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By W. V. Shaw, M.A., M.D. (Oxon.).[1]

From the Wellcome Physiological Research Laboratories.

The clinical features of rheumatic fever, especially the disease as seen in childhood, undoubtedly point to an infectious origin of the disease.

In adults the onset is often sudden, beginning with an acute tonsillitis. Then one joint after another is affected, the inflammation disappearing in one joint and reappearing in another. The arthritis is a constant and prominent feature. The joints are swollen, tender, and painful.

In children the disease is more general and less localised in the tissues of the joints. The morbid process is more widespread and more varied in its chief features. The articular inflammation is slight; it is frequently transitory, and may be absent altogether. On the other hand, lesions of the cardio-vascular system are more prominent. Endocarditis and pericarditis are more frequent, and in more intimate association with the rheumatic process. They must be regarded as primary and essential phenomena of the rheumatism of childhood. Various phases may, however, arise independently, or in various and varying groups. Perhaps most frequently an arthritis is the first evidence of rheumatic infection. In other cases an endocarditis is the initial event, and chorea may sometimes inaugurate the rheumatic process. The series of events is often spread out, and thus "the complete history of a rheumatism becomes the history of a whole childhood" (Cheadle).

In rheumatism the heart generally suffers as a whole, one part perhaps more in one case, another in another case. Endocarditis, pericarditis, and myocarditis occur together; and it may be said that in rheumatism the carditis is general. Often endocarditis occurs first and alone, and pericarditis comes at the end of the fatal series of events.

Acute rheumatism runs a definite course, from the onset of acute tonsillitis to the fatal cardiac lesion. It is a definite disease, with very characteristic lesions, especially in young subjects.

[1] Part of a thesis presented for the degree of M.D. in the University of Oxford.

For acute rheumatism, then, there must be an infectious origin. Only a specific infective process will account for all the lesions of clinical rheumatism, though this view has not been accepted by even the majority of clinical observers. Some indeed maintain that "rheumatism" is a symptom-complex of which the cause is at present unknown; while those who admit its infective origin are not unanimous in their opinion as to the specific infective agent.

The difficulty of defining the exact limits of the rheumatic process, from a clinical standpoint, has led many to discard the term "acute rheumatism," and to include all joint affections under the wide heading of "acute infectious arthritis." In this sense, the arthritis and its attendant clinical features are regarded as a manifestation of the reactions of the organism to a particular variety of infection.

Any attempt at defining the exact nature of the rheumatic process involves an accurate and exhaustive investigation of the true cause of that process.

Many bacteriologists have sought for, and claim to have found, the specific infective agent of rheumatism. Different micro-organisms have been held responsible for the rheumatic process, and even at the present time claims are made for the specific nature of more than one such infective agent.

Klebs, Popow, and Netter all connected rheumatism with the invasion of a streptococcus. Dana obtained a streptococcus from the meninges in a fatal case of rheumatic chorea. All these observers support the microbic origin of the disease, and look on this particular streptococcus as the exciting and infective agent of rheumatic fever.

In 1891 Achalme claimed to have isolated an anærobic bacillus causally connected with rheumatic fever. He admits, however, that his inoculation experiments with animals were not successful. Thiroloix supports and confirms Achalme as to an anærobic bacillus being the causal agent of rheumatic fever. Bettencourt also found a bacillus present in the lesions of rheumatism, thus confirming Achalme and Thiroloix. This observation has been further supported by Hewlett.

In contrast to these views, Hiva claims to have demonstrated that a micrococcus is the specific organism of rheumatic fever. This is confirmed by Triboulet and Cyon, who found a diplococcus in the rheumatic lesions. Apert and Triboulet have found this organism in a series of eleven cases of rheumatic fever.

Further, Wassermann, Westphal, and Malkoff have isolated a diplococcus, growing in chains in fluid media, which they speak of as the specific organism of rheumatic fever. Their work was supported by a series of animal experiments. This organism, isolated by Wassermann, and obtained through Kral, has been used by me for some of the experimental work on rabbits described below. The growth on alkaline media, and appearance as a streptococcus in fluid cultures, is referred to in the *Berl. klin. Wchnschr.*, 1899, p. 644.

Poynton and Paine investigated eighteen cases of acute rheumatism, and isolated the organism from each, thus demonstrating the specific nature of the micrococcus. They further proved the causal connection of the organism with acute rheumatism by a series of animal experiments.

Beaton and Ainley Walker have recently published an investigation of fifteen cases of acute rheumatism. They obtained the specific micrococcus in every case and often in pure culture.

It is with some of the cultures obtained from these latter authors that the later experiments have been carried out.

The microbic origin of acute rheumatism appears from the above evidence to be well established. But, even accepting its infective nature, there are various views on the actual process of the disease.

Summarised, these opinions are the following:—

1. *The bacteriological view.*—The disease is a specific one produced by a specific organism.

(*a*) The specific organism is claimed to have been demonstrated to be a micrococcus by von Leyden, Dana, Triboulet and Apert, Wassermann and Malkoff, FitzMeyer, Poynton and Paine, and Beaton and Ainley Walker, and many others.

(*b*) This is disputed by Achalme, Thiroloix, and other French observers, who claim that the specific organism is a large anærobic bacillus. This view has been supported by Hewlett alone in this country.

2. *The clinical view.*—(*a*) Chvostek, Singer, and others consider that rheumatic fever is not due to a specific infection, but is a particular reaction to a variety of organisms. In fact, rheumatism is considered by them to be merely an attenuated pyæmia, the exciting cause of which may be any one of the pyogenic cocci.

(*b*) Another view is, that simple uncomplicated rheumatism is due to an unknown virus, but the cardio-vascular lesions are produced by a secondary infection with pyogenic cocci.

The difficulty in combating the opposition to the claims of the specific infection produced by the micrococcus of rheumatism is increased by the fact that both Singer and Stengel object to the inoculation test on animals. They hold that the results of inoculation are no proof of the special pathogenic properties of any organism. Indeed, Singer states that rheumatic fever cannot be produced experimentally.

It must be pointed out, however, that the organism has been demonstrated in the tissues from rheumatic cases in man, and that the same organism is found in the lesions of experimentally infected animals.

It is the object of this paper to show that the experimental infection of animals with the micrococcus of rheumatism produces lesions closely agreeing with those of acute rheumatism. That is, accepting the view that the organism described by Netter, Triboulet, Wassermann, Poynton and Paine, and Ainley Walker is the specific causal agent in acute rheumatism, I have carried out a series of experiments to ascertain the special pathogenic features and mode of activity of this organism.

Wassermann's "Streptococcus aus Chorea."

This micrococcus was originally isolated from a case of chorea. The connection between chorea and rheumatism in childhood is very close; hence it is of interest to note that this organism, from a case of chorea, was nearly identical in all its properties and effects with those subsequently investigated from other cases of rheumatism.

The micrococcus grows as a streptococcus, and though usually smaller than the *Streptococcus pyogenes* commonly met with, it might easily be mistaken for it. It grows well on the ordinary nutrient media, but I have found that it grows best on alkaline beef broth and glycerin blood agar.

To grow the organism on blood agar, and yet avoid the injection of portions of fibrin along with the micrococci, I devised a special form of blood agar culture medium. The agar was melted, and allowed to cool to about 42° C. in sterile test-tubes. Into each tube a few drops of sterile blood (sometimes horse's blood and sometimes rabbit's blood) were introduced. The tubes were then sloped and allowed to set. They were subsequently incubated for thirty-six hours, and those that remained sterile set aside for use. I found that, with proper precautions, the blood agar was usually sterile in the majority of the tubes.

The growth of the micrococcus on blood agar was characteristic in several features. After incubation for twenty-four hours at 37° C., small, discrete, colourless, and transparent colonies make their appearance at the site of inoculation. In another twenty-four hours these flatten out somewhat; and when the growth is very active, they may become continuous with one another, forming a film on the surface of the medium. Still further incubation, at 37° C., results in the colonies growing down into the medium.

About the second day or earlier, *i.e.* thirty-six to forty-eight hours after inoculation, the blood agar undergoes a change in colour. The bright red colour is replaced by a dull brown or rusty appearance, or even a greenish-brown tint. This change is most marked when the growth of the micrococci is active and abundant, and it appears to take place more extensively in the rabbit's blood agar than in the horse's blood agar culture medium. The alteration is undoubtedly due to the micrococcus, as it does not occur in similar culture media incubated for the same length of time, but without previous inoculation.

This colour change takes place only in that part of the medium where the organism is actually growing, and is closely analogous to that taking place in cultures of pneumococci growing on blood agar. In the case of several other varieties of micrococci investigated, this alteration in colour does not occur, *e.g.* with the *Streptococcus pyogenes* and the *Streptococcus septicæmiæ*. It is probably due to acid formation and reduction of the oxyhæmoglobin by the micrococci of rheumatism. As proving this point, the spectroscopic examination of the pigment is important. This shows the absence of oxyhæmoglobin in the brown pigment, and the presence of a body with the absorption bands of hæmachromogen.

This micrococcus grows on other solid media, *e.g.* on peptone agar 1 per cent. alkaline to phenolphthalein, on glycerin agar, and on peptone gelatin. The gelatin media are not liquefied. But on all these media the growth, in my hands at least, soon ceases; and inoculations after ten days, from the initial cultivation, have frequently proved sterile.

It will grow, however, in many of the fluid media. In milk, neutral to litmus, it grows fairly well, producing acid and clotting the caseinogen in about two days. In broth, neutral to litmus, it grows, but not rapidly, and with a tendency to die out. In peptone broth, 1 per cent. alkaline to phenolphthalein, it grows rapidly. But perhaps the most suitable fluid medium for the cultivation of this micrococcus is glycerin veal broth, containing 2 per cent. peptone and 1 per cent. alkaline to phenolphthalein. In this medium it grows rapidly, and does not die out for at least one month.

When growing in broth the micrococci form flocculi, and aggregations that tend to settle to the bottom of the containing vessel. The growth in veal broth is usually visible as a flocculent mass after twenty-four hours' incubation. The growth then is extremely active. This is also shown by the

change in the reaction of the broth. In twenty-four hours, from being 1 per cent. alkaline, the micrococcus will change the reaction to neutral.

There is no general turbidity of the broth cultures when the flocculi have settled, contrasting strongly with the appearance of a broth culture of a motile organism, for example, the appearance of a broth culture of the *Bacillus typhosus*. The amber colour of the fresh broth is, however, somewhat reduced after a few days' growth of the micrococcus. The broth is distinctly paler in colour.

The microscopical appearances of this organism vary greatly according to the media upon which it is growing previous to examination. When grown on solid media, *e.g.* blood agar and glycerin agar, for twenty-four hours at 37° C., the micrococci form chains with eight to ten cocci in each chain. In these chains the individual cocci are usually arranged in pairs, though this is not invariably the case. Each coccus is about 0·5 to 1 μ in diameter.

The chains on glycerin agar are frequently shorter than on glycerin blood agar.

Incubated for forty-eight hours at 37° C. the micrococci cease to grow as rapidly as before, and microscopically they now appear as diplobacilli, each bacillus apparently representing an undivided pair of cocci. Longer incubation results in the micrococci undergoing still further involution. They now appear as small bacilli of irregular shape, about 2·5 to 3 μ in length, and 0·5 to 1 μ in thickness. These bacilli are arranged in short chains, or irregularly in twos and threes.

There is no doubt that these bacilli are true involution forms of the micrococci. I have seen them on several occasions, and subcultures have always yielded the micrococcus in pure culture. At the commencement of the investigation these forms were regarded as a contamination of the original culture. Subsequently, however, they were proved to be the specific micrococcus. Poynton has also noticed these bacillary forms.

It may here be noted that similar involution forms have been described by Gordon as occurring in cultures of the *Streptococcus scarlatinæ* when grown on solid media.

In fluid media Wassermann's *Streptococcus aus chorea* grows like a short streptococcus. The chains are usually short, not exceeding six cocci to the chain. These are sometimes grouped as diplococci, especially when the growth is rapid. In this case the intermediate members of the chains are not spherical, but semilunar in appearance, the straight edges of adjacent cocci facing one another. It has been frequently noticed that the terminal members of these chains differ somewhat from the other cocci of the same chain. They are larger, rounder, and more spherical, and they also stain more intensely. When the terminal members have this appearance, it usually happens that some of the intermediate cocci fail to take on the stain, or at all events stain less deeply, as if they had undergone degeneration.

It will be easily understood that an organism with so many and different involution forms is difficult to identify when occurring in the tissues.

The characteristics described above apply, in the first place, to the organism isolated by Wassermann and Westphal, but they apply, without modification, as far as morphology and cultural characteristics go, to the micrococcus kindly sent me by Dr. E. W. A. Walker from Guy's Hospital, and to that sent by Dr. F. J. Poynton from the Hospital for Sick Children, Great Ormond Street.

Dr. Walker isolated the micrococcus from the blood and urine of patients suffering from chorea, endocarditis, subcutaneous nodules, and

arthritis, *i.e.* from patients suffering from "acute rheumatism," as recognised clinically by some of the best observers of the day. Dr. Walker's own results have been referred to above.

Dr. Poynton's culture was obtained by incubating the valve from the mitral orifice of the heart in a fatal case of acute rheumatism. The valve was incubated in bouillon, and gave pure cultures of the micrococcus.

There was one small point of difference in the growth of these cultures. Dr. Walker's micrococcus grew far better on neutral and alkaline peptone agar and on glycerin agar than either Wassermann's or Poynton's organism. The differences in pathogenic properties are noticed in the animal experiments below.

The micrococcus—whether that of Wasserman, Walker, or Poynton—was always subcultured from blood agar to blood agar at intervals of not more than three, or more usually two, days. In this way fresh and active cultures were always available, and the virulence and vegetative activity of the organism were maintained.

For the animal inoculation experiments, cultures forty-eight hours old were used. Explanations will be given as to the life-history of the particular cultures in each case.

The first inoculation experiments were made with the micrococcus obtained from Wassermann in 1902; it was found, however, to be but feebly pathogenic. A few of the rabbits suffered from a transitory arthritis, from which they always recovered. This organism was then subcultured on to rabbit's blood agar, and after a few repeated inoculations was growing freely. After several generations its pathogenic power was much increased, as will be seen from the results of my experiments.

Experimental Results.

Rabbit 1.—This rabbit received four injections into the marginal vein of the ear. It was feared that in this case the total result would be small, but on the eighth day after the last injection the animal was found to be ill and off its feed. There was obvious dyspnœa, increased on attempts at locomotion. Both elbow-joints were painful and swollen. On the next day the animal was worse, and both knee-joints were hot, painful, and swollen. The rabbit became thinner and feebler, and died on the tenth day after the last injection.

An autopsy showed that shoulder-, elbow-, knee-, and carpal-joints were all affected, but that there was only slight beading of the endocardium on the mitral valve flaps. Cultures from the elbow-joint proved positive, the micrococcus being present in pure culture. Slides made from the joint exudations contained large numbers of the specific micrococcus.

Rabbit 2.—This rabbit was injected with the same organism as Rabbit 1, but it had been grown for several generations on rabbit's blood agar. The rabbit received three injections on three successive days. On the fourth day the carpal-joints were enlarged and tender, and two days later the swelling and tenderness had increased. A week later the rabbit received its fourth inoculation, and three days afterwards the left knee was swollen and tender,

obviously affected with acute arthritis. Other three injections were made into the vein of the ear, and *these last three inoculations were from cultures* of the micrococcus from *the elbow-joint of Rabbit* 1. The rabbit died a week after the last injection.

On post-mortem inspection the left knee- and carpal-joints contained fibrinous exudation, and the endocardium of the mitral valve showed several vegetations.

Cultures from the joint exudations were all positive, and microscopical examination showed the presence of the specific micrococcus. But it is to be noted that in this case the endocarditis was more marked and more advanced than in Rabbit 1. Another feature is the recurrence of the arthritis after the fourth injection of the living cultures.

Rabbit 3.—The cultures of Wassermann's micrococcus, from the elbow-joint of Rabbit 1, were grown on rabbit's blood agar from November 10 to November 24, being subcultured every two days. By this means the virulence of the organism was exalted, as will be seen from a consideration of this experiment. The rabbit was inoculated with living cultures, and received three injections in all. There were no signs of arthritis, but the rabbit was obviously ill. It wasted and became more feeble. The heart was affected early, and was feeble and irregular; and the animal died about a fortnight after the last injection.

Post-mortem examination of the animal showed that the infective process had in this case involved the heart almost entirely. There was extreme mitral stenosis, with infarcts in the kidneys and lungs, and dilatation of the left auricle.

The above three cases form a series interesting in itself, showing the increasing virulence of the organism; and also that the more acute the infection, the more serious are the cardiac lesions. They were all inoculated with cultures of Wassermann's streptococcus.

Rabbit 4.—Fearing that the micrococcus used for Rabbit 3, above, might be the *Streptococcus pyogenes*, a rabbit was injected with 10 c.c. of a glycerin veal broth culture intraperitoneally. The immediate result was nil, and for the ten days the rabbit was under observation no pathogenic effect was noticeable.

Rabbit 5.—This animal was injected with living cultures of the micrococcus, isolated by Dr. Walker from a case of endocarditis. It received three injections intravenously, in the first instance. Five days after the last injection the knee-joints were both swollen, hot and tender. A fortnight later the animal was recovering; and the knee-joints, though still slightly swollen, were less obviously affected. Six weeks after the last inoculation it received a further injection of the micrococcus, which had been grown on rabbit's blood agar meanwhile. It died the next day. Post-mortem examination showed that there were old infarcts in the lungs and recent acute pericarditis.

This is a case of a second infection proving rapidly fatal. This is often observed clinically.

Rabbit 6.—The same micrococcus was used in this case. Four inoculations were made in the first instance, resulting in arthritis of the carpal-joints on both sides. In ten days the joint lesions were disappearing; so, six weeks after the last injection, a further inoculation of the micrococcus was carried out intravenously. The rabbit died five days after this further infection. Post-mortem, it was found to have been suffering from pericarditis and recent endocarditis.

Rabbit 6 (*a*).—This animal was similarly infected with Dr. Walker's micrococcus. It had definite arthritis eight days after the first inoculation.

Other two inoculations were made, and it died a month later of well-marked endocarditis.

Rabbit 7.—This rabbit showed definite signs of arthritis after two inoculations of Dr. Walker's second culture. After three weeks' interval it received still further injections, and after five such additional infections there was a fresh attack of arthritis, involving both the knee- and elbow-joints.

This rabbit is still alive, and is now regaining the weight and strength lost during the two attacks of arthritis. On auscultation there are evidences of endocarditis, and there is dyspnœa following attempts at locomotion.

Rabbit 8.—This animal proved more susceptible. Three inoculations caused an acute illness. It died two days after the last injection, of acute pericarditis. Cultures from the pericardium and heart's blood were positive.

Rabbit 9.—This animal only received two injections of cultures of Dr. Walker's micrococcus. No arthritis was perceptible, but the animal was ill and off its feed. It died three days after the last inoculation of acute endocarditis and pneumonia. Cultures from the heart's blood were positive.

Rabbit 10.—As there had been no evidence of arthritis in Rabbits 8 and 9, a different method of inoculation was used in this case. Subcultures from the original cultures obtained from the heart's blood in Rabbit 9 were made in alkaline broth. The fur was clipped off from over both knees. The skin was cleaned with a 1 in 1000 solution of mercuric iodide in KI. This was washed off with methylated spirit. Five c.c. of the broth culture was then injected into the knee-joint on each side, between the condyles of the femur and head of the tibia. The next day there was marked swelling of the joints. The rabbit was obviously ill, and died six days after infection. Both knee-joints contained considerable amounts of fibrinous exudation, and the endocardium was inflamed. Cultures from the heart's blood and knee-joints were all positive.

This result is remarkable. It shows that an acute infection can originate in the knee-joints. It demonstrates, moreover, the specific effect of the micrococcus on the heart, however introduced, when the affection is acute, as in this case.

Rabbit 11.—This animal was also inoculated in the knee-joints. Broth cultures from Rabbit 10 were used, and 5 c.c. were injected into each knee-joint with the same strict precautions as before. The rabbit died two days after infection. Both knee-joints contained fibrinous exudation. But the *elbow- and carpal-joints* were also involved; and there was a small vegetation in the right ventricle of the heart. Slides of the exudation show the presence of the micrcoocci. Cultures were also positive.

Again this demonstrates the peculiar pathogenic powers of this organism, producing, as it does, acute arthritis and endocarditis. It is to be noted that the elbow- and carpal-joints were *not* inoculated, but proved to be affected with the micrococcus at the post-mortem examination.

Rabbit 12.—This rabbit was also inoculated, but intravenously, with the cultures from Rabbit 10. It died three days after the first injection, with endocarditis on the mitral valve. Cultures from the blood of the heart were positive.

This rabbit was inoculated with Dr. Walker's culture after passing through Rabbit 10.

RABBIT 13.—Cultures of Wassermann's micrococcus, grown on blood agar, had been maintained since the injection of Rabbit 3. Six of these were made into an emulsion with sterile normal saline, and injected into the pericardium of this rabbit. Strict precautions were taken to avoid infection from the skin. This animal died seventeen days later with knee-joints, carpal- and elbow-joints all affected. There was also most extensive fibrinous exudation from the parietal and visceral surfaces of the pericardium. Cultures from the pericardium yielded the micrococcus in pure culture.

RABBIT 14.—This rabbit, inoculated intravenously, shows the complete picture of the action of this micrococcus. It died nearly two months from the date of the first infection, and during this time developed arthritis in elbows and knees, and definite signs of endocarditis. These conditions were all demonstrable post-mortem. A further point for notice is that *broth cultures* were used for the inoculations, eliminating any source of error possibly due to the blood agar.

RABBIT 14 (*a*).—This rabbit was injected with cultures of the micrococcus recovered from Rabbit 13. It received only one inoculation, and developed acute arthritis in one knee-joint in seven days. It lived rather more than a fortnight from this, but wasted considerably.

On post-mortem examination there was definite endocarditis, and also extensive fibrinous exudation into the right knee-joint. This is an example of fatal monarticular arthritis, sometimes met with in the rheumatic fever of man. This was produced by Wassermann's micrococcus after passage through Rabbit 13.

RABBIT 15.—Another culture from Dr. Walker was used for the infection of this animal. It received a considerable number of injections, but finally developed arthritis, dying the day after the joint lesions were first noticed. Post-mortem cultures from the knee-joints were positive.

RABBIT 15 (*a*).—This animal, a much younger rabbit than any used in the above series, received only one injection intravenously. Four days afterwards both knees and elbows were swollen. It died nearly a fortnight later with early endocarditis.

RABBIT 16.—This experiment was a control of that on Rabbit 13. It was inoculated intra-pericardially with the micrococcus from Dr. Walker. It died in three days with acute pericarditis, but the knee-, elbow-, and carpal-joints were also affected. Cultures from these were positive. Compare with Rabbits 13 and 14 (*a*).

RABBIT 17.—Wassermann's micrococcus, from the cultures obtained from Rabbit 13, were used in this experiment. The rabbit received only one inoculation, which in this case was made intravenously. The animal died four days after of acute pericarditis, with fibrinous exudations in the right knee, and both carpal-joints.

RABBIT 18.—One inoculation, intravenously, was sufficient in this case also. The rabbit developed definite arthritis in the carpal-joints about a fortnight later. It rapidly became thinner, and died five days after the appearance of the arthritis.

RABBIT 19.—This experiment is worthy of special notice. The rabbit was inoculated with the micrococcus recovered from Rabbit 16. After two injections intravenously it was noticed that the rabbit was suffering from *acute iritis in the left eye*. This was confirmed by several independent observers. It received two other injections subsequently, and died twelve days after the last infection. Post-mortem examination showed the presence of marked

endocarditis. There were vegetations on the tricuspid valve and mitral valve, and extensive recent granulations of the semilunar valves of the aorta. Cultures from the heart, spleen, and anterior chamber of the eye were all positive.

RABBIT 20.—This experiment is confirmatory of that on Rabbit 19. The cultures from the spleen of Rabbit 19 were used for inoculation. The rabbit died thirty hours later with acute dilatation of the heart, and serous effusions in the pericardium, pleuræ, and peritoneum. Cultures from the heart's blood were positive.

RABBIT 21.—This animal received several intravenous injections of the micrococcus. After three injections it had definite arthritis in both carpal-joints. Two more injections increased the amount of swelling in these joints, and also caused arthritis in one knee-joint. A further injection proved fatal. Cultures from the heart's blood, affected joints, bladder, and spleen all proved positive, and were pure cultures of the micrococcus.

RABBIT 22.—This rabbit was a larger and older animal than the majority of those hitherto used for these experiments. Four separate inoculations of the micrococcus, injected intravenously, produced a severe illness. Three days after the last injection it had *well-marked iritis*, with fibrinous exudation into the anterior chamber of the eye. In about a month this condition gradually cleared up. The rabbit was then again injected with cultures of the micrococcus. Again it had an attack of acute iritis. At this stage the rabbit was seen by several independent observers, including an ophthalmic surgeon. They all agreed that the animal was suffering from acute iritis. This condition again gradually cleared up, leaving, however, some iritic adhesions and an opacity of the anterior part of the lens.

RABBIT 23.—This was injected with cultures obtained by Dr. Poynton from a case of acute rheumatism. After three intravenous injections the animal developed acute arthritis in both carpal-joints, and died a week after the last injection. Post-mortem it was found to have also early endocarditis.

RABBIT 24.—This animal had three injections of cultures of Dr. Poynton's micrococcus intravenously. Both carpal- and knee-joints were affected after the third injection. The rabbit died five days after the last injection. Post-mortem, it was found to have extensive exudation into the knee-joints, and this exudation was full of the micrococci; also, there was recent early endocarditis, with beading of the mitral valve flaps.

RABBIT 25.—This animal was infected with cultures obtained from the fatal case of acute rheumatism in the monkeys recorded below. That is, it was infected with the micrococcus obtained from Dr. Poynton and passed through the monkey.

The first inoculation, intravenously, was followed by acute arthritis of the left knee-joint. This developed in two days. The rabbit was then inoculated a second time with the same micrococcus. This infection proved rapidly fatal. Post-mortem, the left knee-joint was found to contain recent fibrinous exudation. Microscopical examination showed this to be full of the micrococci. The mitral valves were the seat of recent endocarditis, and there was a red infarct in the right kidney.

MONKEY EXPERIMENTS.

MONKEY 1.—This monkey was injected intravenously on three different occasions with cultures of the micrococcus. After the first injection it had a transient arthritis in the right knee-joint. Within twelve hours of the last injection (of cultures obtained by Dr. Poynton from a case of acute rheumatism) the monkey was feverish and very ill. After twenty-four hours from

the injection both knee-joints were swollen and tender, and in forty-eight hours the elbow- and carpal-joints were also involved. The monkey was now extremely ill and unable to feed itself. It was kept alive for another twenty-four hours by feeding it with brandy and milk, but during this time it had very obvious dyspnœa. It died just three days after the injection with Dr. Poynton's micrococcus. A careful post-mortem examination was carried out. The pericardium was thickened, and contained a considerable quantity of turbid, sero-fibrinous exudation, in which were floating flakes of lymph. The visceral pericardium was dull and covered with flakes of fibrinous exudation. The heart itself was dilated, and the heart muscle pale and flabby. The endocardium was inflamed, with recent exudation in the mitral valve flaps, and a distinct beading of the free edges. Both knee-joints were distended with fibrinous exudation, which was thick and glairy in appearance. There was similar exudation into the carpal- and elbow-joints. In the lungs there were a few patches of consolidation. The spleen was enlarged, and there were a few small hæmorrhagic infarcts in the kidneys. Cultures were taken from the heart's blood, right and left knee-joints, and pericardial exudation. All the cultures proved positive, yielding pure growths of the micrococcus.

This monkey then had arthritis, pericarditis, myocarditis, and endocarditis, all caused by the micrococcus. These lesions are clinically associated with an attack of acute rheumatism. Indeed, the phenomena exhibited by this monkey during the fatal illness were in every way similar to those of acute rheumatism in man.

Monkey 2.—This monkey was inoculated intravenously on three separate occasions. The first two only caused transitory illnesses. After an interval of one month the monkey was injected with cultures of the micrococcus obtained by Dr. Poynton from a case of acute rheumatism, at the Hospital for Sick Children. The same evening the monkey was ill and feverish. The next day it was worse, and unable to use its legs. In the evening the knee-joints were swollen, hot, and tender. The animal was also feverish and thirsty, and showed a dislike for solid food. On the following day the elbow- and carpal-joints were affected. The monkey was unable to use its hands. The knees also were still swollen and painful. On the fourth day the animal was better and more lively, but the joints were still swollen. On auscultation over the præcordium a distinct systolic murmur was heard, especially close to the left nipple. This murmur was never detected in the healthy animal.

This monkey is still alive. The joints are slowly recovering, and the swelling decreasing.

Those who saw the monkey in the different stages of the acute illness were unanimous in the opinion that it bore a close, unmistakable resemblance to a case of acute rheumatic fever, as seen in children.

Microscopical Examination of the Tissues of the Infected Animals.

1. *Exudation into joints.*—Thin films were made of the exudation after the manner of blood films. These were simultaneously fixed and stained by the L. L. Jenner modification of Romanowsky's eosinate of methylene-blue. The micrococcus was found in all the films examined, being stained a purple-blue. It occurred in short chains sometimes as a diplococcus, but more often as a streptococcus. The specific micro-organism was present in by far the larger numbers in the exudation from joints in the early or acute stage. In such

cases, though there was but little external manifestation, the micrococcus was present in very large numbers, evidently multiplying very rapidly *in situ*. The diplococcus arrangement was more frequent in these acute cases.

The same micrococcus was present in the joint exudation of the more chronic arthritis (*e.g.* Rabbit 1). But here it occurred as a streptococcus, with the swollen terminal cocci mentioned above. Cultivation experiments proved this to be the same micrococcus.

The exudation in the acute cases consists chiefly of the polymorphonuclear corpuscles, which in the rabbit contain fine eosinophile granules. These are not the true coarsely granular eosinophiles, which are also found, but in much smaller numbers. Numbers of large mononuclear cells are also found.

In the chronic arthritis the polymorphonuclear cells undergo degeneration, losing their staining reactions; and mononuclear and basophile cells and connective tissue corpuscles make their appearance.

2. *Pericardial exudation.*—Films of the pericardial exudation were mounted and stained in the same way as in (1) above. In the four cases of extensive fibrinous exudation in the pericardium the micrococcus was present in large numbers, in every way identical with that found in the exudation of the joints. In several others, where the pericardium was injected and inflamed, slides were made of the pericardial fluid, and a few micrococci were found in this also.

The cellular elements in the pericardial exudation are very similar to those present in the exudation of the joints.

3. *Pericardium and endocardium.*—Portions of the heart wall, valve flaps, and papillary muscles were fixed, either in 10 per cent. formalin, or in a solution of 4 per cent. bichromate of potash, containing 1 per cent. of osmic acid. Tissues fixed in the formalin solution for from twelve to twenty-four hours were taken through the spirit, absolute-alcohol, cedarwood-oil, paraffin process; but those placed in the bichromate-osmic acid solution were given four days at least in the fixing fluid, being kept carefully in the dark. Sections were cut in paraffin by the Cambridge rocking microtome. These were flattened out in warm water, and attached to the slides by drying for twelve hours at 40° C.

Carbol thionin, differentiated by alcohol, was found to be the most useful stain, though some tissues gave good results with borax-methylene-blue.

In portions of tissue from the infected rabbits it was easy to recognise the leucocytosis and connective tissue proliferation in the damaged areas of the heart.

Patient search in these areas often resulted in the discovery of a few of the micrococci, singly or in groups. The difficulty of finding them was increased by the extreme tardiness with which they take on the stain, and further by their pleomorphism. The micrococci in the tissue, as in the exudation from the more chronic cases of arthritis, are often swollen and spherical in shape. They occur very rarely in chains, more often in pairs and singly; these cocci are usually not far from the smaller vessels or capillaries of the infected area.

The osmic acid specimens also showed the presence of fatty degeneration of the myocardium. The fat droplets occurred in the cells, usually at either end of rectangular nucleus.

4. *Lungs and pleuræ.* — The lungs were fixed, cut into sections and mounted as above (2).

In rabbits dying of an acute infection with the micrococcus, the specific micro-organism could be demonstrated in the lungs; and there more rapidly than in the tissues of the heart. The micrococci were found in the alveoli, together with an exudation of polymorphonuclear leucocytes and endothelial cells.

In several cases there was a fibrinous exudation between the two layers of the pleura. Films of this exudation, mounted and stained, showed the presence of the specific micrococcus.

4. *Iritis.*—A film was made of the exudation from the iris in Rabbit 20. This showed the micrococci to be present, and cultures were also obtained in a pure state.

These microscopical examinations amply confirm the nature of the lesions produced by the infection of the rabbits with the micrococcus from undoubted cases of rheumatism. In every case the changes observed were associated with the presence of the micro-organism inoculated.

Moreover, there is a marked identity in the lesions produced by the organism of rheumatism obtained from different cases, as a comparison of the various inoculation experiments will show.

The pathological changes in the animals infected by Wassermann's micrococcus are almost identical with those caused by the organism isolated by Ainley Walker (cf. Rabbits 1, 2 and 3, and 5, 6 and 7). The former was isolated from a case of chorea, the latter from cases of endocarditis and pericarditis. Such results all point towards the same conclusion, namely, the identity of the infective process in these cases of (experimental) rheumatism. These results are also confirmed by the lesions found in Monkeys 1 and 2, after infection with the micrococcus isolated by Dr. Poynton.

Another point, fully confirmed by the experiments recorded, is, that this micrococcus from cases (in the human subject) exhibiting various types of the rheumatic process breeds true. Under a variety of circumstances, as in these experiments, it reproduces the lesions clinically associated with acute rheumatism.

Cultures obtained from the different animals infected all show the same cultural and morphological characteristics. Still more important, on inoculation in animals they reproduced the rheumatic process in its chief and essential features.

Bearing on the identity of the organism isolated from the different animals is the gradual rise in virulence of the organism, after a few passages through a susceptible animal, *e.g.* the rabbit (cf. Rabbits 1, 2, and 3). Indeed, in some of the later experiments the infection has proved very acute and rapidly fatal. Even here, however, we have a close parallelism with the acute rheumatism of childhood.

At this point it is necessary to refer to those who object to animal experiments being made a part of the investigation into the etiology of acute rheumatism (cf. Singer and Stengel).

A series of animal experiments, as herein recorded, can leave no doubt of the nature of the infective process, and those who seek to combat this view cannot fairly ignore them. Indeed, to accept the clinical evidence without the experimental, and to use the clinical evidence against the experimental upon which it depends, is logically unsound. An argument based on such data is an instance of the negative use of the well-known fallacy *ignoratio elenchi.*

The experimental method of investigating such a question as the

etiology of rheumatism hardly needs any further defence, but there is one point that has been raised by the critics of the experimental results. It has been objected that the cardiac lesions found and recorded above may sometimes be met with in healthy and apparently normal rabbits. This is difficult to believe, and directly contradicting such a statement is the result of the autopsy of a considerable number of rabbits in some experiments on the use of antiseptics.[1]

All the rabbits in the antiseptic experiments had died of an acute infection. But in no single instance was any abnormal feature of the endocardium discovered; this observation must add weight to the above experiments on the etiology of rheumatism.

Nor can it be said that I have merely produced a condition of pyæmia in the rabbits. The definite and most characteristic illness produced in the two monkeys, and the lesions found after death in one of them, show that true rheumatism can be reproduced by inoculations of this micrococcus.

Summary.

Taking a collective view of the lesions found in the different rabbits, it is evident that Wassermann's micrococcus can be held responsible for arthritis, pericarditis, myocarditis, endocarditis, and pleurisy, with infarction of distant viscera. Similarly the micrococcus of Ainley Walker, and also that of Poynton, produced arthritis, iritis, pericarditis, myocarditis, and endocarditis, with visceral infarcts in infected animals, both rabbits and monkeys.

That is, this organism produced the majority of the lesions clinically associated with an attack of acute rheumatism.

Conclusion.

From this it naturally follows that the particular micro-organism in question, called the *Micrococcus rheumaticus* by Walker, the *Diplococcus rheumaticus* by Poynton, and the *Streptococcus aus chorea* by Wassermann, is the actual infective and causal agent of acute rheumatism.

Acute rheumatism is therefore of microbic origin, and the actual causal agent is a micrococcus closely resembling the *Streptococcus pyoyenes* in its chief characteristics.

[1] *Journ. Hyg.*, April 1903.

A—Knee joint, serofibrinous exudation.

B—Knee joint laid open, showing exudation.

C—Shoulder and D—Elbow joint, showing arthritis and tenosynovitis.

Fig. 1

A—Knee joint, from case of experimental rheumatic fever, showing arthritis.

B—Patella.

C—Exudation into joint.

D—Exudation into subligamentous bursa.

E—Tibia.

F—Normal knee joint.

Fig. 2

A—Hæmorrhagic infarcts of kidney.

B—Hæmorrhagic nephritis with small infarcts.

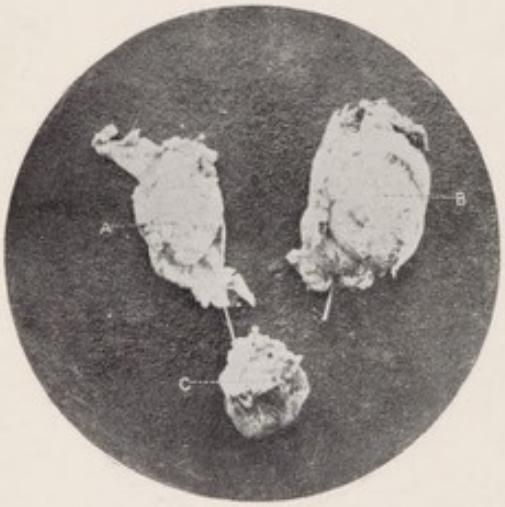
C—'Nutmeg' liver, from case of heart x, Fig. 6

Fig. 3

A—Fibrous nodule on chorda tendinea.

B—Inflammatory thickening on mitral valve.

Fig. 4



A—Pericarditis.

B—Pericarditis, extensive exudation of serofibrinous material.

C—Endocarditis of mitral valve.

Fig. 5

A—Granulations on mitral valve.

B—Left auricle laid open.

Fig. 6—Heart x