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THE ETIOLOGY OF ACUTE RHEUMATISM AND ALLIED CONDITIONS.

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[PRELIMINARY COMMUNICATION.]

CONSIDERABLE attention has been directed in the last few years to the question of the precise causation of the group of closely-related conditions of disease which may be broadly spoken of under the term "rheumatic," and which are recognized by clinicians generally as constituting the various manifestations (in different tissues and under different conditions) of a single and definite morbid process. Under this heading it is only necessary to mention here acute rheumatism with its associated arthritis, pericarditis, myocarditis, endocarditis, and subcutaneous nodules, chorea, some forms of tonsillitis, and a considerable proportion of cases of ulcerative endocarditis. From the clinical standpoint the diseased conditions in question have all the characters of an infective malady, and not infrequently present the picture of what may be reasonably termed an attenuated septicaemia. Yet they maintain in general a distinct identity which justified the expectation that they would be found to have a specific causal agent—probably a micro-organism, but one distinct from those of ordinary septicaemia.

Numerous investigations have been carried out in the endeavour to determine this infective agent; and a large number of different organisms have been described by bacteriologists and others as the specific cause of rheumatism, though often on quite insufficient evidence. But attention has been definitely focused in the last few years upon a micrococcus first described by Triboulet in 1898, and then by Wassermann in 1899, by the important and extended observations carried out by Paine and Poynton upon the association of this organism with the morbid lesions of rheumatic fever, and its specific action when inoculated into animals. Paine and Poynton have obtained this micro-organism from no fewer than 18 cases of acute rheumatism, and consider it to be the specific causal agent in this infection.

The present investigation was begun a year ago with the object of determining, primarily, to what extent the original observations of Triboulet and Wassermann, and those of Paine and Poynton in this country could be repeated. In the interval a considerable number of communications on the subject have appeared in the foreign literature, but no confirmatory results have yet been published in England.

We are now able to state, as the result of our own observations on the subject, that we agree entirely with the assertion that a micrococcus is constantly associated with acute rheumatic lesions, and is the causal agent in their production. And we believe the coccus which we have isolated from rheumatic cases to be identical with that obtained by Triboulet, Wassermann, Paine and Poynton, Meyer, and several others. At the outset certain important differences appeared between the organism as we found it and the original description given by Paine and Poynton; but with their later accounts of its staining and cultural characters we are, in general, fully in agreement. It may be added that the same micro-organism is obtainable from cases of chorea, and on injection into animals may produce a typical attack of acute rheumatism, just as do cultures from rheumatic fever cases.

Up to the present time we have obtained the micrococcus from some 15 cases—namely, from 8 cases of acute rheumatism, 3 cases of chorea, and 4 cases of acute endocarditis in rheumatic subjects. The general nature of the cases examined and the particular source from which the coccus was obtained in each are shown in the following summary:

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Summary of Cases.

Case.	Source of Culture.	Remarks.
1. Acute rheumatism with pericarditis and endocarditis	Post-mortem heart's blood	—
2. Acute rheumatism	Fluid from knee-joint <i>intra vitam</i>	Cocci very few, growth very feeble.
3. Acute rheumatism with endocarditis	Post-mortem heart's blood	Inoculations made in rabbits with positive results.
4. Subacute rheumatism, mitral stenosis	Urine	—
5. Acute rheumatism	Urine	—
6. Acute rheumatism, nodules, morbus cordis	Blood from venesection	—
7. Acute rheumatism	Blood from ear and urine	—
8. Subacute rheumatism, morbus cordis, adherent pericardium	Post-mortem heart's blood	—
9. Chorea, heart failure	Post-mortem heart's blood	Inoculations made in rabbits with positive results.
10. Chorea	Urine	Inoculations made in rabbits with positive results.
11. Chorea, heart failure	Post-mortem heart's blood	—
12. Fungating endocarditis	Post-mortem heart's blood	Inoculations made in rabbits with positive results.
13. Fungating endocarditis	Post-mortem heart's blood	—
14. Acute endocarditis	Blood from ear	—
15. Acute endocarditis	Blood from ear	—

In the majority of cases we obtained the organism in a pure condition in the original cultures. Sometimes in the *post-mortem* specimens it was associated with the bacterium coli; and twice in specimens isolated from small quantities of blood (0.2 to 0.5 c.cm.), obtained by puncturing the ear with a Pakes's needle, it was contaminated with a staphylococcus albus. The subcultures used in animal experiments, however, were all derived from cases in which the original cultivations had yielded the micrococcus in a pure culture.

Microscopical and Staining Characters.

The organism is a tiny micrococcus, arranged in pairs and short chains in films from ordinary subcultures.

It retains the stain by Gram and in the Weigert modification of this method.

It stains well with all the ordinary dyes.

It is not capsulated.

It is not agglomerated either by the serum of convalescent rabbits or of man.

This organism has been classed by Paine and Poynton as a diplococcus; by the German workers it is usually called a streptococcus. So far as our own observations go, the pairing of the cocci is usually very marked in recent cultures, and more especially in the original cultivations, and in body fluids or on agar-agar; and chains may be quite infrequent in the films. Under cultivation the arrangement in chains becomes more marked, and cultures grown in milk or in a highly alkaline bouillon frequently present long chains, which in some cases may extend across one or more fields of the microscope with a $\frac{1}{2}$ oil immersion objective. When growing in the tissues—as, for example, in endocarditic vegetations in the human subject—the majority of the cocci are seen to be in pairs, but sections not infrequently show chains of half-a-dozen individuals or more.

Whether the micrococcus be spoken of as a diplococcus or a streptococcus does not appear to be of any great importance, and might be regarded merely as a matter of words.

But we think that the persistent application of the former term to an organism which is at present indistinguishable on culture media from an ordinary streptococcus, and which in cultures often shows a marked tendency to grow out into chains, has probably tended to delay considerably its general recognition by bacteriologists; for we have no hesitation in saying that without animal experiments it would almost certainly be diagnosed as an ordinary streptococcus by any one to whom cultures and stained preparations were submitted without comment. Thus, in Pakes's report on cases of ulcerative endocarditis streptococci were found in more than 80 per cent. of the cases examined. We therefore prefer to speak of it as the "micrococcus rheumaticus."

Cultural Characters.

Since the cultural characters of the micrococcus do not appear to have been very fully worked out, or at any rate have not been fully published in this country, it may be well to quote the results of our examination of a specimen from a

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Table of Cultural Characters.

Culture Medium.	Coccus from Acute Rheumatism.	Coccus from Chorea.	Streptococcus from Septicaemia.
Bouillon	24 hours, white flocculent precipitate in clear fluid 3 days, ditto 5 days, ditto; no indol	24 hours, white precipitate, fine deposit on side of tube, fluid clear 2 days, ditto 5 days, ditto; no indol	24 hours, flocculent precipitate hanging to side of tube, fluid clear. 4 days, ditto 8 days, ditto; no indol.
Glucose formate bouillon	24 hours, slight acidity 3 days, acid, no gas	24 hours, acid, no gas 2 days, ditto 5 days, ditto	24 hours, neutral, with flocculent precipitate. 2 days, slight acidity 4 days, ditto 8 days, sediment, slight acidity.
Peptone water	—	24 hours, slight precipitate, fluid clear 2 days, ditto 5 days, ditto; no indol	24 hours, no visible growth. 2 days, slight precipitate. 4 days, ditto; no indol.
Dextrose bouillon	24 hours, slight acidity, flocculent precipitate 3 days, ditto 5 days, ditto	24 hours, acid, good growth, deposit on sides and bottom of tube 2 days, ditto 5 days, ditto; fluid clear	24 hours, flocculent precipitate, (?) a trace of acid 2 days, ditto; distinct acidity 4 days, ditto 8 days, precipitate, very acid
Saccharose bouillon	24 hours, acid, flocculent precipitate 3 days, ditto 5 days, ditto	24 hours, acid, good growth, deposit on sides and bottom of tube 2 days, ditto 5 days, ditto; deposit at bottom, fluid clear	24 hours, flocculent precipitate, neutral 2 days, distinctly acid 4 days, ditto 8 days, ditto
Glycerine bouillon	24 hours, slight acidity, flocculent precipitate 2 days, acid 4 days, do. 5 days, do.	24 hours, acid, precipitate at bottom 2 days, ditto 5 days, ditto	24 hours, fair flocculent precipitate, fluid clear 2 days, slightly acid 4 days, acid 8 days, ditto
Nitrate bouillon	24 hours good flocculent precipitate, no nitrite	24 hours, precipitate, fluid clear, no nitrites	24 hours, fair flocculent precipitate, fluid clear, no nitrites
Lead bouillon	24 hours, flocculent precipitate, no H ₂ S 3 days, ditto 5 days, ditto	24 hours, good growth, fine, suspended in clear fluid, no H ₂ S 2 days, ditto; precipitate 5 days, ditto	24 hours, flocculent suspended precipitate, no H ₂ S. 2 days, ditto 4 days, ditto 8 days, ditto
Gelatine (stab)	2 days, fair growth along track of needle, colonies discrete at bottom, no liquefaction 5 days, thin growth along track, no liquefaction	24 hours, fine growth along track of needle 2 days, ditto 4 days, ditto; growth greater, no liquefaction	2 days, no visible growth. 4 days, fine, thin, translucent, discrete colonies. 8 days, ditto; no gas, no liquefaction.
" (streak)	—	24 hours, fine, thin, transparent growth 2 days, ditto 4 days, growth greater, no liquefaction	2 days, no growth. 4 days, very slight growth.
" (shake)	2 days, full of minute colonies, no gas, no liquefaction 5 days, ditto	24 hours, fine growth 2 days, ditto; no liquefaction, no gas	2 days, no growth. 4 days, dotted throughout with fine colonies. 8 days, ditto; no gas, no liquefaction.
Agar streak	24 hours, small, semitransparent, non-spreading, slightly-raised colonies 3 days, ditto 5 days, colonies spreading a little	24 hours, fine, translucent, slightly-raised colonies 2 days, ditto 5 days, ditto	24 hours, semitransparent, discrete, minute, point-like colonies. 2 days, colonies larger size of pin's head, whiter and denser. 4 days, ditto
Blood serum	24 hours, very small transparent, slightly-raised colonies 3 days, ditto 5 days, ditto	24 hours, fine, small, transparent colonies 2 days, ditto 4 days, ditto	24 hours, whitish, moist, semitransparent colonies, larger than on agar. 2 days, 4 days, and 8 days, ditto
Litmus milk	24 hours, acid, no clot 2 days, acid, slight clot 5 days, acid, clotted	24 hours, acid, no clot 2 days, acid, beginning to clot 5 days, acid, clotted	24 hours, slightly acid, no clot. 2 days more acid, no clot. 4 days acid, no clot. 8 days, acid, clotted.
Potato	No visible growth	No visible growth	No visible growth.
Anaërobic (in glucose formate bouillon)	24 hours, slight flocculent precipitate on side of tube, no gas 3 days, deposit at bottom, no gas 5 days, ditto	24 hours, slight precipitate, no gas 2 days, ditto 5 days, ditto	24 hours, no visible growth. 2 days, ditto

case of acute rheumatism and of one from a case of chorea in parallel columns compared with a streptococcus isolated from an ordinary case of septicaemia.

These are the details of a parallel test. But it may be stated that the rheumatic coccus when grown in fluid media of marked alkalinity not infrequently produces slight turbidity of the medium. When grown upon the surface of blood agar it gradually produces to a slight extent the change observed by Eyre and Washbourn in the growths of pneumococci on the same medium—namely, an alteration of the red blood pigment around the colonies, leading to the production of a rusty brown or chocolate colour.

From an inspection of the table, it appears that there is nothing in the ordinary cultural characters of the organism which distinguishes it from any other streptococcus.

Is this Micrococcus an Ordinary Streptococcus?

Marmorek has found that if a fluid medium in which streptococci of human origin have been grown be filtered free from micro-organisms and again employed as a culture medium, bacteria generally can flourish in the fluid, while streptococci of human origin are entirely unable to multiply in it.

Such a fluid, therefore, may be used as a test solution to determine whether a given organism obtained from man is identical with ordinary human streptococci or is different from them. And Marmorek, using this method, has recently

published the conclusion that all the streptococci found in infections in the human subject are identical, though he allows that that of scarlatina exhibits a slight differentiation.

We have applied this method to two specimens of the micrococcus rheumaticus on two occasions with positive results. In both trials both specimens grew freely in a medium of filtered streptococcus bouillon, exhibiting considerable chain formation. From this it would follow that the micrococcus of rheumatism is specifically different from the ordinary pyogenic streptococci of human origin. And, if the result be confirmed by other workers, the specificity of the micrococcus rheumaticus will be established.

Growth in Alkaline Media: Acid Formation.

The organism grows well in alkaline media, and can be grown in media of high alkalinity. We have found it capable of growth in bouillon with an alkalinity of — 25 on Eyre's notation. If a series of bouillon tubes with alkalinity of 0, — 5, — 10, — 15, — 20, and — 25 respectively be inoculated and incubated at 37° C., the growth at the end of eighteen hours is good and approximately equal to naked-eye examination in the tubes 0, — 5, and — 10; in the — 15 tube it is slight but definite; and in the other two growth is determinable on microscopic examination. Twenty-four hours later the growth in bouillon of an alkalinity of — 15 has become as copious as that in the three tubes of lower alkalinity, while that in the tubes at — 20 and — 25 remains indeter-

minable by the naked eye. Later more active growth occurs in these tubes also; apparently assisted by the reduction of alkalinity in the medium due to the formation of acid by the micro-organism.

We have grown it in a large quantity (a litre) of bouillon which contained $\frac{1}{2}$ gram of sodium hydrate to the litre; that is to say having an alkalinity of — 25 on Eyre's notation (35 degrees more alkaline than ordinary standard media). In this case the production of acid was such that the culture was only faintly alkaline to litmus at the end of six days' growth. The nature of the acid formed is quite unknown. Mr. Ryffel, of Guy's Hospital, has kindly undertaken to assist us in identifying it. Up to the present, however, an exact analysis has not been possible. It apparently is not a lactic acid—a fact of interest in view of the old experiments of Richardson on dogs.

Animal Experiments.

We have examined the action of this organism in the living body on seventeen rabbits with positive results, and have obtained the following effects from inoculations into the venous system, namely, fever and wasting, mono-arthritis, polyarthritis, paresis of limbs, pericarditis, endocarditis, septicæmia, and death. Some of our rabbits have had two or three attacks of acute arthritis from successive inoculations; and one still under observation and in excellent condition has had four distinct attacks following as many different injections of the micro-organism.

The cultures which we used for the inoculations were all from growth on alkaline glycerine agar of about twenty-four hours' incubation. Paine and Poynton state that they employ blood-agar cultures for the purpose of inoculation; and this undoubtedly forms a better culture medium. There are, however, obvious objections to the intravenous injection into animals of material scraped from a number of culture tubes whose surface is covered with fine shreds of fibrin. Some shreds are almost certain to be injected, and are likely to become entangled in the chordæ tendineæ of the right heart, or to form emboli within the lungs with consequent infarction. Accordingly we decided not to use blood-agar cultures for the animal experiments.

We have been able to obtain quite copious growth on the medium we employ, but a blood medium appears to be necessary for the maintenance of the vitality and virulence of the micrococcus for any length of time. We are now trying blood-agar made by a special method, namely, by adding a quantity of sterile defibrinated blood to melted agar tubes when they have cooled to about 40° C. The tubes are then sloped and rapidly solidified. A perfectly smooth agar surface impregnated with unaltered blood is thus obtained, and the medium is, so far as we have tested it, extremely satisfactory and convenient.

The amount of culture necessary to obtain results depends on the activity of the organism employed. Paine and Poynton found that they required about six blood-agar cultures of their specimens. With cultures of the organism recently isolated from the human subject a similar number of glycerine-agar cultures has in our hands invariably caused death within forty-eight hours in inoculated rabbits; and we have obtained arthritis and even death with as little as a single agar culture, especially after passage of the organism through the rabbit.

We do not propose to give the protocols of our experiments at length in the present paper. But the results which we have obtained with the four specimens of the micrococcus used for inoculations may be summarized as follows:

1. *Large Doses.*—Six or more agar cultures cause death within some forty-eight hours of the time of inoculation. *Post mortem* we have usually found a large pericardial effusion, and sometimes pleural effusion also; there is a marked engorgement of the heart, which appears to be dilated, and there are sometimes beady vegetations upon the mitral valve, and occasionally on the tricuspid segments also. The joints are very hyperæmic, and may contain a clear effusion. The organism is found in pure culture in the blood, in all the exudations, and in the urine. It is also seen in sections of the heart valves (mitral) and synovial membranes of the joints.

2. *Moderately Large Doses.*—Death may occur in three to seven days or as late as three weeks after the inoculation. *Post mortem* in the earlier cases there was found a clear or blood-stained pericardial effusion and sometimes also effusion in the pleural cavities. There is effusion in the joints and tendon sheaths which may be clear or turbid, and in some joints is flaky or definitely purulent. From all the effusions

the organism can be isolated in pure culture, and also from the heart blood.

3. *Small doses* of the original organism or of cultures from the heart or joints of animals killed under 1 and 2 above, give rise on inoculation to acute arthritis. This may affect only one joint but is more usually a polyarthritis passing from joint to joint in a characteristic manner, and tending to involve especially the wrists, ankles, and knees. These cases tend to make complete recovery. After recovery a fresh inoculation usually induces a fresh attack, which may affect the same or different joints from those affected in the previous attack. The arthritis usually develops in from three to six days from the date of inoculation.

Up to the present we have been unable to obtain chronic valvular disease of the heart in rabbits from inoculations of the micrococcus. Nor have we seen fungating endocarditis follow infection, though very striking examples of this have been obtained by Paine and Paynton. But Dr. Vernon Shaw, to whom we sent some of our cultures, allows us to state that one of these has in his hands produced endocarditis and visceral infarctions in an inoculated animal.

We have not seen a true chorea result from our inoculations into the blood stream, although a number of quite young animals have been used. Our cultures, even from chorea in man, have up to the present given us only acute rheumatism in animals. On one occasion, however, we have observed violent spasmodic movements of the head upon the second day after inoculation, in an animal which had developed a mono-arthritis within twenty-four hours of its inoculation, and died on the third day with beady vegetations on the mitral valve; and in another animal, which suffered from polyarthritis, and eventually recovered completely, there was for several days paralysis of both hind limbs.

Some emphasis has been laid by various observers on the non-suppurative character of the lesions following inoculations of this micrococcus. This we do not regard as of particular importance. An intravenous inoculation is the least likely to result in local suppuration, but under suitable conditions the arthritis which results may become undoubtedly purulent. Pus is occasionally seen, moreover, in rheumatic lesions in the human subject; and that the rheumatic coccus can, like any other pathogenetic bacterium, give rise under suitable conditions to suppuration is evidenced by the fact that we have produced a suppurative peritonitis in the rabbit by an intraperitoneal inoculation, and have twice seen the formation of a subcutaneous abscess after inoculation in the subcutaneous tissue of the abdomen. Moreover, on two occasions, when a small amount of the intravenous injection had escaped into the subcutaneous tissue of the ear, we subsequently saw a local suppuration and a polyarthritis both present in the animal together and both recovering. Small subcutaneous inoculations, however, usually give rise to a local infiltration which goes on to the formation of a fibrous nodule in the subcutaneous tissue.

Conclusion.—As a result of our experiments we agree with those observers who maintain that a particular micro-organism is constantly associated with acute rheumatic lesions; that this micro-organism can be cultivated on artificial media outside the living body; and that on inoculation into animals it gives rise to the characteristic lesions of the disease, and can again be isolated from those lesions.

Culturally this organism resembles a streptococcus, but that it is specifically different from the ordinary streptococci is rendered probable by our results on the application of the test of Marmorek.

Accordingly, we conclude that the bacterial specificity of acute rheumatism has been satisfactorily established. Its toxic specificity remains to be investigated; and is at present under examination.

Our thanks are due especially to Dr. Newton Pitt for much assistance in obtaining material for our investigation, and also for many valuable suggestions during the course of our experiments.

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A PSYCHOLOGICAL RESEARCH LABORATORY AT BUDA-PESTH. —A psychological research laboratory has been established in Buda-Pesth. It is under the direction of Dr. Paul Ranschburg and the Minister of Public Worship and Education, Dr. Julius Wlassics, has recognized it as an integral part of the Royal Hungarian Paediatric Institute, which is now housed in a fine set of buildings.