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NOTE ON THE DISCOVERY OF A MICRO-ORGANISM IN MALTA FEVER

SURGEON DAVID BRUCE

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The Practitioner,

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EDITED BY

T. LAUDER BRUNTON, M.D., F.R.C.P., F.R.S.,

Assistant Physician and Lecturer on Materia Medica and Therapeutics at St. Bartholomew's Hospital;

DONALD MACALISTER, M.A., M.D., B.Sc., F.R.C.P.,

Fellow and Medical Lecturer, St. John's College, Cambridge; Physician to Addenbrooke's Hospital; and University Lecturer in Medicine;

AND

J. MITCHELL BRUCE, M.A., M.D., F.R.C.P.,

Physician and Lecturer on Therapeutics at Charing Cross Hospital.

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NOTE ON THE DISCOVERY OF A MICRO-ORGANISM IN MALTA FEVER.

BY SURGEON DAVID BRUCE, M.B. EDIN.

Station Hospital, Valetta, Malta.

This fever, which does not appear to occur in England, has a wide distribution in the Mediterranean. It is identical with the Rock Fever of Gibraltar, the Neapolitan Fever of Naples, the Country Fever of Constantinople, and the New Fever of Crete. It is also known by various other names, such as Adeno-typhoid, Intermittent-typhoid, Gastric, and Bilious Remittent Fever, &c. From private correspondence I find that it occurs also at Cagliari in Sardinia, Catania in Sicily, Smyrna, and Tunis. On further investigation I have no doubt this list could be much enlarged, and this fever found to extend further eastward, and many of the so-called remittent fevers of India found to be identical with it.

Although a considerable number of communications on the clinical aspects of this fever have been published, there is, as far as I am aware, no notice regarding the presence of microorganisms in the organs of fatal cases.

Before describing the cases in which I have found a special form of micro-organism, I may briefly sketch the prominent features of the fever. It is a disease of long duration,

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ninety-one cases treated in this hospital during 1886 having an average stay in hospital of 85.5 days. The fever, which often runs high, is continued, remittent, and intermittent in type. In this, however, there is no regularity, one case being almost continued throughout, another almost purely intermittent. Some cases begin with a markedly intermittent type of temperature curve, and pass into the continued; whilst others again begin as continued and pass into the intermittent. In several severe cases which I have watched from beginning to end, there was a long irregular elevation of temperature, only reaching normal limits about the ninetieth day. An undulatory curve is frequently observed, the undulations being separated by a period of apyrexia. These waves are usually longer (twenty to thirty days) at the beginning of the disease, the relapse-waves ten to fifteen days, and the period of apyrexia often about ten days. Sometimes these undulations persist for a long time: in one case I observed two well-marked waves between the 120th and 160th days of the disease.

Besides the rise in temperature, the spleen is usually found to be enlarged, and the patient suffers from profuse sweating and sudamina. The bowels are as a rule confined. There is a tendency to the development of some bronchitic affection. Relapses are almost invariable, accompanied or followed by pains of a rheumatic or neuralgic character, sometimes swelling of joints, or orchitis. The mortality is as a rule exceedingly small. In the above-cited ninety-one cases there did not occur a single death. In some years, however, the disease appears to be more virulent. In the present year, for example, up to the middle of July, as many as nine deaths from this fever have occurred among the British soldiery in Malta. On post-mortem examination these cases showed enlargement and congestion of the spleen and other internal organs, but no appearance of any glandular enlargement or lesion in the intestines. Microscopically the condition of the liver, spleen, and kidneys was found to be very similar to what obtains in enteric fever, scarlet fever, and other micro-organismal diseases.

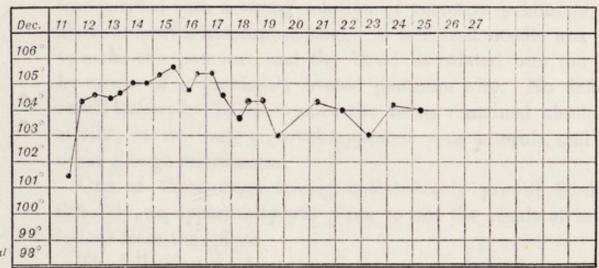
The following five cases were examined for micro-organisms, the first by means of stained sections, the remaining four by inoculation into tubes containing Agar-agar nutrient jelly. ninety-one cases treated in this baspital during 1886 having an average stay in hospital of 85° days. The fever, which often runs high, is consigned, remittent and intermittent in type, In this however, where is no regularity, one case being almost continued throughout another almost proof, intermittent. Some cases begin with a markedly intermittent intermittent turn curve, and pass into the continued; whilst others again begin as continued and pass into the intermittent. In several severe cases which I have watched from beginning to end, there was a long irregular elevation of temperature, only reaching normal limits about the emetited day. An undulatory curve is frequently observed, the undulations being separated by a period of interesting the beginning of the disease, the relapse-waves ton to fifteen days, and the period of approxia often about ten days. Sometimes these modulations points for a long time; in one case I days, and the period of approxia of an long time; in one case I days, each the disease the lighth and 100th the disease.

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The following five cases were examined for micro-organisms, the first by means of stained sections, the remaining four by inoculation into tubes containing Agar-agar nutrient jelly. Case No. I.—Private J. R., South Yorks. Regiment, aged 20 years. Admitted to hospital 11th December, 1886; died at 7.10 a.m., 26th December, 1886. The following chart shows the course of the disease:—



Normal

The post-mortem examination was held nine hours after death, when small portions of spleen were at once placed in a large quantity of absolute alcohol. Afterwards these portions of tissue were impregnated with paraffin, and very thin sections cut by means of a Cambridge rocking microtome. On staining these sections by Gram's method, and also by an opaque watery solution of methylene-blue for twenty-four hours, and examining by means of a ½ Zeiss oil immersion and Abbe condenser, enormous numbers of single micrococci were seen scattered through the tissues. Dr. Sims Woodhead, pathologist to the Edinburgh Royal Infirmary, kindly assisted me in the examination of these sections, and was of opinion that the micrococci would be found to have some causal relationship to the disease.

On returning from leave of absence at the end of May last, in conjunction with Dr. Caruana-Scicluna, Government analyst, I prepared sterilized tubes of Agar-agar nutrient jelly. It was our intention to work conjointly at the distribution of bacteria in the air in different parts of Malta. I must here thank Dr. Caruana-Scicluna for the very great assistance he has given me, not only in preparing sterilized fluids, but also in supplying apparatus, &c.; in fact, without his co-operation the following results could not have been attained.

Having in hospital about this time many severe cases of

Case No. 1—Private J. R., South Yorks Regiment, aged 20 years Admitted to hospital 11th December, 1880; died at 7.10 a.m., 26th December, 1880. The following chart shows the course of the disease:

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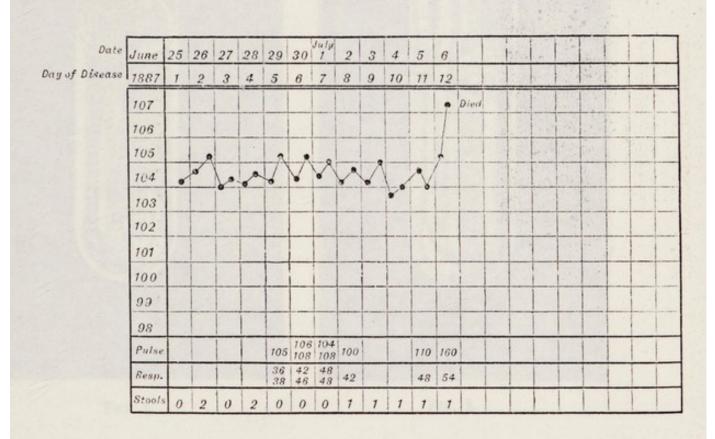
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Malta fever, Dr. Caruana-Scicluna and I made some thirty or forty inoculations into sterilized tubes of Agar-agar jelly with blood taken from the tip of the finger of ten of the most severe cases. The following was the method of procedure:—

The finger, having been well washed with soap and hot water, was purified with a strong solution of corrosive sublimate, and afterwards with absolute alcohol. A sterilized needle was used to make the puncture, and the blood inoculated into the jelly by means of a previously heated platinum wire. No definite result followed. In most cases the tubes remained absolutely sterile; in two only a growth appeared—one a torula, and the other a large micrococcus.

One of these cases having unfortunately proved fatal on 6th July, an opportunity was given to test the result of inoculations from the spleen.

The following chart represents the course of the fever :-



Case II.—Private H. D., 1st R.H., aged 24, admitted 25th June, 1887; died 5.30 p.m., 6th July, 1887.

As it is absolutely necessary in hot climates, from sanitary considerations, to do post-mortem examinations as soon as possible after death, I at once performed the duty. The spleen was first NOTE ON THE DECOFERY OF A

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As it is absolutely necessary in hot climates, from canitary considerations, to do post-mortem examinations as soon as possible after death, I at once performed the duty. The spleen was first

removed and immediately wrapped in a cotton cloth saturated with a solution of corrosive sublimate (1 in 100).

As it had been found by experiment that the air of the mortuary was peculiarly rich in germs, and that inoculations made there were almost certain to become contaminated, I removed the spleen to a small room in my quarters, the door and window of which had been kept shut for some time, so as to have a still

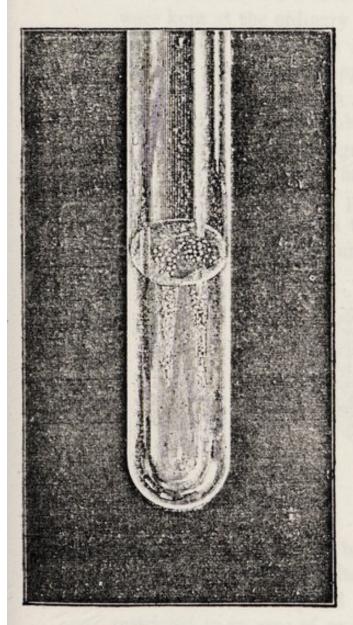




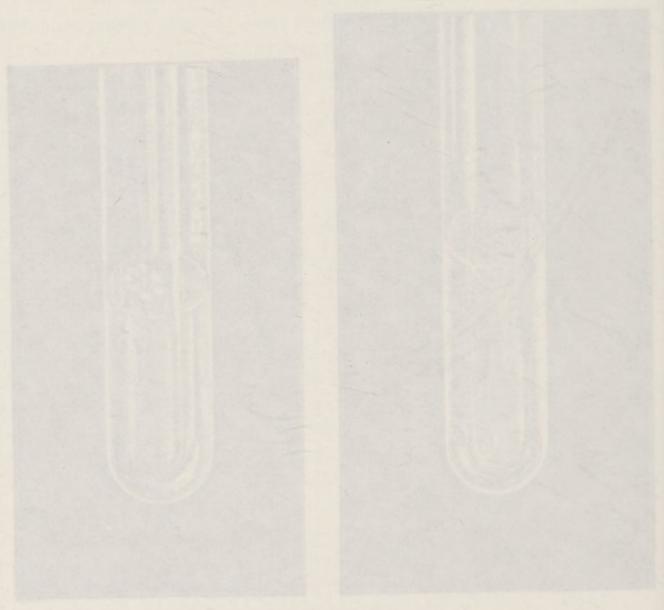
Fig. 1.

Fig. 2.

condition of the atmosphere. Here I inoculated eight tubes containing sterilized Agar-agar nutrient jelly. The usual precautions were taken. Three extensive cuts, the second in a plane at right angles to the first, and the third at right angles to the second and parallel to the first, were made by three thin-bladed knives, previously thoroughly sterilized by holding them

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in the flame of a spirit-lamp. A platinum needle, heated to redness before each inoculation, was used to convey a small portion of the spleen pulp to the solid jelly. These inoculations were made at 6.30 p.m., and the tubes remained at the ordinary temperature of the air until 11 a.m. on the following day, when six tubes were placed in the incubator in Dr. Caruana-Scicluna's laboratory. This incubator has a constant temperature of 37° C. Two tubes were kept at the ordinary temperature of the air. In all these eight tubes one and the same growth appeared. This growth was slow in making its appearance. It appeared simultaneously in all the tubes placed in the incubator after a period of sixtyeight hours. The two tubes kept at the ordinary temperature of the air only showed signs of growth at the end of 168 hours. The growth appeared at first as minute pearly-white spots scattered round the point of puncture. Small round colonies could be seen along the needle track. After several days these spots grew somewhat larger, and joined to form a rosette-shaped

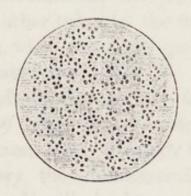


Fig. 3.

colony on the surface. There is no tendency to liquefaction of the nutrient jelly, and after the lapse of twenty-one days the colonies have not spread over the whole surface, but remain restricted to an area of, on an average, '25 of an inch. Fig. 1 represents the appearance of the growth on the fifth day, and Fig. 2 on the twenty-first day after inoculation.

When a minute portion taken from one of these colonies is placed in a drop of sterilized water and examined under a high power, innumerable small micrococci are seen. They are very active, and dance about—as a rule singly, sometimes in pairs, rarely in short chains. All the tubes were examined, and found

in the flame of a spirit-lamp. A platinum needle heated to reduce house how the seal portion of the spices paths of the solid jelly. These ineculations were made the spices paths to the solid jelly. These ineculations were made at 0.30 p.m., and the index remained at the ordinary tomporature of the mir matil 11 a.m. on the following day when six tubes were placed in the incubator in Dr. Carmana-Sciclum's laboratory. The mire hand in the incubator in Dr. Carmana-Sciclum's laboratory were kept at the politicary temperature of 37 C. Two tubes were kept at the politicary temperature of the air. In all these sight index can and the same growth appeared. This growth wire slow in making its appearance. It appeared singultaneously finall the tubes pixed in the incubator after a period of sixty-mire slow. The two tubes kept at the ordinary temperature of the air day shows at any and the point of penetures. Simil round colories could for sear along the needle treely. After several days these spots scarters along the needle treely. After several days these spots grow grow along the needle treely. After several days these spots grow grow along the needle treely. After several days these spots grow grow along the needle treely. After several days these spots grow grow along the needle treely. After several days these spots grow grow along the needle treely. After several days these spots grow grow accommendant larger, and foined to form a resette-shaped grow grow accommendant larger, and foined to form a resette-shaped



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to contain the same organism. Fig. 3 represents the micrococcus stained with methyl-violet and magnified about 700 diameters.

CASE III.—A. B., aged 24, admitted 26th June, 1887; died 11th July, 1887.

In this case I had no opportunity of making a post-mortem examination. I succeeded, however, in making inoculations into six tubes containing Agar-agar, in the following manner. Seven hours after death, having previously cleansed the skin over the region of the spleen by means of a strong solution of corrosive sublimate and absolute alcohol, I drew off a small portion of the splenic pulp by means of a sterilized trocar and cannula. Next morning five of these tubes were placed in the incubator, and one left at the ordinary temperature of the air. On the following morning, thirty-six hours after inoculation, a growth appeared in one of the tubes placed in the incubator. On examination this growth proved to be a large non-motile micro-organism, quite different from the one previously found. No growth appeared in the other tubes in the incubator, until the end of eighty-four hours, when the same growth appeared as that in the tubes inoculated from Case II. In the sixth tube, also, which had been left at the ordinary temperature of the room, at the end of 110 hours the characteristic colonies were seen. In the third case, then, although the system of inoculation was unsatisfactory, the characteristic micrococcus grew in five tubes out of six. It will also be noted in these two cases that the tubes placed in the incubator showed signs of growth after the same time, viz. eighty-four hours. The more rapid growth of Case III. kept at the ordinary temperature of the air, is to be accounted for by the greater heat of the weather.

Case IV.—Private B. E., aged 23, admitted to hospital 8th July, 1887; died at 3.30 p.m. 15th July 1887.

Inoculations from the spleen of this case were made in precisely the same manner as in Case II., except that the door and window of the room were left open during the operation. Six tubes of Agar-agar jelly were inoculated. Two of these at the end of forty-three hours showed growths, which proved to be large non-motile cells. The remaining four tubes at the end of to contain the same negonism. Fig. 3 represents the micro-

Case III -A. B. aged 24, schmitted 20th June, 15 37; died

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sixty-seven hours showed the characteristic growth, similar to that found in Cases II. and III. This period of sixty-seven hours instead of eighty-four hours is to be accounted for by the fact that in this case the tubes were placed in the incubator immediately after inoculation.

The following chart represents the course of the disease

Date .	July	8	9	10	11	12	13	14	15		25				-	1	1	1		1	
y of Disease		1	2	3	4	5	6	7	8							-				1	
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	109°								• 3		p.m			1		1		I		1	1
	108					100			• 2.	30	p.m								1	1	
	107								2		p.m						1	-	I	-	
	106								• 12	45	p.m						1	1			
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	Pulse		100		96	110	120	160		130		1			1	-	-	T		-	
	Resp.		1		30	28		1	44	48											
	Stools	0	0	2	0	0	0	2							1			1	1	-	

Case V.—Private I. C., aged 22, admitted to hospital on 30th June 1887; died 23rd July 1887.

In this case before inoculating any tubes, I did what I had not thought of doing in the previous cases, that is, I examined directly the splenic pulp for the living and moving microbes. The result was, I confess, somewhat startling. A minute portion of the pulp was removed by means of a clean needle and placed on a slide in a drop of water, distilled the moment before. On placing the slide under a magnifying power of

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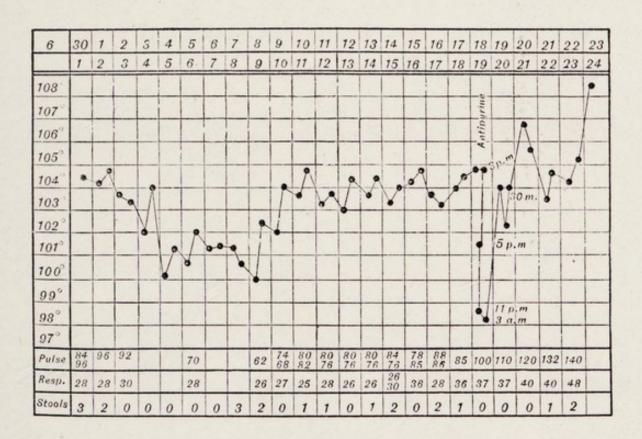
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500 diameters, the field of the microscope was literally crowded with myriads of micrococci dancing about in the most active manner. They appeared to be of the same size and appearance as those examined from previous cultures.

Two tubes out of six having become contaminated in Case IV., I used the utmost diligence to prevent the same thing occurring in this last case. The door and window of the room were kept tightly closed, and the extra precaution was taken of inoculating the tubes under a carbolic spray (1 to 40). The six tubes inoculated from the spleen showed no signs of growth for ninety-five hours, when in all of them there appeared the characteristic colonies. This lengthened period of ninety-five hours was probably due to the fact that the gas-jet used to heat the incubator had been accidentally turned out, and the accident not discovered for twenty-four hours.

The following chart represents the course of the disease.



From a consideration of the above facts I think it will appear to be sufficiently proved: (a) that there exists in the spleen of cases of Malta fever a definite micro-organism; and (b) that this micro-organism can be cultivated outside the human body. On

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the latter point I may remark that I have already cultivated four successive generations. It now remains to be seen what effect, if any, this micro-organism has on healthy animals; what are the conditions of temperature, &c., under which it flourishes; where it is to be found; how it gains entrance to its human host; and many other points. All of these will take a long time to investigate. I have therefore published this preliminary note in order to draw the attention of other workers to what seems to me to be an attractive field.

Wil. SHORM-DREE TAXABLES TO THE TEREBER one the conditions of temperature, we analer which it flourishes ;