

## ETIOLOGY OF OROYA FEVER.

### IV. THE EFFECT OF INOCULATION OF ANTHROPOID APES WITH BARTONELLA BACILLIFORMIS.

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PLATES 25 TO 28.

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It has been reported in previous papers that the intravenous inoculation of young *rhesus* monkeys with a strain of *Bartonella bacilliformis* isolated from a case of Oroya fever gives rise to two essential manifestations of Oroya fever, namely, protracted fever, and typical localization of the parasite within the red blood cells (Fig. 1), while the introduction of the organism intradermally induces skin lesions which are indistinguishable from those of verruga peruviana.<sup>1</sup> It has been shown also that after intradermal inoculation the organism may pass into the circulation and invade the red blood cells, that it may give rise to an anemic condition comparable with that of Oroya fever, except for the absence of appreciable numbers of nucleated red cells in the blood stream, and that it may induce a generalized eruption similar to that of human verruga.<sup>2</sup> In most instances, however, the inoculated monkeys have shown little or no anemia, and comparatively few erythrocytes are invaded by the parasite, while the verruga nodules have usually arisen only at the sites of inoculation into the skin.

Since the invasion of the red corpuscles by the bacilliform organisms is the most characteristic sign of Oroya fever, and the formation of nodules by the proliferation of endothelial cells is equally typical of verruga, the findings described indicated that the organism cultivated was probably the cause of both these conditions. Nevertheless, it

<sup>1</sup> Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1925, xliii, 851.

<sup>2</sup> Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.

was desirable to reproduce, if possible, the complete clinical picture of Oroya fever,—the extreme anemia of pernicious type, and the invasion of large numbers of the erythrocytes by the microorganism, such as takes place in human Oroya fever (Fig. 2). The opportunity presented itself of studying the effect of the inoculation of two anthropoid apes with cultures and passage strains of *Bartonella bacilliformis*, and the experiments were undertaken in the hope that these animals, because of their close phylogenetic relationship to man,<sup>3,4</sup> might prove sufficiently susceptible to the infection to manifest all of the severe symptoms to which the parasite gives rise in man, either of the type of Oroya fever, or that of verruga. As the protocols show, however, they manifested only slight constitutional reaction to the inoculations. The erythrocytes were invaded by the organisms to a small extent only, and there was little or no anemia.<sup>5</sup> The skin lesions, while of the characteristic verruga type, remained localized at or near the sites of introduction of the microorganisms; there was no spontaneous generalized skin eruption such as is observed in human verruga, and in rare instances in *rhesus* monkeys.

A young female chimpanzee (*Pan leucoprymnus*), said to be about 4 years old, was inoculated on Jan. 29, 1926, with the suspension of a nodule excised\* from the eyebrow of *M. rhesus* 3<sup>1</sup> on the same day, and also with cultures, grown on leptospira medium, representing the second generation from the original human blood and the first generation from the blood of *M. rhesus* 1.<sup>1</sup> The suspension was inoculated intradermally into the right eyebrow and by scarification on the right side of the abdomen, while on the left eyebrow and left side of the abdomen a mixture of the suspension with cultures was introduced part intradermally and part subcutaneously. The quantities injected were 0.2 to 0.3 cc. 5 cc. of the mixture were injected subcutaneously into the right lower portion of the abdomen, above the inguinal region, the site of inoculation being carefully massaged after the injection.

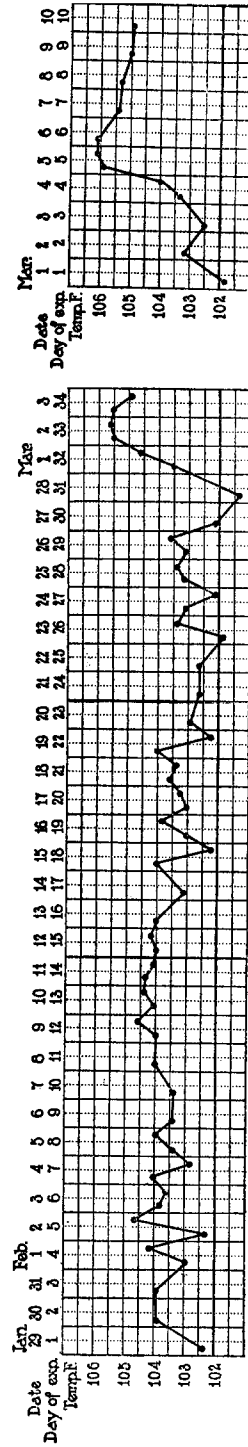
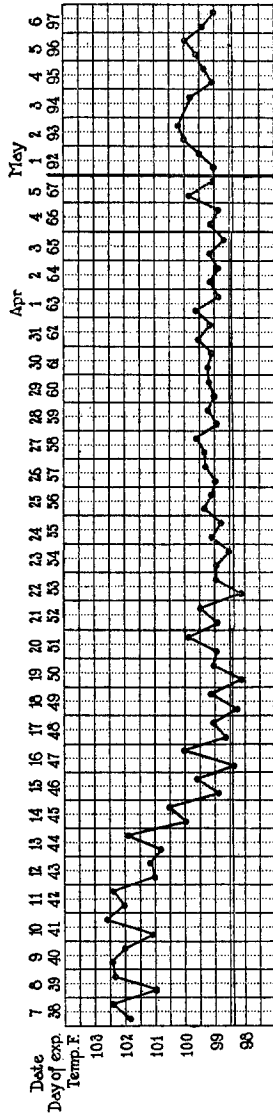
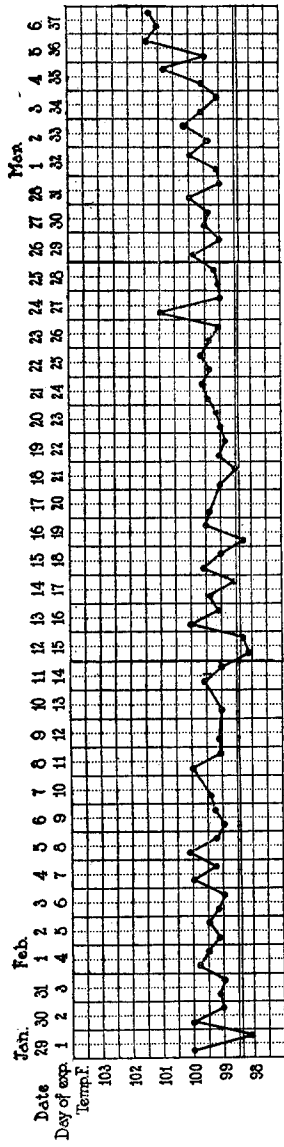
The normal temperature of the chimpanzee, as shown by daily records covering a period of several weeks, varied between 98.6°F. and 99.6°; occasionally the afternoon (4 p.m.) temperature was as high as 100°. Blood counts made before inoculation on Jan. 29 gave the following results: Erythrocytes 5,520,000, leuco-

<sup>3</sup> Nuttall, G. H. F., Blood immunity and blood relationship, Cambridge, 1904.

<sup>4</sup> Landsteiner, K., and Miller, C. P., *J. Exp. Med.*, 1925, xlii, 853.

<sup>5</sup> The hematological studies were by Dr. J. H. Bauer.

\* All operations were performed under ether anesthesia.



TEXT-FIG. 1. Chimpanzee.

TEXT-FIG. 2. *M. rhesus* 14.

TEXT-FIG. 3. *M. rhesus* 21.

cytes 7,810, hemoglobin 80 per cent.<sup>6</sup> The inoculations were made between 12 noon and 1 p.m.

*Jan. 30.* Animal appeared as usual; appetite good. Slight irritation at the sites of scarification. *Jan. 31.* No change, except that the lines of scarification were slightly raised. *Feb. 3.* Some swelling at the sites of intradermal injection, but no reddening. *Feb. 4.* Animal appeared perfectly well. Erythrocytes 5,680,000, leucocytes 8,190, hemoglobin 85 per cent.

*Feb. 8.* The abdominal wall showed definite edema, but the animal ate well and appeared normal. Erythrocytes 5,335,000, leucocytes 11,900, hemoglobin 85 per cent. The blood yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10, but routine examination of film preparations failed to demonstrate cells containing the organism. *Feb. 9.* Distinct induration at the sites of intradermal inoculation on eyebrows and abdomen, but no reddening. *Feb. 12.* Abdominal nodule at site of intradermal and subcutaneous injection increased in size ( $3 \times 5$  mm.); overlying skin edematous. There was a small red spot at the most prominent portion of the nodule. Animal still in excellent condition. *Feb. 15.* Reddening of abdominal nodule had spread peripherally; a tumor of irregular contour and uneven surface was felt under the tense, somewhat edematous skin. The tumor was firm, and the skin was partly adherent to it; there was no softening or fluctuation on palpation. Scarified areas on abdomen showed erythematous dots, but there was no definite change in the appearance of the scarified areas on the eyebrows. The nodules induced by the intradermal injections measured  $4 \times 4$  mm. The induration was more subcutaneous than intradermal, and at the points of needle insertion the skin was pinkish.

*Feb. 19.* Abdominal nodule  $1.5 \times 2$  cm. in size, deep seated, irregular to palpation, and partly adherent to the skin, which appeared somewhat yellowish. Abdomen less edematous. Nodules on eyebrows slightly increased in size; overlying skin normal in color. *Feb. 22.* Abdominal nodule measured  $5 \times 6$  cm. and stood out prominently above the abdominal wall. It seemed to consist of several lobulous nodules of varying size. The skin showed a definitely yellowish tint on one side. The lymph glands in the axillary and inguinal regions and at the flexor side of both elbow joints were markedly swollen.

*Feb. 23.* The abdominal nodule was harder and perhaps somewhat larger (Fig. 13). The lines of scarification on the right abdominal wall were bright red, considerably raised, and indurated (Fig. 9). Erythrocytes 6,234,000, leucocytes 12,000, hemoglobin 90 per cent. The blood yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10,000, and the organism was demonstrated in small numbers in the red cells. *Feb. 25.* The intradermal nodules on the eyebrows (Fig. 8) were red at the apices. The other lesions had increased in size and intensity.

*Mar. 1.* The animal had shown no constitutional reaction and appeared to be perfectly well. Erythrocytes 5,120,000, hemoglobin 85 per cent. Cultures of

<sup>6</sup> All the hemoglobin estimates were made with Sahli's hemoglobinometer.

undiluted blood positive. Routine examination of blood films failed to reveal blood corpuscles containing *Bartonella bacilliformis*. A portion of the abdominal nodule (Fig. 3) was excised for examination. It showed characteristic proliferation of endothelial cells (Fig. 14), and *Bartonella bacilliformis* was demonstrable in the cytoplasm of the cells (Figs. 5, 15). Cultivation of a suspension of the nodular tissue yielded growth of the organism. A portion of the skin lesion on the scarified area of the abdomen (Fig. 9) was also excised. It was similar in structure to the abdominal nodule (Figs. 10, 11) and yielded a culture of *Bartonella bacilliformis*.

For more than four weeks the blood count, temperature, appetite, and general health and disposition of the animal had remained apparently normal. There were so few microorganisms in the blood on Mar. 1 that undiluted citrated blood in amounts less than 0.2 cc. failed to yield growth, and demonstration of red corpuscles containing *Bartonella bacilliformis* was not possible by the ordinary routine examination of one or two film preparations. The effect of the inoculation had become mainly local, although the lymph glands were still much enlarged.

At this point it seemed desirable to determine the effect of reinoculating the animal with a virulent passage strain from a *rhesus* monkey. *M. rhesus* 14 (Text-fig. 2), which had been inoculated in the same way as the chimpanzee and at the same time, had developed large nodules on eyebrow and abdomen by Feb. 23 (Figs. 16, 20), and the one at the former site had been excised on Feb. 27 for examination. The abdominal nodule (Figs. 21, 22) was excised on Mar. 1, and suspensions of both nodules and of a piece of skin from one of the scarified areas on the abdomen (Figs. 18, 19) were mixed with cultures of *Bartonella bacilliformis* and inoculated intravenously into the chimpanzee. The same material was injected into *M. rhesus* 21 as a test of its virulence.

An accident intervened at this time. *M. rhesus* 14, which had served as the control for the first inoculation of the chimpanzee and was showing far more active and rapidly progressing local and constitutional reactions, had been used in some experiments to determine whether or not *Bartonella bacilliformis* could be transmitted by insects. Thirty ticks (*Dermacentor andersoni*, believed to be free from spotted fever) were allowed to feed on the animal from Feb. 25 to Feb. 27.

All became attached. The animal had been having irregular fever (104°F. or slightly above) for the past 4 weeks, and the unintentional infection with spotted fever by tick feeding escaped attention for a time. When the animal died on Mar. 4, however, it became evident that death was due to spotted fever, and that *M. rhesus* 21 (Text-fig. 3) and the chimpanzee had received on Mar. 1 Rocky Mountain spotted fever virus as well as *Bartonella bacilliformis*. *M. rhesus* 21 died of typical spotted fever within 10 days (4 days incubation, 6 days of high fever, 106°F. during the first 2 days). Notwithstanding the presence of the spotted fever infection, the blood of Monkeys 14 and 21 both yielded cultures of *Bartonella bacilliformis*, that of *M. rhesus* 21 in a dilution of 1:100,000. Injections into guinea pigs of the blood and of suspensions of the nodular tissue from Monkey 14 induced typical fatal spotted fever.

*Spotted Fever in the Chimpanzee.*—As the chart shows (Text-fig. 1), the spotted fever infection (Mar. 1 to 15) gave rise in the chimpanzee to a very different temperature curve from that of the pure infection with *Bartonella bacilliformis* (Jan. 29 to Mar. 1). After an incubation period of 5 days the temperature rose to 101°F., then gradually to 102.5°F., then fell to a slightly subnormal level, and remained there for several days. The fever was relatively mild, as compared with that usually observed in spotted fever infection in man, *Macacus rhesus* monkeys, and guinea pigs; in these animals spotted fever gives rise to a temperature of 105–106° lasting 7 to 10 days. The chimpanzee had become inactive on the 5th day, was disinclined to move about, depressed, and indifferent to food. This condition continued for 5 days (until Mar. 9). By this time the accident had become known through the death of *M. rhesus* 14, and 20 cc. of pooled spotted fever antiserum from hyperimmunized rabbits were given intravenously to the chimpanzee and also to the control *rhesus* (No. 21). In the *rhesus* the injection had no effect; the animal died the following day. In the chimpanzee the character of the fever before and after the administration of the serum appeared to be the same, and the recognition of curative effect is difficult unless the short duration of the fever is assumed to have been due to the effect of the serum. In *rhesus* monkeys and guinea pigs, however, the serum has not been known to influence the course of fever unless given during the incubation period.<sup>7</sup> At all events, the chimpanzee recovered very rapidly and probably without aid of the antiserum.

During the period of spotted fever infection, when the animal was very ill, all the nodules, including the reddened skin lesions, became paler and smaller,

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<sup>7</sup> Noguchi, H., *J. Exp. Med.*, 1923, xxxvii, 383.

and the face of the animal was cyanotic, though the color had returned within a week after the time of injection of the serum.

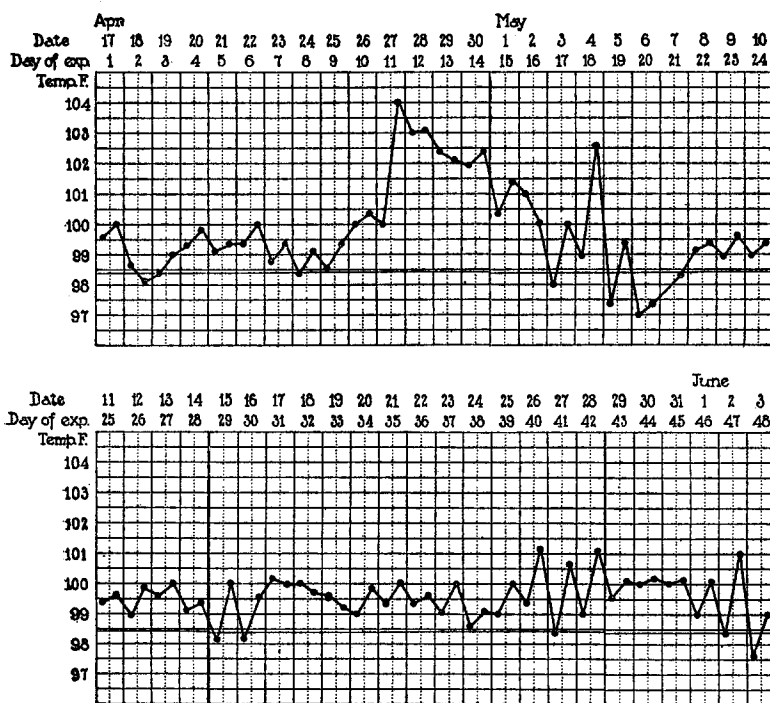
*Mar. 1*, 1 p.m. Intravenous injection of 1.5 cc. of a mixture of suspension of nodular tissue of *M. rhesus* 14 (removed Feb. 27 and Mar. 1) and cultures of *Bartonella bacilliformis*. *Mar. 5*. The animal ate very little and was inactive. The nodules on the eyebrows were still large (Fig. 12). *Mar. 6*. Temperature 101°F. *Mar. 7*. Temperature 102° a.m., 102.5° p.m. *Mar. 8*. Temperature 101° a.m., 102.5° p.m. Animal inactive, apathetic, appetite poor. The abdominal wounds, where the lesions had been removed, had partly opened, and the surrounding tissues were edematous. *Mar. 9*. Temperature 102.5° a.m., 102° p.m. The animal was very ill. The nodules were pale and cyanotic. Erythrocytes 4,856,000, hemoglobin 80 per cent. 20 cc. spotted fever antiserum given intravenously at 12 noon.

*Mar. 10*. Temperature 101° a.m., 102.5° p.m. The color had returned to the nodules, and the animal was somewhat more active. Appetite improved. *Mar. 11*. Temperature 102° a.m., 102.4° p.m. The animal was more active, and the color of the face and nodules more nearly normal. *Mar. 12*. Temperature 101° a.m., 101.2° p.m. Eyelids edematous. Erythrocytes 4,288,000, hemoglobin 65 per cent. Blood yielded growth of *Bartonella bacilliformis* in dilution of 1:100. *Mar. 13*. Temperature 101° a.m., 101.8° p.m. The monkey had removed some of the stitches from the wounds caused by excision of the lesions; the wounds were healing satisfactorily, however. *Mar. 14*. Temperature 100.5° a.m., 100.5° p.m. *Mar. 15*. Temperature 99° a.m., 99.5° p.m. Nodules smaller and paler, not cyanotic. Erythrocytes 3,488,000, hemoglobin 60 per cent. Blood culture negative. One of the inguinal glands on the left side was removed for examination. The suspension yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10. *Mar. 17*. Temperature 98.4° a.m., 99° p.m. The animal was very active. Erythrocytes 4,344,000, hemoglobin 65 per cent. *Mar. 20*. Temperature 99.5° a.m., 100° p.m. The remaining nodules had diminished considerably in size and the overlying skin was of normal color. *Mar. 25 to 31*. Temperature normal. The skin lesions had healed and showed slight brownish pigmentation. The site of the abdominal nodule was practically flat, and the induration had disappeared. The nodules on the eyebrows were reddish but small (3 × 4 mm.).

*Apr. 2*. The nodules on the eyebrows were much smaller. The lymph nodes had also decreased in size considerably. *Apr. 6*. Blood culture negative. Erythrocytes 4,456,000, hemoglobin 75 per cent. *May 1*. The nodules had practically disappeared. Neither the blood nor the lymph yielded cultures of *Bartonella bacilliformis*. *May 3*. Erythrocytes 5,800,000, hemoglobin 85 per cent. The animal had completely recovered.

*The Behavior of Bartonella bacilliformis in the Ourang-Utan.*

The response of the ourang-utan to the inoculation of *Bartonella bacilliformis* was similar to that of the chimpanzee, but in this instance there was a moderate febrile reaction (Text-fig. 4) which began 10 days after inoculation and lasted about a week. On the day before the onset of fever *Bartonella bacilliformis* was demonstrable



TEXT-FIG. 4. Ourang-utan.

by culture in a 1:100 dilution of the blood, but a week later the titer of the blood was only 1:10. The local lesions, while characteristic of those induced by the organism, did not approach in severity those usually observed in *rhesus* monkeys. The largest nodules and the slowest to pass away were two which arose subcutaneously at the sites of attempted intravenous inoculation into the basilic vein of the left arm (Fig. 24), and which remained prominent for 2 months.



They had disappeared, however, by Aug. 15 (4 months after inoculation).

The ourang-utan (*Pongo pygmaeus*), a young female (probably 2 to 3 years old), was inoculated Apr. 17, 1926. The right eyebrow was scarified and smeared with a piece of nodule just excised from *M. rhesus* 23 and was intradermally inoculated, on the inner aspect, with 0.2 to 0.3 cc. of a suspension of the same tissue. 0.2 cc. of the suspension was injected intradermally into the shaved skin of the right upper abdominal wall, and about 1 cc. was smeared over a scarified area on the right lower side of the abdomen. The left side of the abdomen was inoculated similarly at two sites with a mixture of cultures derived from the blood of *M. rhesus* 7 and *M. rhesus* 18. The mixture included cultures grown on leptospira medium for 10 days and blood agar cultures 6 days old which contained a large number of motile organisms.

The animal received intravenously a mixture containing 6 cc. of the cultures, 1 cc. of the suspension of nodular tissue of *M. rhesus* 23, and 3 cc. of citrated blood from each of two monkeys, *M. rhesus* 11 and *M. rhesus* 24. Two attempts to introduce the syringe needle into the basilic vein of the left arm having failed, the material was inoculated into the corresponding vein of the right arm. A small amount of material from the syringe had, however, been unintentionally introduced subcutaneously into the left arm, and two large subcutaneous nodules later developed at the sites of the attempted intravenous injections.

A blood count made on Apr. 2, 1926, had shown 5,940,000 erythrocytes per c.mm., 12,200 leucocytes, and 85 per cent hemoglobin. The normal temperature of the animal had been taken daily at 11 a.m. and at 4 p.m. for a period of several weeks. It varied from 98.6°F. to 99.6° and was rarely as high as 100° in the afternoon.

Blood taken on Apr. 26, 9 days after inoculation, yielded growth of *Bartonella bacilliformis* in a dilution of 1:100, and a few cells containing the organism were found in film preparations. The erythrocytes at this time numbered 5,392,000, and the hemoglobin was 70 per cent. For the 10 days following inoculation the temperature remained within normal limits, but on the afternoon of Apr. 27 it rose to 104°, and on Apr. 28 it was 103° both morning and afternoon.

Apr. 29. Temperature 102.4° a.m., 102.2° p.m. Appetite poor. Lymph glands enlarged. Slight induration at site of needle puncture on right arm. Erythrocytes 4,880,000, hemoglobin 75 per cent. Apr. 30. Temperature 102° a.m., 102.4° p.m. Animal quiet and apathetic. May 1. Temperature 100.4° a.m., 101.4° p.m. May 2. Temperature 101° a.m., 100° p.m. May 3. Temperature 98° a.m., 100° p.m. Diarrhea. Blood yielded growth of *Bartonella bacilliformis* in dilution of 1:10. Erythrocytes 4,808,000, hemoglobin 80 per cent. Slight induration at sites of intradermal injection on each eyebrow.

May 4. Temperature 99° a.m., 102.6° p.m. May 5. Temperature 97.4° a.m., 99.4° p.m. May 6. Temperature 97° a.m., 97.4° p.m. Distinct nodules

at sites of inoculation on eyebrows and areas of induration at the sites of the attempted intravenous injections on the left arm.

From this time on the temperature varied within normal limits. On May 11 the erythrocytes numbered 5,632,000, and the hemoglobin was 80 per cent. Blood taken on May 17 yielded cultures of *Bartonella bacilliformis*. The induration at the site of intradermal inoculation on the right eyebrow had disappeared by May 13, but the adjacent lines of scarification had become distinctly raised and reddish, and there was a distinct nodule, 5 × 6 mm., at the site of intradermal inoculation on the left eyebrow. The area of scarification on the lower right side of the abdomen also showed activity, being markedly raised and reddish. *May 20.* Distinct nodules on left side of abdomen at sites of inoculation of cultures. One of these was excised, and also a piece of skin from the scarified area on the lower right abdomen. Both showed the characteristic structure of lesions induced on the skin by *Bartonella bacilliformis*, and the organisms were demonstrated in sections of the tissue (Figs. 6, 25, and 26).

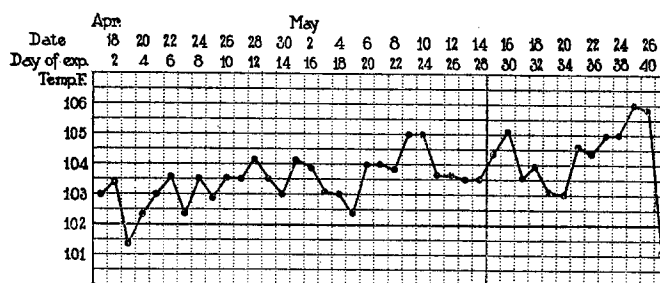
*May 23.* One of the nodules on the left arm measured 2.5 × 3 cm., the other 5 × 5 mm. (Fig. 24). The nodule on the left eyebrow was about 8 × 9 mm., and there were numerous small reddish papules on the scarified area of the right eyebrow (Fig. 23). All the lesions continued to increase in size until about June 1, when the photographs shown in Figs. 23 and 24 were taken. *June 2.* Blood culture negative for *Bartonella bacilliformis*. Erythrocytes 5,512,000, hemoglobin 80 per cent, leucocytes 11,600. Polymorphonuclears 33.3 per cent, small lymphocytes 45.25 per cent, large lymphocytes 3.8 per cent, large mononuclears 11.0 per cent, transitionals 1.0 per cent, eosinophils 0.7 per cent, mast cells 4.75 per cent.

The monkey scratched the nodule on the left eyebrow until on June 8 she had succeeded in opening it and on June 10 had removed nearly half of it. The remaining portion was excised on June 10 for examination and culture (Fig. 4). The papules on the right eyebrow had become dry and scaly at this time, and the abdominal lesions had practically disappeared.

In the *rhesus* monkey, inoculated with the same material as the orang-utan to serve as a control, the local reactions were less severe than those often observed in this species, but there was a tendency to generalization of the skin lesions, and the systemic reaction was very severe. Within 7 days after inoculation the sites of intradermal injection on the eyebrows were definitely indurated, and there was a slight spontaneous eruption on the abdomen. The temperature rose on the 11th day after inoculation, and there was continuous high temperature (104–106°F.) after the 20th day (Text-fig. 5). The hemoglobin at the time of death, 40 days after inoculation, was 15 per cent, and the erythrocytes numbered 1,176,000.

*M. rhesus* 37 was injected intravenously (saphenous vein) on Apr. 17, 1926, with the same mixture of cultures and nodular suspension which the ourangutan had received. The right eyebrow was inoculated intradermally and by scarification with the suspension of the nodule of *M. rhesus* 23, the left in the same way with the mixture used for intravenous injection. The shaved skin of the lower right side of the abdomen was inoculated by scarification with the nodule suspension of *M. rhesus* 23, the lower left side with a suspension of nodular tissue of *M. rhesus* 18. The latter suspension was also inoculated by scarification on two sites on the upper abdomen.

Apr. 23. Slight induration at the sites of intradermal inoculation on the eyebrows. Apr. 24. Indurations on the eyebrows definite; slight spontaneous eruption on abdomen. Apr. 28. Temperature 104.2°F. Distinct nodule at site of intravenous injection on the right leg and red papules at all four sites of scarification on abdomen. Apr. 29. Blood yielded growth of *Bartonella bacilliformis* in dilution of 1:10. Erythrocytes 5,180,000, hemoglobin 70 per cent.



TEXT-FIG. 5. *M. rhesus* 37.

May 1. Temperature 104.2°. May 3. Nodule on left eyebrow 2 × 3 mm. May 7. Lines of scarification on right eyebrow slightly raised. Nodules on left eyebrow and on leg increased in size. May 12. Erythrocytes 5,536,000, hemoglobin 75 per cent. May 19. Blood culture negative.

From May 19 to May 24 this animal was used for the feeding of nineteen ticks (*Dermacentor variabilis*). On May 20 there was a spontaneous papular eruption on the abdomen. The nodules on the eyebrows had not increased in size, but the one on the right leg was 4 × 5 mm. On May 26, when the temperature was 105.8° the animal was used for the feeding of ten bedbugs, which were removed the following morning. May 27. Temperature 101°; animal very ill, cyanotic. May 28. Died.

*Autopsy*.—Profuse hemorrhage on the abdominal wall and in the omentum but no free blood in the peritoneal cavity. Peritoneal fluid yellowish, slightly turbid, contained a few red cells. Intestines smooth and glistening, no evidence of peritonitis. Lungs whitish gray, extremely anemic, but otherwise normal.

No fluid in the pericardium, no evidence of pericarditis. Heart muscle heavily congested and very hemorrhagic. Liver very pale and soft but showed no gross pathological changes. Spleen normal in size but very dark in color and much harder than normal. Kidneys normal in appearance. There were a few dark bluish enlarged lymph nodes in the mesenterium and one in the large intestine, but otherwise the intestinal tract appeared normal. Axillary and inguinal lymph glands very slightly enlarged. One small nodule on the left eyebrow and another larger one on the back of the left leg. Both on section showed the presence of *Bartonella bacilliformis*.

Erythrocytes 1,176,000, leucocytes 3,200, hemoglobin 15 per cent. Polymorphonuclear leucocytes 50.7 per cent, small lymphocytes 31.1 per cent, large lymphocytes 3.2 per cent, large mononuclears 11.8 per cent, transitionals 1.9 per cent, eosinophils 0.8 per cent, mast cells (basophils) 1.25 per cent, megablasts 1.0 per cent.

The red cells stained very irregularly, a large number of them remaining practically unstained, while about 25 per cent showed marked polychromatophilia. There were a large number of microcytes and a few macrocytes, mostly of irregular shape. No nucleated red cells were found. A few red cells contained *Bartonella bacilliformis*. There were a few threads of fibrin in the smears but no platelets.

The majority of the polymorphonuclear leucocytes had become so changed that the cytoplasm had practically disappeared. The nucleus was still intact. Most of the lymphocytes were extremely small, about half the size of the red cells.

#### SUMMARY.

The inoculation of a chimpanzee with cultures and a passage strain of *Bartonella bacilliformis* induced local reactions which, while definite and characteristic, progressed less rapidly and were much less striking than those in the control *rhesus* monkey. *Bartonella bacilliformis* was demonstrated in the blood corpuscles with difficulty, and the fever was slight compared with the high and persistent fever of the *rhesus* monkey. In both the swelling of the lymph glands was an early symptom and constantly present. Definite anemia developed in the chimpanzee only after accidental infection with Rocky Mountain spotted fever and may have been due to either one or both infections, though it disappeared when the blood had become negative by culture for *Bartonella bacilliformis* and the local lesions had disappeared. Incidentally, the chimpanzee was found in this one instance to be less susceptible to the spotted fever than *Macacus rhesus* and guinea pigs.

In the ourang-utan, also, *Bartonella bacilliformis* induced a mild systemic and local infection. A rise of temperature occurred 10 days after inoculation, and fever continued for a week, though it was decidedly less severe than that in the control *rhesus*. The lesions induced by scarification were less definite than those which arose at the sites of intradermal inoculation. *Bartonella bacilliformis* was recovered from the blood on the 9th and on the 16th days after inoculation and from nodules excised on the 33rd and 53rd days. A few erythrocytes containing the organism were demonstrated in stained smears, but prolonged search was required to find them.

The symptoms and lesions observed in the chimpanzee and ourang-utan as a result of infection with *Bartonella bacilliformis* are far milder than those of *rhesus* monkeys and show less resemblance to human Oroya fever or verruga.

## EXPLANATION OF PLATES.

## PLATE 25.

FIG. 1. *Bartonella bacilliformis* in the erythrocytes of *Macacus rhesus*. Selected cells from film preparations of the blood of *M. rhesus* 25 and *M. rhesus* 30. Giemsa's stain.  $\times 1,750$ .

FIG. 2. *Bartonella bacilliformis* in the erythrocytes of Case S. A. 15, from which the organism was isolated. Giemsa's stain.  $\times 1,750$ .

FIG. 3. The nodule excised from the abdomen of the chimpanzee 31 days after inoculation. Natural size and color.

FIG. 4. The nodule excised from the eyebrow of the ourang-utan 53 days after inoculation. Natural size and color.

FIG. 5. *Bartonella bacilliformis* in a section of the chimpanzee nodule shown in Fig. 3. Giemsa's stain.  $\times 1,750$ .

FIG. 6. *Bartonella bacilliformis* in a section of a nodule of the ourang-utan, excised 33 days after inoculation. Giemsa's stain.  $\times 1,750$ .

FIG. 7. *Bartonella bacilliformis* in a section of a spontaneous nodule of *M. rhesus* 25 excised 23 days after inoculation. For comparison. Giemsa's stain.  $\times 1,750$ .

## PLATE 26.

FIG. 8. Appearance of the nodules on the eyebrows of the chimpanzee 27 days after inoculation.  $1/2$  natural size.

FIG. 9. The skin lesion on the right side of the abdomen of the chimpanzee as it appeared 25 days after inoculation.

FIG. 10. Section of the lesion shown in Fig. 9, which was excised 32 days after inoculation. Giemsa's stain.  $\times 182$ .

FIG. 11. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.

FIG. 12. The appearance of the nodules on the eyebrow of the chimpanzee 31 days after inoculation.

FIG. 13. The abdominal nodule of the chimpanzee as it appeared 25 days after inoculation.

FIG. 14. Section of the nodule shown in Fig. 13, which was excised 32 days after inoculation. Giemsa's stain.  $\times 182$ .

FIG. 15. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.

#### PLATE 27.

FIG. 16. Appearance of the nodules on the eyebrows of *M. rhesus* 14, 23 days after inoculation. Natural size.

FIG. 17. The skin lesions on the abdomen of *M. rhesus* 14, 23 days after inoculation. Natural size.

FIG. 18. Section of one of the skin lesions shown in Fig. 17, excised 23 days after inoculation. Giemsa's stain.  $\times 182$ .

FIG. 19. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.

FIG. 20. The abdominal nodule of *M. rhesus* 14, 23 days after inoculation. Natural size.

FIG. 21. Section of the nodule shown in Fig. 20, which was excised 28 days after inoculation. Giemsa's stain.  $\times 182$ .

FIG. 22. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.

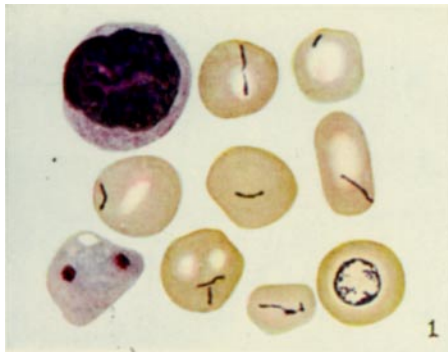
#### PLATE 28.

FIG. 23. Appearance of the skin lesions on the eyebrows of the orang-utan 43 days after inoculation.  $2/5$  natural size.

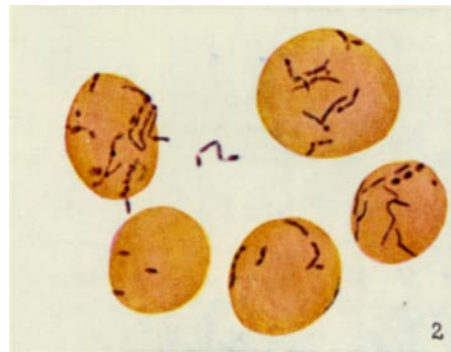
FIG. 24. The large subcutaneous nodules which arose on the left arm of the orang-utan at the site of unsuccessful attempts to enter the basilic vein at the time of inoculation. Photograph taken 43 days after inoculation.  $2/5$  natural size.

FIG. 25. Section of the abdominal nodule of the orang-utan, excised 40 days after inoculation. Giemsa's stain.  $\times 182$ .

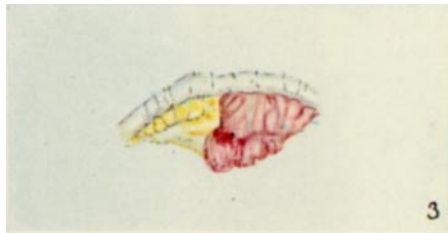
FIG. 26. The same section at a magnification of 1,000 times, showing the presence of *Bartonella bacilliformis*.



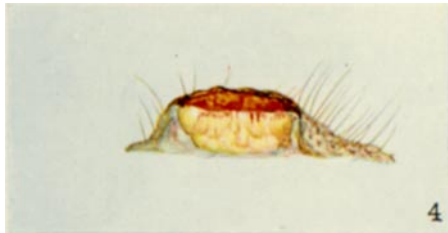
Blood of *M. rhesus*.



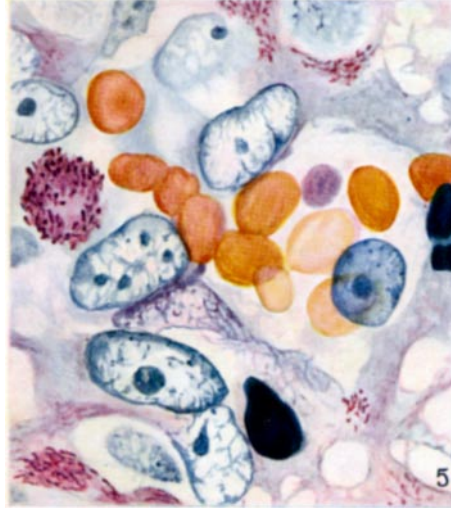
Human blood.



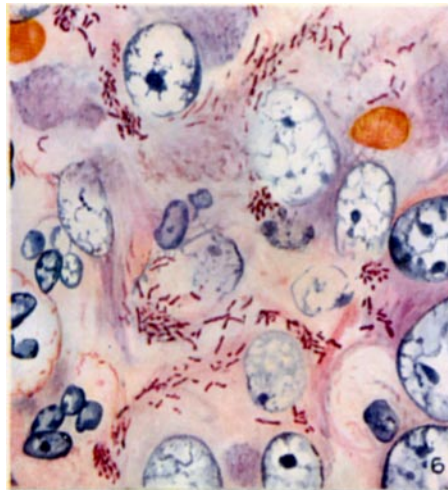
Abdominal nodule, chimpanzee.



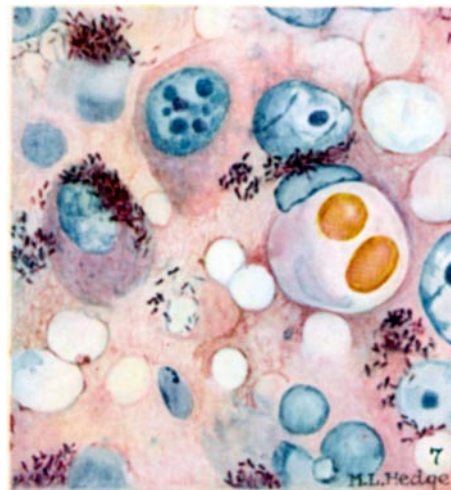
Eyebrow nodule, ourang-utan.



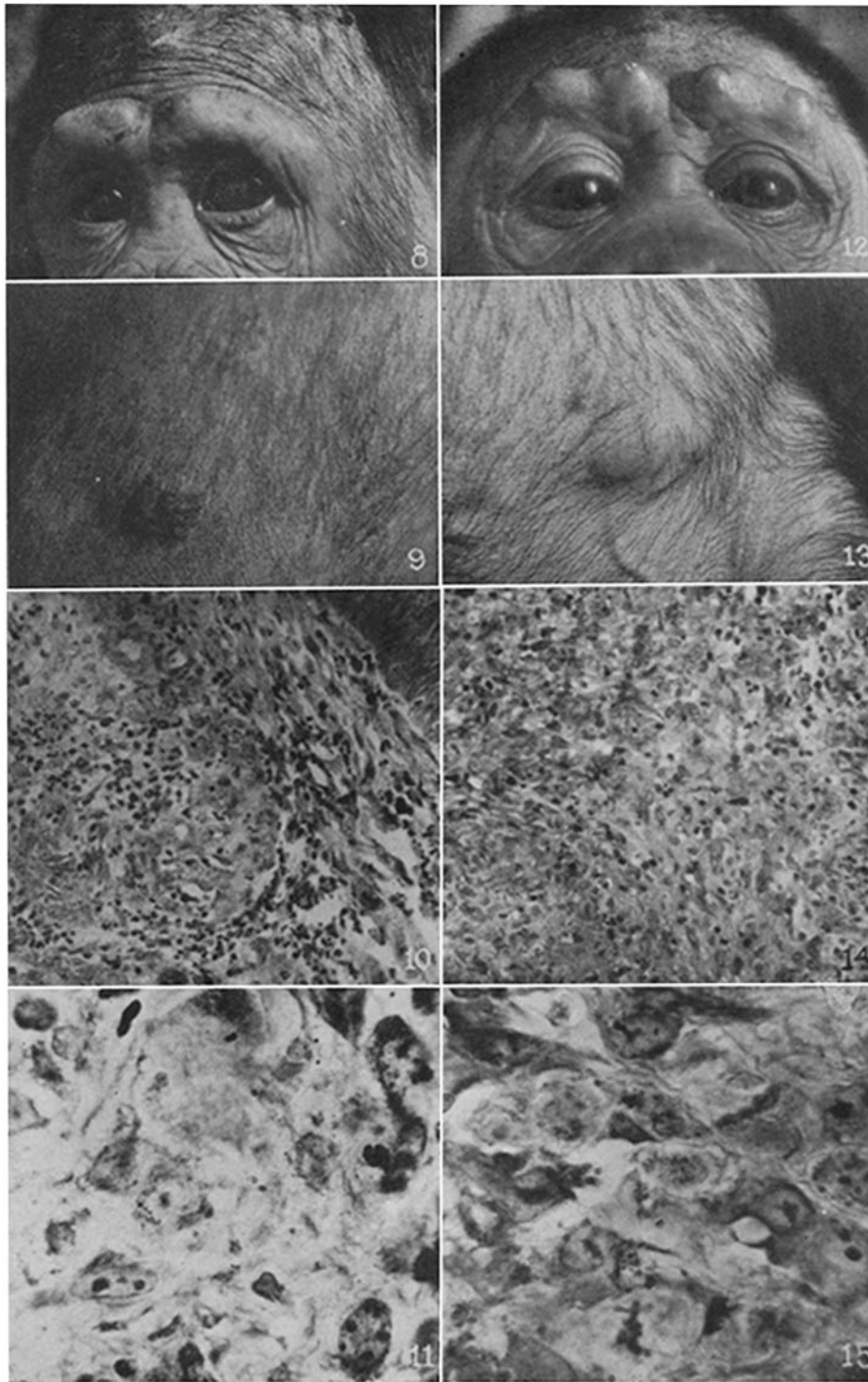
*Bartonella* in chimpanzee nodule.



*Bartonella* in ourang-utan nodule.

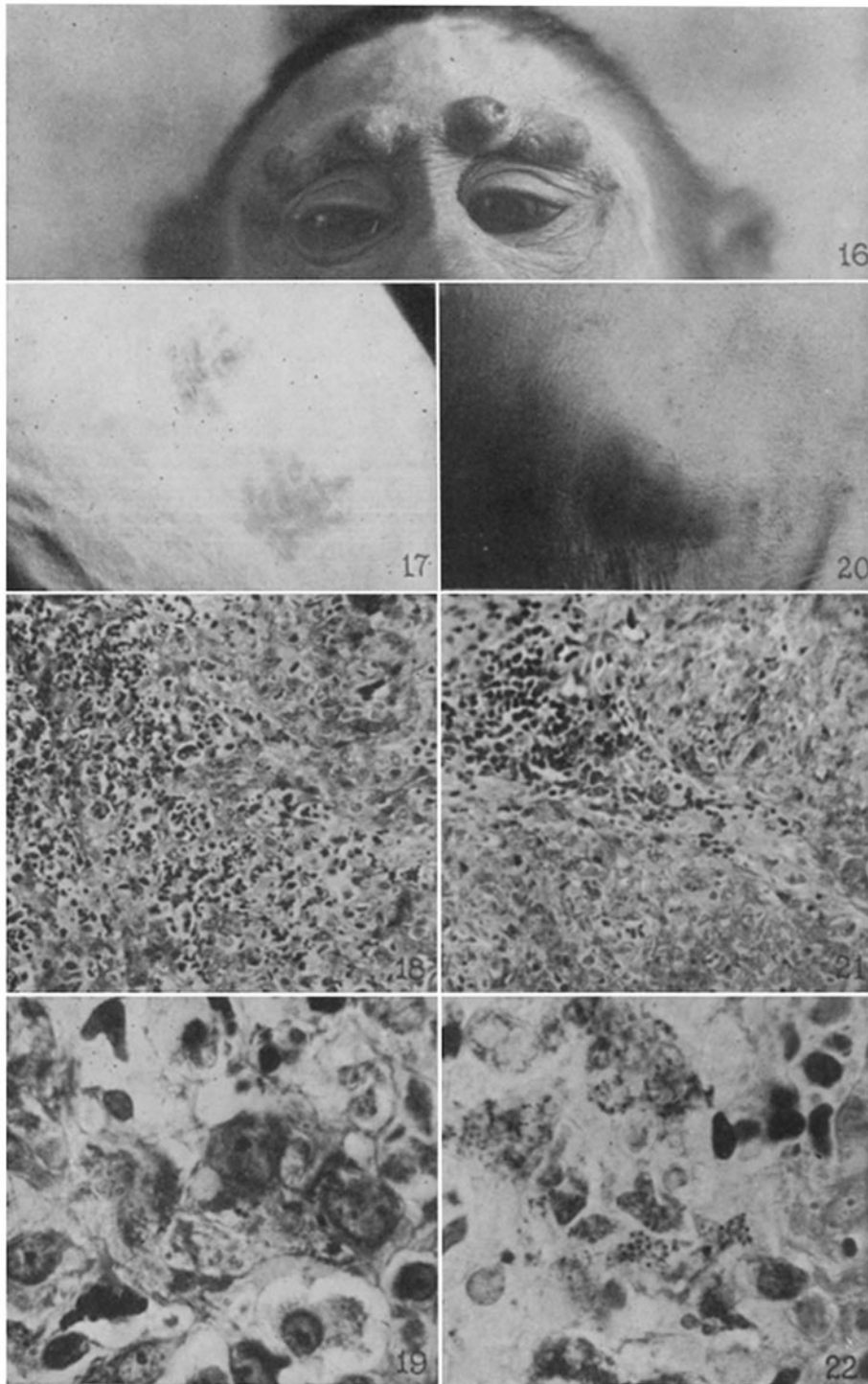


*Bartonella* in nodule of *M. rhesus* 25.

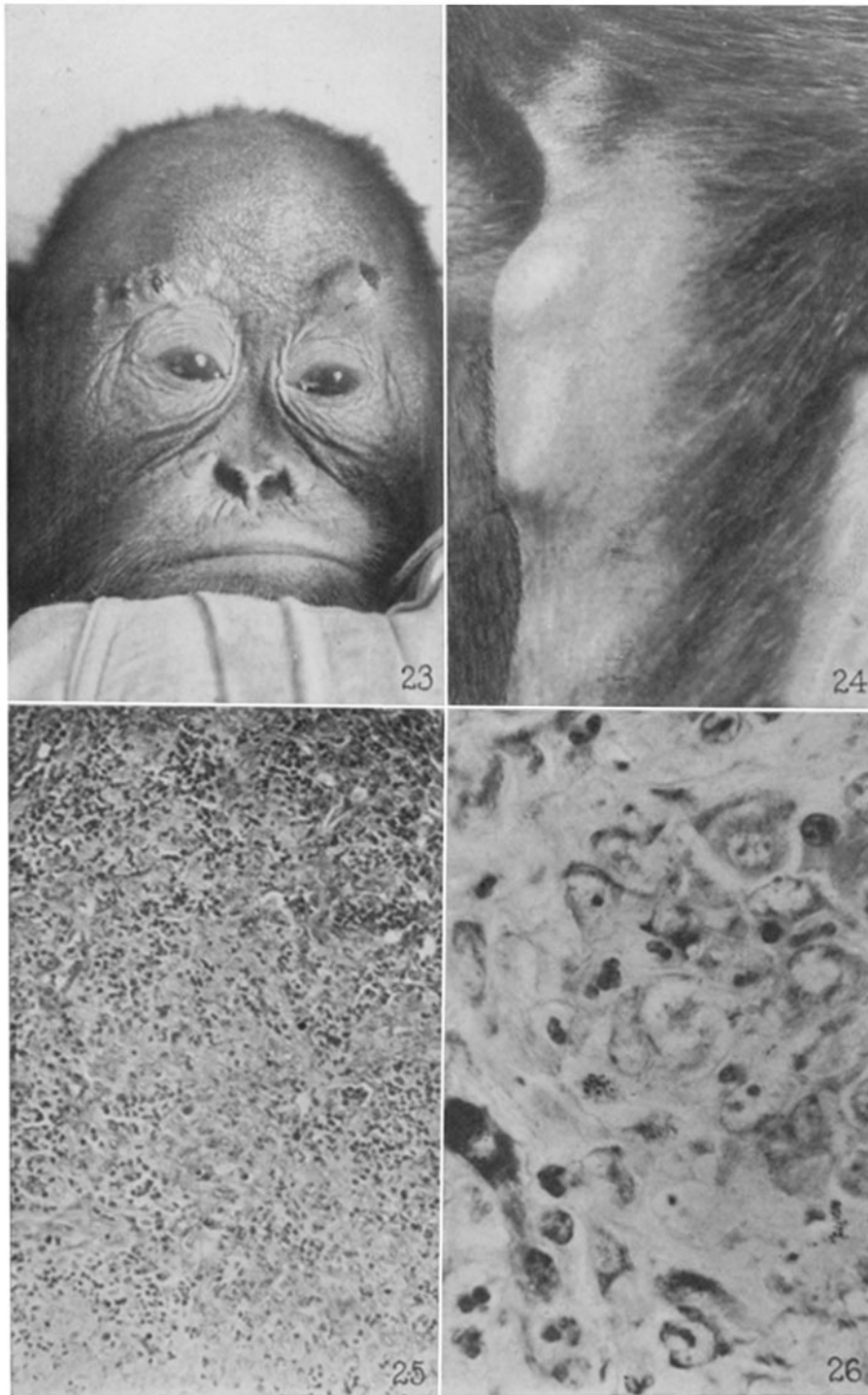


(Noguchi: Etiology of Oroya fever. IV.)





(Noguchi: Etiology of Oroya fever. IV.)



(Noguchi: Etiology of Oroya fever. IV )