II	No clumps or very few.
Streptococcus III	No clumps or very few.
Streptococcus IV	No clumps or very few.
Streptococcus V	A few clumps.

II. (*Control*) *Normal Rabbit Serum:* Plus emulsion in peptone broth of the separate organisms gave the following reactions:

Micrococcus rheumaticus	Hardly any change.
Streptococcus I	Nil.
Streptococcus II	Nil.
Streptococcus III	Nil.
Streptococcus IV	Nil.
Streptococcus V	A few clumps but not nearly so marked as in immune serum.

III. Serum of Rabbit Immunized with Streptococcus I.

Plus emulsion in peptone broth of M.rh.	x ?	Very few clumps
Plus emulsion in peptone broth of Strepto. I	xxx	
Plus emulsion in peptone broth of Strepto. II	x	
Plus emulsion in peptone broth of Strepto. III	xxx	
Plus emulsion in peptone broth of Strepto. IV	xx	
Plus emulsion in peptone broth of Strepto. V	xxx	

IV. Serum of Rabbit Immunized with Streptococcus II.

Plus emulsion in peptone broth of M.rh.	x ?
Plus emulsion in peptone broth of Strepto. I	xxx
Plus emulsion in peptone broth of Strepto. II	xx
Plus emulsion in peptone broth of Strepto. III	xx
Plus emulsion in peptone broth of Strepto. IV	xx
Plus emulsion in peptone broth of Strepto. V	xxxx

V. Serum of Rabbit Immunized with Streptococcus III.

Plus emulsion in peptone broth of M.rh.	x	
Plus emulsion in peptone broth of Strepto. I	xxx	
Plus emulsion in peptone broth of Strepto. II	xx	
Plus emulsion in peptone broth of Strepto. III	xxxx	
Plus emulsion in peptone broth of Strepto. IV	xx	
Plus emulsion in peptone broth of Strepto. V	xxx	

VI. Serum of Rabbit Immunized with Streptococcus IV.

Plus emulsion in peptone broth of M.rh.	x	Very slight; one large clump at bottom of tube.
Plus emulsion in peptone broth of Strepto. I	xx	
Plus emulsion in peptone broth of Strepto. II	x	
Plus emulsion in peptone broth of Strepto. III	xx	
Plus emulsion in peptone broth of Strepto. IV	xx	But some very large clumps.
Plus emulsion in peptone broth of Strepto. V	xx	

VII. Serum of Rabbit Immunized with Streptococcus V.

Plus emulsion in peptone broth of M.rh.	Nil.	
Plus emulsion in peptone broth of Strepto. I	xx	
Plus emulsion in peptone broth of Strepto. II	x	
Plus emulsion in peptone broth of Strepto. III	xxx	
Plus emulsion in peptone broth of Strepto. IV	x	Very slight.
Plus emulsion in peptone broth of Strepto. V	x	

VIII. Serum of Rabbit Immunized with Micrococcus rheumaticus.

Plus emulsion in peptone broth of M.rh.	xx	Big clumps; bigger than with Strepto. I.
Plus emulsion in peptone broth of Strepto. I	xx	
Plus emulsion in peptone broth of Strepto. II	Nil.	
Plus emulsion in peptone broth of Strepto. III	x	
Plus emulsion in peptone broth of Strepto. IV	Nil.	
Plus emulsion in peptone broth of Strepto. V	x ?	

The absence of the organisms from the blood and joint exudations during life. Poynton and Paine and Walker and Beaton record quite frequently successful cultivations from the blood and the exudates in the joints during life. Philipp,[8] in twenty-four cases of acute articular rheumatism, attempted cultivations twenty-one times from the blood and six times from the joint exudate, and twice from the blood and twice from the joints in chronic rheumatism. No bacteria were cultivated, though various kinds of media were used. Cole[9] reports that for the last three years, in prac-

tically all cases of acute rheumatism treated in the Johns Hopkins Hospital, routine cultures have been made from the blood and from the joints whenever any effusion was present. All the cultures were negative.

My own experience in this connection has been very limited. I have examined three cases of acute rheumatism—and all of them post-mortem. In all of them tubes inoculated from the blood remained sterile. In only one case cultures were made from the exudate in the joint, and these also were negative. In the three cases, however, the organism was grown from pieces of the synovial membrane. Several of the tubes inoculated with pieces of synovial membrane also remained sterile.

The results of my culture experiments, and also of microscopic examination, indicate that the organism may be missed altogether in cases where the arthritis is the principal manifestation of the disease, unless several different areas of the synovial membranes are examined.

No doubt in some cases, and especially in severe attacks and those with vegetative endocarditis, the organisms may be found in the blood at certain stages of the disease, but in most of the ordinary cases the organisms appear to be localized and probably produce their results by a toxin. Unless these localized areas are examined there is no possibility of getting cultivations.

Rabbit 9 is of extreme interest in this connection as in it has been reproduced a condition which is the common one in acute rheumatism in the human subject, *i. e.,* a localized bacterial infection in the synovial membranes and secondary effects the result of a toxine. By definite experiment, I have thus shown a complete picture of a case of infection with *Micrococcus rheumaticus* where joint exudation and blood are sterile. Unfortunately, I have, through want of time, not yet been able to repeat this experiment.

Further, the "interstitial nephritis" seen in several of the rabbits, shows the secondary results of the toxine. This also came under my notice late in the research, and has opened up a field which will require careful investigation before any definite conclusion can be drawn from it.

CONCLUSION.

The conclusions I would draw from this work are merely those stated in a former paper.

1. The results obtained by injections of streptococci are different from those produced by *Micrococcus rheumaticus.*

2. *Micrococcus rheumaticus* cannot be regarded as an attenuated streptococcus, nor acute rheumatism as an attenuated streptococcal pyæmia.

3. In uncomplicated cases of acute rheumatism the organism may not be found in the blood or in the joint exudates.

EXPLANATION OF PLATE VI.

FIGURE 1. Synovial Membrane (Rabbit 9) showing localized inflammatory areas (*a*) in which the bacteria were found.

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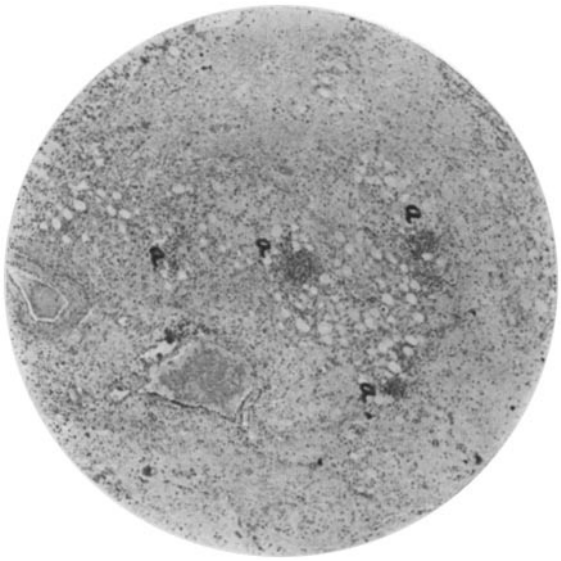

FIG. 1.