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ETIOLOGY OF OROYA FEVER.

III. THE BEHAVIOR OF *BARTONELLA BACILLIFORMIS* IN *MACACUS RHEBUS*.

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PLATES 22 TO 24.

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As reported in a previous paper,¹ a strain of *Bartonella bacilliformis* isolated from the blood of a fatal case of Oroya fever when inoculated into young *Macacus rhesus* monkeys induced a protracted course of intermittent fever, resembling that observed in some human cases of Oroya fever. The microorganism was demonstrated in the red cells of these animals and recovered in culture, but the clinical manifestations in monkeys differed from those of the human disease in that none of the animals showed the severe anemia so characteristic of Oroya fever. It was also shown that intradermal inoculation of the cultures into the shaved skin of the eyebrows of the monkeys gave rise to a granulomatous nodule rich in capillaries, in which the microorganisms could be demonstrated by section and by culture. The nodules presented a marked resemblance to those of *verruca peruviana*.

Further study of the behavior of *Bartonella bacilliformis* in *rhesus* monkeys, particularly with reference to the effects of passage strains, has brought to light a number of additional facts, which throw some light on the variety of manifestations of *Bartonella* infection in man.

The protocols are presented in chronological order, and the extraordinary variability in response to inoculation of the parasite is illustrated by all of them. The severity of infection in most instances is referable probably to enhanced virulence of the parasite through adaptation to the animal. In Monkey 5 we have an example of severe systemic infection induced by local inoculation. In Monkey 6 intra-

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

venous and intraperitoneal inoculation gave rise to a fatal systemic infection, which was not accompanied by local lesions, and which, except for the slight degree of anemia present, resembled a case of simple Oroya fever; the fever was severe (104–105°C.) and continuous, and the parasites were present in the blood in high titer and also in the lymph glands, spleen, and bone marrow. Monkey 7 was inoculated intradermally and intravenously with both passage virus and cultures, and the reaction was constitutionally as well as locally severe; *Bartonella bacilliformis* was isolated from blood diluted 1:10,000,000, from nodular tissue diluted 1:10,000, and also from the lymph glands, spleen, and bone marrow. Monkey 8, on the other hand, while receiving considerable amounts of passage virus both locally and intravenously, manifested no local and only mild systemic symptoms. Monkey 18 is of special interest as an illustration of a severe type of verruga such as may result from the inoculation of monkeys with human verruga tissues,^{2,3} while the striking fact brought out in the case of Monkey 25 is the simultaneous occurrence of severe symptoms of both verruga and Oroya fever, typical verruga nodules appearing spontaneously at sites remote from those of local inoculation. These appearances had not been observed by previous investigators in monkeys inoculated with human verruga tissues.

Quantitative estimates of the number of *Bartonella bacilliformis* present at any given time in the blood are expressed in terms of the highest dilution of the blood from which cultures of the organism could be obtained. The culture method is the only satisfactory test of the presence of the parasite. The use of monkeys is obviously not practicable for quantitative determination and would not, moreover, be reliable because of the variation in susceptibility to *Bartonella* infection. Careful microscopical examination of stained blood films was not, of course, neglected, but when one considers that citrated blood of as high titer as 1:100,000 would show only 1 cell invaded by the parasite among 3,000 cells (1 cc. of citrated blood containing approximately 3 billion red cells), the difficulties of this method of demonstrating the organism become evident.

² Jadassohn, G., and Seiffert, G., *Z. Hyg. u. Infektionskrankh.*, 1910, lxvi, 247.

³ Mayer, M., Rocha Lima, H., and Werner, H., *Münch. med. Woch.*, 1913, lx, 739.

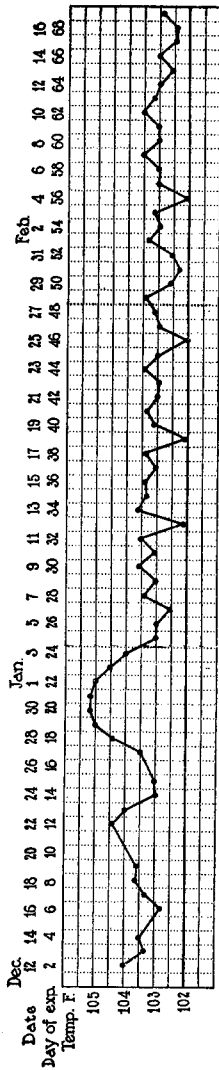
The Invasion of the Blood by B. bacilliformis after Intradermal Inoculation.

The ease with which *Bartonella bacilliformis* may enter the general circulation after intradermal inoculation is illustrated in the case of *M. rhesus* 5.

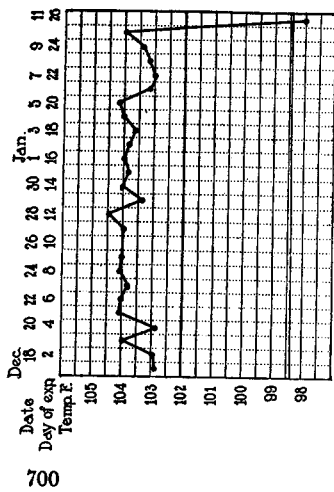
M. rhesus 5 (Text-fig. 1). A young monkey, about 2,000 gm.—a size which has been found most satisfactory for experimental work with *Bartonella bacilliformis*—was inoculated, Dec. 11, 1925, intradermally on the shaved left eyebrow, with 0.2 cc. of a saline suspension of pooled cultures which on dark-field examination showed 3 to 4 motile microorganisms per field. The mixture included 1 cc. of condensation water from a 9 day old culture on a blood agar slant, derived from the original human blood, and 0.3 cc., in 20 cc. of 0.9 per cent saline, of a 10 day old culture, derived from the blood of *M. rhesus* 1, and grown on leptospira medium at 25°C. Cultures made with the blood withdrawn on that date (11 days after inoculation) revealed the presence of *Bartonella bacilliformis* in a titer of 1:100,000, and in stained preparations the parasites were demonstrated in small numbers in the erythrocytes. Cultures made on Dec. 28, 1925, and on Jan. 5, 12, and 25, 1926, also yielded positive results. The animal showed the highest fever during this period, the temperature being continuously at 105°F. from Dec. 29 to Jan. 1. The last two specimens of blood had a titer of only 1:10, and the parasites could no longer be demonstrated in stained smears, hence the number of organisms in the blood stream had diminished rapidly within a period of 24 days. The site of intradermal inoculation on the left eyebrow, however, began to show induration nearly 42 days after injection; on Jan. 22 it was about 2 × 3 mm. in area and was slightly raised; on Feb. 1 it measured 6 × 6 mm., and on Feb. 4 about 9 × 9 mm. It was at first pale and semitransparent, but firm to the touch; then it became a deep rose-red and finally cherry-like in form and color. During the following 10 to 12 days it remained nearly stationary, but later it showed slight excoriation at the apex and was removed* for transfer, culture, and histological studies. It proved to be infective for *rhesus* monkeys, yielded a pure culture of *Bartonella bacilliformis*, and on section showed the characteristic endothelial proliferation with intracellular localization of the organism.

Blood cultures made on Feb. 9 and 17 yielded growth of *Bartonella bacilliformis* in 0.1 cc. of a 1:10 dilution. At no time after subsidence of the early violent febrile reaction had the animal's temperature been higher than 103.6°F. The animal was killed under ether anesthesia on Feb. 17, 1926, 68 days after inoculation, in order that the distribution of the microorganisms and the character of the specific lesions might be ascertained.

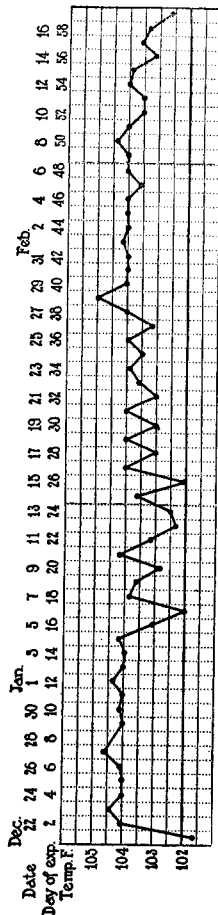
* All operations were performed under ether anesthesia.



TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIG. 3.

Autopsy.—Spleen enlarged and granular on surface, culture +. Lymph glands everywhere enlarged, culture +. Heart blood culture +. Bone marrow (femur) reddish, culture +. Lungs normal, culture negative. Liver normal, culture negative. Kidney normal, culture lost because of contamination.

With the cooperation of Dr. J. H. Bauer, erythrocyte counts and hemoglobin estimates⁴ were made on several occasions. The results follow:

Jan. 12, 1926 (32 days after inoculation). Erythrocytes 6,169,000. Hemoglobin 82 per cent.

Jan. 25, 1926 (45 days after inoculation). Erythrocytes 5,901,000. Hemoglobin 75 per cent.

Feb. 15, 1926 (66 days after inoculation). Erythrocytes 5,248,000. Hemoglobin 70 per cent.

Feb. 17, 1926 (68 days after inoculation). Erythrocytes 4,856,000. Hemoglobin 70 per cent.

The chronic nature of the infection is clearly indicated by the persistence of the parasites in the peripheral blood, bone marrow, lymph nodes, and spleen. The number of microorganisms demonstrated by stained preparations in the blood corpuscles was never large at any time, even during the febrile or "septicemic" period. There was little sign of anemia until the latter part of the experiment, when the number of red cells seemed definitely to have diminished, notwithstanding the marked fall in temperature and the scarcity of *Bartonella bacilliformis* in the blood at this time.

Variability in the Effect of Inoculation of Bartonella bacilliformis into Macacus rhesus.

The outcome of inoculation of a given pathogenic microorganism must depend in part on the virulence of the strain and in part on the degree of susceptibility of the host. In the case of a newly isolated microorganism the effect of successive animal passages on virulence is problematic; the pathogenic property may be either weakened or enhanced by continued passage. Experiments have just been described in which a second passage of *B. bacilliformis* through *Macacus rhesus* indicated a decided increase in virulence of the organism for this species of animal. The present experiment has to do with the effects of further direct passages in *rhesus* monkeys.

⁴Dr. J. H. Bauer was kind enough to make the blood counts on all the animals of this report. The hemoglobin estimates were made by means of the Sahli hemoglobinometer.

As the protocol shows, intravenous and intraperitoneal inoculation of a young monkey (*M. rhesus* 6) with the citrated blood from infected monkeys of the same species led to an immediate and severe febrile reaction to which the animal succumbed after 3 weeks of continuous fever of about 104°F.

M. rhesus 6 (Text-fig. 2) received 2 cc. of citrated blood from *M. rhesus* 2 intravenously on Dec. 17, 1925, and 5 cc. of a mixture of citrated blood from Monkeys 2, 3, and 4 intraperitoneally on Dec. 18. The temperature rose to 104°F. within 48 hours, dropped to 103° for 1 day, returned to 104°, and remained at about that point until the 20th day. It was 103° on the 21st to 24th days and 104° on the 25th, but on the 26th day it fell suddenly to 98° (subnormal). The animal was cyanotic, cold, and rigid and died during ether anesthesia.

Blood cultures made on the 4th, 12th, 18th, and 26th days after inoculation yielded the following results:

Dec. 21, 1925.	Positive with blood diluted 1:100,000.
“ 28, “	“ (untitrated).
Jan. 3, 1926.	Positive with blood diluted 1:100,000.
“ 11, “	“ “ “ “ 1:10,000.
“ “ “	“ “ plasma “ 1:10.

The erythrocyte count on Jan. 3, 1926 (17th day), was 4,760,000, hemoglobin 75 per cent. Examination of organs at autopsy revealed enlargement of the spleen and of the lymph glands generally. The other organs appeared normal. *Bartonella bacilliformis* was obtained in pure culture from blood, spleen, and lymph nodes.

The striking phenomena in the foregoing experiment were the absence of localized lesions and the sudden collapse of the animal. The latter can hardly have been due to the insignificant anemia that had developed. The persistent presence of *Bartonella bacilliformis* in large numbers in the blood and organs may have resulted in a toxemia.

That different individual animals react differently to practically the same infective material is demonstrated in the following experiment.

M. rhesus 7 (Text-fig. 3) was inoculated intravenously on Dec. 21, 1925, with 5 cc. of mixed citrate blood from Monkeys 2, 3, and 4. At the same time intradermal inoculations were made on the right eyebrow with 0.2 cc. of an emulsion of the nodule from Monkey 4 and on the left with 0.2 cc. of a young culture, grown on leptospira medium, of blood from Monkey 2. Adjacent areas on each eyebrow were scarified and smeared with the same material which had been injected intradermally into that eyebrow.

In the case of this animal observation was especially directed to (1) the quantitative fluctuation of *Bartonella bacilliformis* in the blood, (2) the course of the local nodular reactions, and (3) the effect of the infection upon the red corpuscles and hemoglobin, this portion of the study being carried out by Dr. Bauer.

The results of blood culture were as follows:

Dec. 24, 1925 (3 days after inoculation). Positive with blood diluted 1:10,000,000.

Dec. 28, 1925 (7 days after inoculation). Positive (not titrated).

Jan. 4, 1926 (14 days after inoculation). Positive with blood diluted 1:1,000,000.

Jan. 18, 1926 (28 days after inoculation). Positive with blood diluted 1:10.

“ 29, “ (39 “ “ “). “ “ “ “ 1:10.

Feb. 9, “ (50 “ “ “). “ (not titrated).

“ 17, “ (58 “ “ “ “). “ (“ “).

The relatively large number of microorganisms found in the blood withdrawn about 72 hours after intravenous inoculation indicates a strikingly rapid multiplication of the parasites. During the 11 days there would appear to have been a gradual diminution in the number of organisms, and the findings point to an astonishing elimination in the following 2 weeks, since on Jan. 18 *Bartonella bacilliformis* could not be cultivated from blood in dilutions higher than 1:10. The titer was still 1:10, 40 days after inoculation, and the organisms were present in the circulation after 58 days.

In this instance, as in many others, *Bartonella bacilliformis* was demonstrable in small numbers of erythrocytes when the blood titer was high. But as the titer fell to 1:10, examination of film preparations yielded negative results.

The intradermal inoculation of the culture gave rise to a definite nodule (2 × 3 mm.) within 11 days. The lesion gradually increased in size (to 4 × 6 mm.) and had assumed a rose color when it was excised for study on Jan. 8, 1925 (19th day after inoculation). No lesion developed at the site where the culture was inoculated by scarification. The nodule at the site of intradermal inoculation of the nodule emulsion from Monkey 4 became noticeable only after 20 days, and at the same time a small area of induration appeared on the adjacent scarified area. The nodule and indurated area became gradually redder and larger and were removed for study 29 days after inoculation.

fig. 4). During the first 12 days the temperature rose as high as 106°F., and during the second and third periods it fluctuated about 104° for about 10 days. Although the temperature was 104° on the day following inoculation, the febrile reaction seems to have become definite on the 6th day.

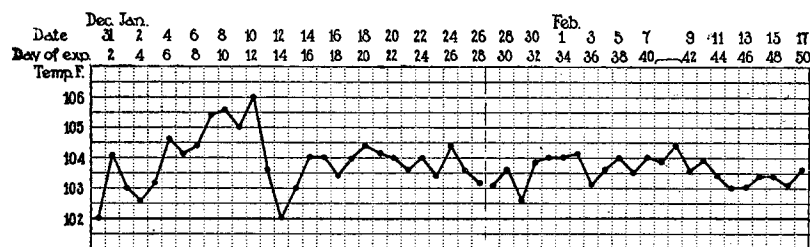
Four blood cultures were made during life and one post mortem, with the following results:

Jan. 4, 1926 (5 days after inoculation). Positive with blood diluted 1:10.
 " 8, " (9 " " "). " " undiluted blood.
 " 18, " (19 " " "). " " blood diluted 1:1,000.
 Feb. 3, " (35 " " "). " " " " 1:10.
 " 17, " (49 " " "). Negative.

Blood counts showed no anemia:

Jan. 8, 1926 (9 days after inoculation). Erythrocytes 5,506,000. Hemoglobin 80 per cent.

Feb. 3, 1926 (35 days after inoculation). Erythrocytes 5,414,000. Hemoglobin 80 per cent.



TEXT-FIG. 4.

Feb. 17, 1926 (49 days after inoculation). Erythrocytes 5,496,000. Hemoglobin 80 per cent.

The animal was killed by etherization on Feb. 17, 1926, for examination. Autopsy revealed marked enlargement of the lymph glands. No other changes were noticeable. The spleen, blood, and bone marrow failed to yield cultures, but growth was obtained from the emulsion of the lymph nodes.

As a result of the inoculations on the eyebrows, four tiny indurated areas appeared within 9 to 10 days but did not enlarge and within 18 days had practically disappeared. A red spot of pin-point size was noticed on the scarified area after 29 days, but it also disappeared in another week.

While the nodule employed for inoculation in this instance probably contained fewer organisms than the one removed from Monkey 4 earlier in its evolution, yet the suspension from it yielded a pure growth of *Bartonella bacilliformis* in a 1:10 dilution, and a large number

of organisms were found in sections. Loss of virulence may have resulted from the final etherization of Monkey 4.

The comparatively mild infection, accompanied by severe febrile reactions, seems to have been due to a higher resistance of this particular animal, but the chronic nature of the *Bartonella* infection is indicated even in this instance by the persistence of the microorganisms in the lymph glands.

The Reproduction of Striking Clinical Features of Oroya Fever and Verruga.

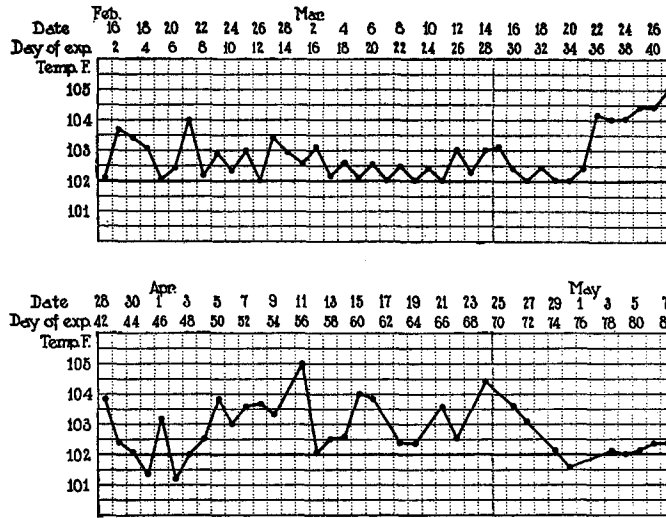
Up to this point in the work, the manifestations by monkeys of infection with *Bartonella bacilliformis* resembled human Oroya fever or verruga in essentials, *i.e.*, in the typical localization of the organism and in the characteristic skin lesions, respectively, but the severe anemia of Oroya fever and the general eruption of verruga had not as yet been reproduced in monkeys.

In Monkey 18, however, the local lesions, while not general or spontaneous, attained extraordinary size and resembled those described by previous investigators as resulting from the inoculation of suspensions of human verruga tissues into monkeys. Monkey 25 developed the spontaneous generalized eruption characteristic of verruga and also severe symptoms of Oroya fever (anemia, presence of *Bartonella bacilliformis* in considerable numbers in the erythrocytes). Although the period of fever was very brief in this animal, the manifestations of infection were similar to those of certain human cases in which Oroya fever and verruga are simultaneously present.⁵

M. rhesus 18 (Text-fig. 5) was inoculated on Feb. 15, 1926, with passage virus (suspension of nodular tissue) from Monkey 5. The shaved left eyebrow received two intradermal injections of the emulsion, and the shaved right eyebrow was scarified and smeared with the same material. The skin of the abdomen was shaved, and on the left side two intradermal injections were made of the emulsion, while on the right two scarified areas (about 3 × 3 cm. each) were spread with the same material. Induration at the sites of inoculation became evident in about 14 days and were definite after 26 days (Fig. 1). The lesions steadily increased in size, and 36 days after inoculation the largest of the nodules on the scarified areas of the right eyebrow measured 2.5 × 2 cm. and was 1 cm. high

⁵ Odriozola, E., *La maladie de Carrion*, Paris, 1896.

(Fig. 7), while the two round subcutaneous nodules on the left abdominal wall stood out about 1 cm. and showed a reddish spot at the point of insertion of the syringe needle (Fig. 8). The lesions were of maximum size 59 days after inoculation (Fig. 2) and remained stationary for about 14 days. Retrogression was slow, but at the time of writing (99 days after inoculation) they are pale, fibrous, and considerably decreased in size, as shown in Figs. 3 and 11. No ulceration or softening of the lesions could be detected at any time. The examination of a nodule excised from the right eyebrow 26 days after inoculation revealed the characteristic granulomatous structure with proliferation of endothelial cells (Fig. 9), in the cytoplasm of which were found varying numbers of *Bartonella bacilliformis*, usually in clumps (Fig. 10).



TEXT-FIG. 5.

Bartonella bacilliformis was demonstrated in sections of the lesions and isolated in culture from emulsions of the tissue. It was recovered from the blood of the monkey on the following occasions:

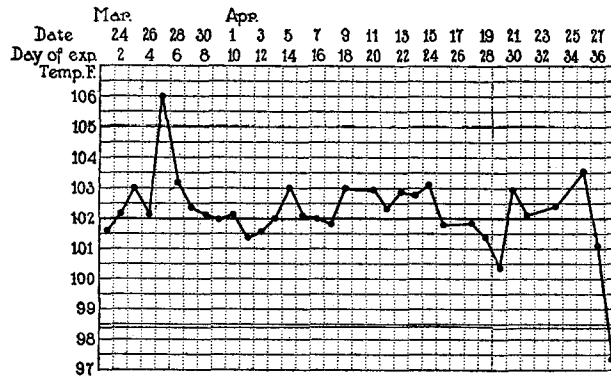
- Mar. 5, 1926 (18 days after inoculation). Positive in blood diluted 1:100,000.
- “ 18, “ (31 “ “ “). “ “ “ “ 1:100,000.
- “ 27, “ (40 “ “ “). “ “ “ “ 1:10.

Comparison of blood counts made on the day of inoculation and 60 days later show that there was a definite decrease in the number of red cells during the course of illness:

- Feb. 15, 1926. Erythrocytes 5,356,000. Hemoglobin 90 per cent.
- Apr. 16, “ “ 4,120,000. “ 60 “ “

One of the most striking features of the experimental *Barionella* lesions is the slow continuous formation of granulomatous tissue on a scarified surface of the skin, which results in an enormous accumulation of endothelial cells and subsequent capillary formation, a process apparently identical with that which takes place in the lesions of verruga in man.

M. rhesus 25 (Text-fig. 6) was inoculated intravenously on Mar. 23, 1926, with 6 cc. of a saline suspension of cultures grown on blood slants for 10 days at 25°C. At the same time the shaved right eyebrow was inoculated intradermally as well as by scarification with a suspension of nodular tissue from *M. rhesus* 18. The left eyebrow was similarly inoculated with culture. This animal had



TEXT-FIG. 6.

only 1 day of high temperature, Mar. 27, when 106°F. was recorded; otherwise the course of disease was practically afebrile.

The first sign of infection in this instance was a peculiar condition of the upper eyelids, the edges of which were irregularly thickened, and perhaps a little pinkish on Apr. 7, 15 days after inoculation. Within a week numerous miliary, cherry-red nodules appeared around the eyes on both sides, and distinct nodules became noticeable at the site of intradermal inoculation as well as on the scarified areas. The animal presented a striking aspect (Fig. 4). These lesions, and several miliary nodules scattered over the body, especially near the right inguinal surface (Fig. 5) gradually became larger within the next 5 or 6 days and partly confluent. On the posterior surface of the left leg nodules of varying size appeared within 3 weeks after inoculation (Fig. 6) and remained until death. The eyes were virtually closed 36 days after inoculation (Apr. 28, 1926), when the animal died (Fig. 12). One of the spontaneous nodules was removed for cultural

and histological examination on Apr. 15. It showed the usual endothelial proliferation (Fig. 13), and large numbers of *Bartonella bacilliformis* were present (Fig. 14).

The number of red corpuscles infected with *Bartonella bacilliformis* was larger than in any of the monkeys previously studied, cells invaded by the parasites being readily demonstrable in film preparations. Curiously enough, however, the detection of the organism was not accomplished, even by the culture method, during the 48 hours preceding death.

Mar. 27 (5 days after inoculation). Positive with blood diluted 1:1,000.

Apr. 8 (18 " " "). " " " " 1:100,000.

" 16 (26 " " "). " " " " 1:1,000,000.

The results of blood counts by Dr. Bauer were as follows:

Mar. 27, 1926 (4 days after inoculation). Erythrocytes 5,288,000. Hemoglobin 85 per cent.

Apr. 20, 1926 (28 days after inoculation). Erythrocytes 3,240,000. Hemoglobin 45 per cent.

Apr. 22, 1926 (30 days after inoculation). Erythrocytes 2,992,000. Hemoglobin 40 per cent.

Apr. 24, 1926 (32 days after inoculation). Erythrocytes 3,368,000. Hemoglobin 40 per cent.

Apr. 26, 1926 (34 days after inoculation). Erythrocytes 2,736,000. Hemoglobin 35 per cent.

Apr. 27, 1926 (35 days after inoculation). Erythrocytes 2,120,000. Hemoglobin 30 per cent.

Apr. 28, 1926 (36 days after inoculation). Erythrocytes 1,624,000. Hemoglobin 25 per cent.

Autopsy.—Very much emaciated, wax-yellow. The nodules on the eyebrows remained unopened, but the lesions on the lids had partly ulcerated, and the tissues had become somewhat necrotic. The eyes were almost closed. There was some seropurulent discharge from the ulcerated nodules, which were nearly confluent. Corneæ not affected. Spontaneous miliary nodules were found on the abdomen, thighs, and legs, the posterior surfaces of both legs, in particular, being covered with cutaneous or subcutaneous nodules of varying size (Fig. 6), some adherent to the fascia. Some nodules were hemorrhagic.

There was exudative pericarditis, due to the presence of minute Gram-negative, motile bacilli in enormous numbers, especially within the polymorphonuclear leucocytes. The heart muscles were pale and flabby, but the pleuræ did not appear to be involved. Lungs normal. Liver perhaps enlarged, and pale. Spleen somewhat enlarged and soft. Stomach empty, normal. Intestines and mesentery: General lymphatic system hypertrophied everywhere. Other organs normal. Bone marrow (femur) dark red.

The findings described suggest a terminal secondary bacterial pericarditis, which may have been the result of transfer by the animal

of infective material from the ulcerated eyelid to a needle wound caused by heart puncture. Notwithstanding this late stage invasion, the essential features of the course of illness in this animal were, I believe, the result of local and constitutional infection with *Bartonella bacilliformis*.

SUMMARY.

The experiments reported here were carried on in the main with passage strains of *Bartonella bacilliformis*, and the results indicate that the virulence of the organism has been considerably enhanced by passage through susceptible animals. While the animals of the earlier experimental series showed no anemia, some of the present group manifested a definite reduction in the number of red cells and in hemoglobin, and in one instance (*M. rhesus* 25) anemia was of the extreme type so often associated with Oroya fever in man. The anemic condition appeared to be secondary in character, however, nucleated red cells being few in number. In this animal also *Bartonella bacilliformis* was readily demonstrated in the erythrocytes by means of stained smears, though the number of cells invaded by the parasites was by no means so great as in the human infection.

In most instances of experimental *Bartonella* infection so far induced the demonstration of the parasites by ordinary routine examination of stained film preparations is possible only when the titer of the blood exceeds 1:1,000. Prolonged search of many slides has not been attempted, however. The number of microorganisms in the blood, as shown by culture tests of ascending dilutions, was in most instances highest (1:100,000 to 1:10,000,000) during the early period of the infection coincident usually with the period of highest fever, falling to a titer of 1:10 during the last half of the disease. In one of the fatally infected monkeys, however, the titer increased from 1:10 on the 4th day to 1:1,000,000 on the 24th day. The titer of the blood was equally great in Monkeys 5 and 6, although the former was inoculated locally, the other intravenously and intraperitoneally. The largest proportion of infected red cells was found in Monkey 25, while the blood titer, as shown by culture test, was highest in Monkey 7.

The febrile reaction varied in the animals of this series from a severe continuous fever of 104–105°F., lasting 2 to 3 months, in one instance,

with a remittance during the 3rd to 5th weeks, to the acute high fever (106°F.) of 1 day's duration in the fatally infected monkey, No. 25. The more usual reaction, however, is an irregular course of moderate fever with one or more periods of high temperature (105°).

Bartonella bacilliformis was constantly demonstrated, both microscopically and by culture tests, in the lymph glands of animals sacrificed 2 to 3 months after inoculation, and in two of three instances it was present also in the spleen, bone marrow, and heart blood. In the case of *M. rhesus* 6, which died 26 days after inoculation, the microorganism was obtained also in culture from the lymph glands, spleen, and heart blood taken at autopsy. In the other animal which died, a terminal bacterial infection, while not obscuring the effects of the *Bartonella* infection, made it impossible to isolate the parasite from either blood or tissues.

The skin lesions, whether of the nodular type, induced by introduction of the virus intradermally or by application to the scarified skin, or of the miliary character occurring spontaneously as a result of systemic infection, always yielded cultures of *Bartonella bacilliformis*, and stained sections of such lesions revealed the parasites in large numbers in their characteristic situation in the endothelial cells.

A chronic, systemic infection, in which the lymph glands are enlarged and *Bartonella bacilliformis* is present in the blood in high titer, may be induced by local inoculation, as shown in the case of *M. rhesus* 5.

The local lesions induced in one instance by introduction of a passage strain, both intradermally and by scarification, attained within 2 months extraordinary size, the nodules arising at adjacent sites of inoculation on the right eyebrow having coalesced into a large pedunculated mass which overhung the eye. This type of reaction had not been observed hitherto in the course of the present study but has been described by earlier investigators as a result of the inoculation of monkeys with human verruga tissues.

The striking fact brought out in the present study is the variety of responses to inoculation which animals of the same species may manifest. The clinical features of the infection may be typical of Oroya fever or may resemble those of verruga peruviana, and in *M. rhesus* 25 we have an instance of a type of infection in which the

characteristic phenomena of both conditions are simultaneously present. Whether the appearance will resemble those of the one or the other condition appears to depend on the susceptibility of the individual as well as on the virulence of the organism. Moreover, it seems probable that different degrees of resistance to the invasion of the parasite on the part of the blood cells, internal organs, or skin of a given animal may determine the predominant clinical manifestations of the infection. The factor of variation in susceptibility of different individuals or different tissues of the same individual would account for the variety of types of human *Bartonella* infection.⁶

EXPLANATION OF PLATES.

PLATE 22.

All figures natural size.

FIG. 1. *M. rhesus* 18, 26 days after inoculation. The nodule on the inner aspect of the left eyebrow was subsequently removed for examination.

FIG. 2. *M. rhesus* 18, 59 days after inoculation, when the nodules had reached their maximum development. The nodules on the right side had been induced by inoculation by scarification; there was no softening or ulceration of these lesions at any time. The nodule on the left side was induced by intradermic inoculation.

FIG. 3. *M. rhesus* 18, 99 days after inoculation, when the nodules had retrogressed considerably and the animal was decidedly paler.

FIG. 4. *M. rhesus* 25, 23 days after inoculation. The indurations which had developed at the sites of inoculation on the inner aspect of each eyebrow were almost eclipsed by the numerous spontaneous deep red nodules which had appeared about the eyes.

FIG. 5. *M. rhesus* 25, showing the spontaneous nodules which developed in the inguinal region, 29 days after inoculation.

FIG. 6. *M. rhesus* 25, showing the spontaneous nodules which had appeared on the posterior surface of the legs 29 days after inoculation.

PLATE 23.

FIG. 7. *M. rhesus* 18, 36 days after inoculation, showing the appearance of the lesions at the sites of intradermal inoculation and of inoculation by scarification. Natural size.

FIG. 8. *M. rhesus* 18, 36 days after inoculation, showing the lesions on the abdomen. The verrucous lesions on the right side were induced by scarification,

⁶ Arce, J., La enfermedad de Carrion, Lima, 1920.

the two round subcutaneous nodules on the left side by intradermal and partly subcutaneous inoculation. Natural size.

FIG. 9. Section of nodule removed from eyebrow of *M. rhesus* 18, 26 days after inoculation. Giemsa's stain. $\times 182$.

FIG. 10. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.

FIG. 11. *M. rhesus* 18, 99 days after inoculation, showing the appearance of the receding lesions on the abdomen. One of the nodules at the sites of intradermal inoculation had been removed.

PLATE 24.

FIG. 12. *M. rhesus* 25, at the time of death 36 days after inoculation.

FIG. 13. Section of spontaneous nodule of *M. rhesus* 25, removed 23 days after inoculation. Giemsa stain. $\times 182$.

FIG. 14. The same section at a magnification of 1,000 times, showing the presence of masses of *Bartonella bacilliformis*.



FIG. 1. *M. rhesus* 18, 26 days after inoculation.

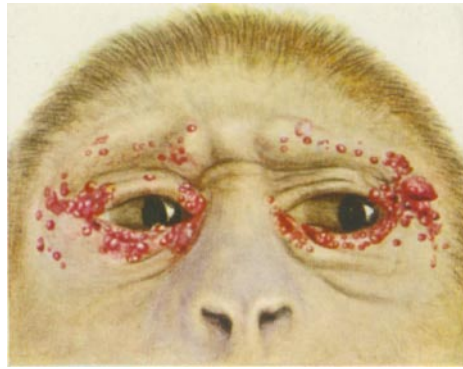


FIG. 4. *M. rhesus* 25, 23 days after inoculation.



FIG. 2. *M. rhesus* 18, 59 days after inoculation.



FIG. 5. *M. rhesus* 25 (groin), 29 days after inoculation.

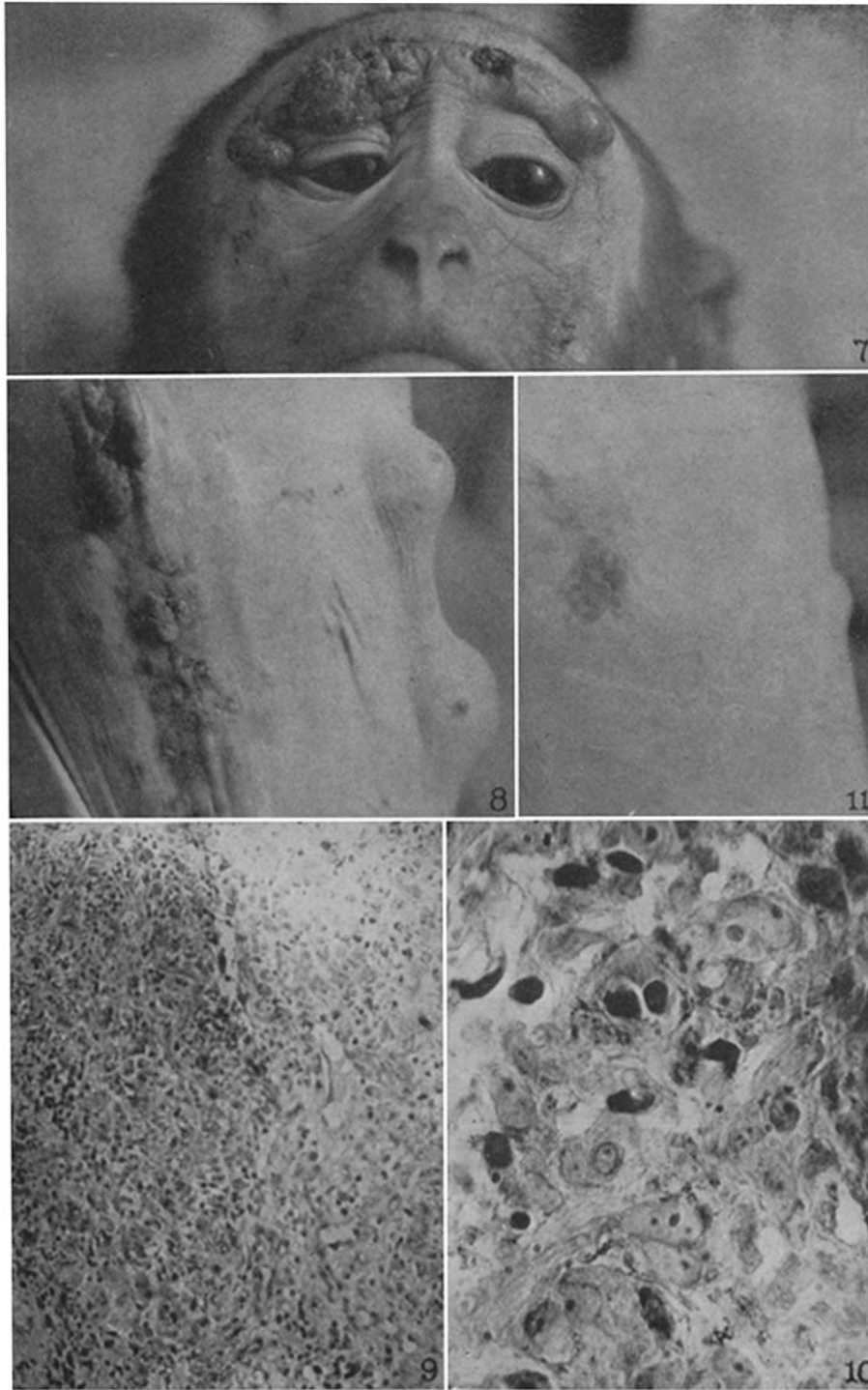


FIG. 3. *M. rhesus* 18, 99 days after inoculation.

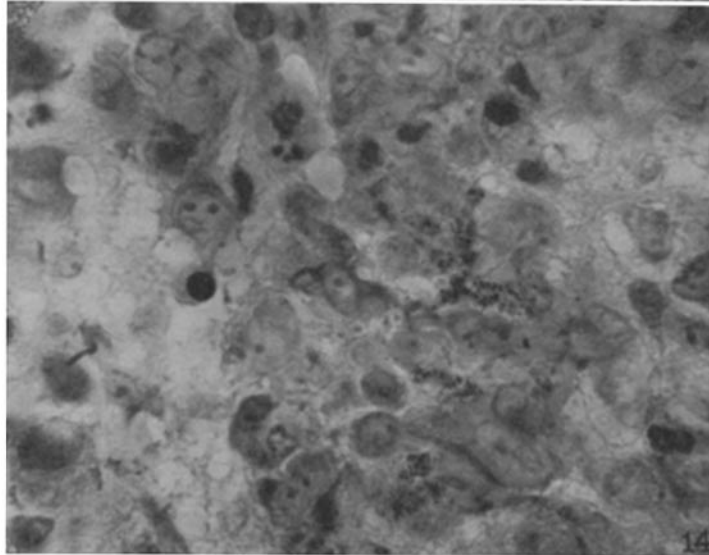
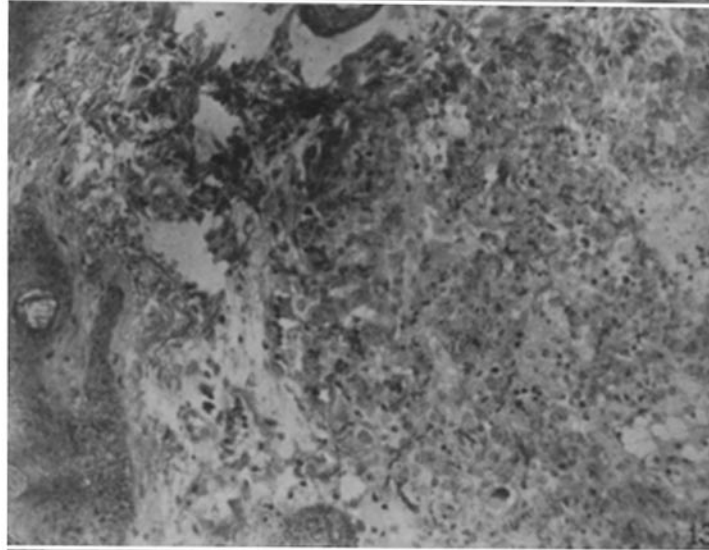


FIG. 6. *M. rhesus* 25 (leg), 29 days after inoculation.

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