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ETIOLOGY OF OROYA FEVER.

II. Viability of Bartonella bacilliformis in Cultures and in the Preserved Blood and an Excised Nodule of Macacus rhesus.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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Pure cultures of *Bartonella bacilliformis* were first obtained from a specimen of human blood in citrate solution, transported from Lima, Peru, to New York City in the refrigerator.[1] The first successful cultures were made on October 3, and the second on October 20, 1925, the blood being 28 and 43 days old, respectively, when used for the purpose of cultivation. The fact that pure cultures were obtained on both occasions indicated a surprising ability of *Bartonella bacilliformis* to exist under modified conditions and suggested experiments on the viability of the organism.

Viability of Bartonella bacilliformis in Infective Blood.

Table I shows the results of viability tests made with citrated blood of infected monkeys (*M. rhesus*) kept constantly at 4°C. All of the fifteen specimens, which were derived from seven monkeys infected with cultures of *Bartonella bacilliformis* or monkey passage strains, yielded cultures of the organism when tested after periods of refrigeration varying from 24 to 152 days following withdrawal from the animals. In most instances the number of organisms was considerably reduced, as is evident from the fact that none of the preserved specimens yielded growth in dilutions higher than 1:10, whereas the titer in some instances had been 1:100,000 at the time of withdrawal.

In testing the effect of room temperature upon similar material, two specimens of citrated blood, derived from *M. rhesus* 8[2] and *M.*

[1] Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, xliii, 851.

[2] Noguchi, H., *J. Exp. Med.*, 1926, xliv (in press).

rhesus 12,[3] were employed (Table II). The temperature of the laboratory where the samples of blood were kept varied from 15° to 22°C., and the tall test-tubes serving as containers were stoppered with cotton plugs and were not protected from the light. There was still a trace of what appeared to be hemoglobin in the supernatant portion of the plasma after 45 days, but the general tone had become brownish

TABLE I.

Viability Tests of Bartonella bacilliformis in Citrated Blood Kept at 4°C.

Source of blood	Date of withdrawal	Result of test on Jan. 29, 1926	Days after withdrawal	Result of test on Apr. 12, 1926	Days after withdrawal
Rhesus 1	Nov. 11, 1925	+	79	+	152
" "	Dec. 8, "	+	32	+	104
" 2	" 18, "	+	42	+	114
" "	Jan. 4, 1926	+	25	+	97
" 3	" " "			+	97
" "	" 25, "			+	86
" "	Feb. 6, "			+	74
" 4	Dec. 18, 1925			+	114
" 5	" 22, "			+	110
" "	Jan. 12, 1926			+	89
" 6	Dec. 28, 1925			+	104
" "	Jan. 4, 1926	+	25		
" 7	" " "	+	24		
" 8	Dec. 18, 1925	+	42		

TABLE II.

Viability of Bartonella bacilliformis in Citrated Blood Kept at Room Temperature.

Source of blood	Date of withdrawal	Titer of fresh blood	Result of test on Mar. 20, 1926 (after 45 days)	Titer of blood on Apr. 12, 1926 (after 67 days)
Rhesus 8	Feb. 3, 1926	1:10	+	>1:10,000,000
" 12	" " "	1:10	+	>1:10,000,000

when examination was made 67 days after withdrawal of the blood. As the table shows, the titer of the preserved blood specimens was at least 1:10,000,000, whereas the titer of the fresh blood in each instance had been only 1:10, that is, multiplication had taken place at room temperature. Film preparations made from the specimens showed

[3] Noguchi, H., *J. Exp. Med.*, 1926, xliv (in press).

the organisms to be in small clumps scattered among the corpuscles. There was no special tendency to localization within or around the erythrocytes, such as is evident in the blood of human beings suffering from Oroya fever.

Viability of Bartonella bacilliformis in the Nodule from an Animal Experimentally Infected.

Bartonella bacilliformis as it occurs in the local lesions of infected animals is exclusively an intracellular parasite, being found usually in the cytoplasm, occasionally within the nuclei, of proliferating endothelial cells (clasmatocytes). In actively growing nodules the number of microorganisms is very large.

TABLE III.

Viability of Bartonella bacilliformis in the Excised Nodule and Emulsion at 4°C. and at Room Temperature.

Nodule excised on Mar. 1, 1926	Unground tissue		Emulsion	
	4°C.	Room	4°C.	Room
Mar. 7 (7 days).....	+	+	+	+
" 14 (14 ").....	+	+	+	+
" 28 (28 ").....	+	+	+	+
Apr. 4 (35 ").....	+	−	+	−
" 25 (56 ").....	+	−	+	−

The suspension made by triturating with citrate solution a portion of the large subcutaneous nodule that developed on the abdominal wall of *M. rhesus* 14[2] following the injection of a mixture of culture and passage strain yielded a culture of *Bartonella bacilliformis* in a dilution of 1:100,000. This material was used for determining the viability of the organism in the tissue of the excised nodule. The results, which are recorded in Table III, indicate that *Bartonella bacilliformis* survives in an untriturated piece of excised nodule or in the suspension of the tissue for more than 56 days at 4°C. At room temperature, on the other hand, the organisms had died out after a period of 35 days, though growth had been obtained from the suspension after 28 days. The autolysis of tissue taking place at the higher temperature may have been detrimental to the organism, or there may have been

an exhaustion of nutrient substances. It was found, however, that the addition, at the beginning of the experiment, of about 10 per cent of fresh rabbit or horse serum and a trace of hemoglobin to the tubes containing the suspension not only prevented the death of the organism but actually induced growth.

Viability of Bartonella bacilliformis in Cultures.

There are two culture media on which *Bartonella bacilliformis* grows well,[1] the so called leptospira medium and blood agar slants. The hydrogen ion concentration most suitable for growth is pH 7.8 to 8, and it is advisable to adjust the medium to this reaction. It has been shown also, through the cooperation of Dr. J. H. Bauer, that the

TABLE IV.

Viability of Bartonella bacilliformis in Culture on Leptospira Medium.

No. of tube	Temperature	No. of days	Result of transplant	No. of tube	Temperature	No. of days	Result of transplant
	°C.				°C.		
1	25	53	+	9	37	37	+
2	25	57	+	10	37	37	+
3	25	63	+	11	37	47	+
4	25	64	−	12	37	47	−
5	25	75	+	13	37	50	+
6	25	80	+	14	37	60	−
7	25	95	+	15	37	62	−
8	25	120	+	16	37	65	−

substitution of Huntoon's hormone broth or Meyer's peptic digest broth for the ordinary meat infusion broth as the basis of these media has a decidedly favorable influence upon the growth of the organism.

One of the peculiarities of *Bartonella bacilliformis* in culture is that it soon reaches the limit of growth at 37°C., though multiplication continues steadily at 25°C. On leptospira medium the organisms remain viable longer than on blood agar slants, growth progressing for a month or more in the former case, while in the latter the maximum seems to be reached in about 14 days at 25°C., and then only when the evaporation of the medium is retarded by soaking the cotton plugs with paraffin or closing the tubes with rubber stoppers. As a rule *Bartonella bacilliformis* remains motile on blood agar for 10 days, occa-

sionally as long as 14 days, but it is usually non-motile when grown on the semisolid medium. Microscopic examination alone is not sufficient for the detection of viability, therefore; cultural tests must also be made.

Many culture tubes having been preserved from the time of the isolation of *Bartonella bacilliformis* in October, 1925, it was possible to make a series of viability tests, the results of which are recorded in Table IV. In order to retard evaporation, the cultures on leptospira medium intended for preservation were covered with a layer 2 cm. deep of sterile paraffin oil.

As the table shows, a culture of *Bartonella bacilliformis* on leptospira medium at 25°C. remained transferable for a period of at least 120 days, while tubes kept at 37°C. longer than 50 days no longer gave growths on new medium. Culture tubes removed to a refrigerator at 4°C. after their maximum growth had been reached (28 days at 25°C.) were found to be still viable at the end of 4 months.

The viability of the organism on blood agar slants is very inconstant. If the plug closing the tube is not practically impermeable the surface quickly dries, and the organisms cease to multiply and degenerate within a fortnight. On a slant at 25°C. containing sufficient condensation water and closed with a nearly air-tight stopper the organisms remain motile for about 2 weeks and transferable for a month or more. The stopper should not be made absolutely air-tight, because *Bartonella bacilliformis* does not grow in the absence of oxygen.[1] As already stated, a slant culture kept at 37°C. dies out in about 10 days.

SUMMARY.

Fifteen specimens of citrated blood from seven monkeys infected with *Bartonella bacilliformis* were kept at 4°C. for periods of 24 to 152 days, and at the end of each period were tested for viability by the cultural method. All yielded cultures, although there was a considerable reduction in the number of living organisms, as shown by titration.

Two specimens of citrated blood, from infected monkeys, which had been kept for 45 days at room temperature yielded growth when a drop of each was inoculated into leptospira medium; while after 67

days at the same temperature 0.1 cc. of a 1:10,000,000 dilution of each specimen was sufficient to yield growth. Since the original titer of the blood had been only 1:10, it is evident that *Bartonella bacilliformis* had multiplied considerably under the conditions. Smears of the specimens showed clumps of organisms among the corpuscles but no intracorpuscular multiplication.

Bartonella bacilliformis survived in the excised nodule from a monkey for at least 56 days at 4°C., and for 28 days at room temperature, when a piece of the tissue was covered with citrate-saline solution or ground up in it. The death of *Bartonella bacilliformis* at room temperature under these conditions may be due to the effect of autolysis of the tissue or to a lack of nutrient substances. The suspension alone is not a suitable culture medium, but a trace of hemoglobin and about 10 per cent of fresh horse or rabbit serum make it a favorable one.

The viability of *Bartonella bacilliformis* was tested in cultures kept at 25°, 37°, and 4°C. for varying periods. At 25°C. cultures on leptospira medium remained transferable after 120 days, and when placed in the refrigerator at the time of maximum growth (after 28 days at 25°C.) they were still viable at the end of 4 months. The viability of cultures on blood agar slants depends to a considerable extent upon the care with which the surface of the medium is protected from drying. Under favorable conditions and at 25°C. the organisms remain motile for about 2 weeks and transferable for a month or longer. Cultures on either of these media die out after 50 days at 37°C.

The data presented suggest that it may be fruitful to make a study by cultural methods of pathological material brought from distant parts of the world, even when many days or weeks have elapsed since it was procured.