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THE ETIOLOGY OF VERRUGA PERUANA.

By HIDEYO NOGUCHI, M.D.

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

PLATES 2 TO 4.

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The experimental investigation to be reported in this paper was made possible through the cooperation of Professor Oswaldo Hercelles, of the University of Lima, who was kind enough to obtain for me selected material for study, and I wish at the outset to acknowledge my indebtedness to him and my appreciation of his courtesy. I wish to express my thanks also to Professor E. Campodónico, of the University of Lima, through whose courtesy the material was forwarded from Lima to New York.

There are many similarities between the conditions known as verruga peruana and Oroya fever. Their geographical distribution, which is rather curiously limited to certain narrow valleys on the western slopes of the Andes Mountains, between 6° and 13° south latitude, is practically the same and is of the type shown by infections conveyed by certain ectoparasites. Fever and anemia are considerably more acute and severe in Oroya fever, but both are present in verruga. Verruga is predominantly a chronic infection, and its conspicuous feature is the characteristic nodular eruption on the skin which lasts for a period of a few weeks to one of many months. Oroya fever, on the other hand, while sometimes accompanied or followed by a similar skin eruption, is distinguished by an acute course of extreme anemia, during which a specific intracellular parasite, *Bartonella bacilliformis*, is abundantly present in the red blood corpuscles. This microorganism has rarely been seen in the blood in cases of benign verruga.

In 1910 Jadassohn and Seiffert[1] showed that verruga peruana could be transmitted to monkeys by local inoculation of suspensions

[1] Jadassohn and Seiffert, G., *Z. Hyg. u. Infectionskrankh.*, 1910, lxvi, 247.

of tissue from human verruga lesions, and their results have received confirmation from Mayer, Rocha-Lima, and Werner,[2] and from the Commission of the Harvard School of Tropical Medicine.[3]

Oroya fever has not thus far been directly transmitted to animals, but by means of a culture recently isolated from a fatal case of the disease[4] it has been shown that young *rhesus* monkeys (about 2,000 gm. body weight) are susceptible to infection with *Bartonella bacilliformis*, though usually not to the same degree as man, and that in the infected animals the parasite is demonstrable in its characteristic situation in the erythrocytes. In some animals which are less resistant to infection, the extreme anemia of Oroya fever has been reproduced.[5] The striking fact brought out in the inoculation experiments, however, was the dual nature of the infection induced, for characteristic signs of both Oroya fever and verruga were observed in the animals, with occasionally a marked favoring of one or the other type of disease, as the virulence of the strain became enhanced by adaptation to the experimental animal. Systemic manifestations—moderate or marked anemia, fever, and high titer of *Bartonella bacilliformis* in the blood, bone marrow, lymph nodes, and spleen—were in general more severe in animals inoculated intravenously or intraperitoneally, and death occurred only in animals so inoculated, but the invasion of the blood by the parasite was found to take place also after local inoculation. The tendency toward the production of verrucous lesions was more marked, as a rule, when the virus was introduced locally, and the lesions usually arose only at the sites of inoculation, as is the case when monkeys are inoculated directly with human verruga material.[1,2] In one instance, however, in which passage virus was injected both locally and intravenously, a spontaneous general eruption typical of severe human verruga arose on various parts of the skin, remote from the sites of inoculation. The skin lesions of the animals, whether local or

[2] Mayer, M., Rocha-Lima, H., and Werner, H., *Münch. med. Woch.*, 1913, lx, 739.

[3] Strong, R. P., Tyzzer, E. E., Sellards, A. W., Brues, C. T., and Gastiaburú, J. C., Report of first expedition to South America, 1913, Harvard School of Tropical Medicine, Cambridge, 1915.

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

[5] Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.

spontaneous in occurrence, in all instances yielded cultures of *Bartonella bacilliformis*, and stained sections of all such lesions revealed the parasites in large numbers in a characteristic endothelial situation.[5]

These findings, when taken in relation to the historical and epidemiological data with regard to the two conditions,[6] suggested very strongly that Oroya fever and verruga peruana were both due to *Bartonella bacilliformis*, the considerable variations in the manifestations of infection being a result of the differences in resistance among individuals or among different tissues of the same individual. It was desirable, however, to approach the problem of the etiology of verruga directly, by means of the cultural and experimental methods used for the isolation of *Bartonella bacilliformis* from Oroya fever.

For the purpose of comparative study subcutaneous nodules were removed from each of two cases of verruga in the Dos de Mayo Hospital, Lima, placed in tubes, and covered with citrate-saline solution (2 per cent sodium citrate in 0.9 per cent sodium chloride). The tubes were sealed, abundant air space being allowed, and were placed in the ship's refrigerator during the journey to New York, a period of 14 days. We were fortunate in having transportation so arranged that the tissues were shipped shortly after their excision from the patients and were received in the laboratory the same day that they arrived in New York (April 7, 1926). Each specimen was immediately examined microscopically, saline suspensions were made, and inoculated into culture media and experimental animals, and a portion of each tissue was fixed in Regaud's fluid for histological study.

The nodule of Case P. 5 measured about 8 × 12 × 6 mm. and was light pinkish in color, with the exception of a small portion to which the skin was still attached. The nodule was still firm, except for slight softening along the cut surfaces. The citrate solution was slightly turbid, owing to liberation of tissue elements, but there was no putrefactive odor.

Minute Gram-negative bacilli were present, occurring singly, in pairs, and in larger groups, as well as in masses (Fig. 18). Sections stained with Giemsa's and Gram's solutions showed masses of the organisms scattered through the tissue (Fig. 18), the general structure of which was still well preserved (Fig. 17) notwithstanding loss of cellular elements through autolysis.

[6] Odriozola, E., La maladie de Carrion, Paris, 1896.

The nodule from Case J. 45 was not as well preserved. The tissue was soft and friable, but there was no sign of bacterial putrefaction. It contained numerous Gram-negative bacilli similar to those found in the specimen from Case P. 5 and in addition a few small Gram-positive diplococci. In sections the bacilli were found in the nodular tissue and the cocci near and on the skin.

Two species of minute Gram-negative microorganisms were isolated from the tissue of Case P. 5, one indirectly, from monkeys inoculated with suspensions of the tissue, and one by direct cultivation. The former is pathogenic for young *Macacus rhesus* monkeys and in every respect has proved identical with the strain of *Bartonella bacilliformis* previously obtained from the blood of a case of Oroya fever. The latter, although present also in the J. 45 tissue, as shown by cultivation experiments, is apparently a non-specific invader; it has failed to induce characteristic local or systemic infection, and it grows within 24 hours on ordinary agar slants, while the pathogenic organism grows slowly and only on leptospira medium or blood agar slants. Our interest, therefore, is in the pathogenic strain obtained by inoculation of the P. 5 material.

Transmission of Verruga Peruana to Monkeys with the Excised Nodular Tissue of Case P. 5.

Transmission of verruga peruana to monkeys, as stated earlier in this paper, has repeatedly been accomplished by previous investigators. In the present instance, however, the tissue had been excised from the patients more than 2 weeks previous to inoculation.

One young and one adult *Macacus rhesus* were available on April 7. The young one was used for inoculation of the more promising material, that is, the P. 5 nodule. Another young monkey, obtained on April 10, was also inoculated with the P. 5 material. Owing to the scarcity of suitable animals, the inoculations with the J. 45 material could not be repeated.

The suspension of the P. 5 nodule induced definite systemic or local infection in both animals inoculated; the full grown monkey receiving inoculation of the J. 45 material showed no symptoms of infection.

M. rhesus 33, inoculated Apr. 7, 1926, with a saline suspension of the nodule P. 5, intradermally on the shaved right eyebrow and by scarification on the left eyebrow.

2 cc. of the suspension were also injected into the saphenous vein of the right leg. After 48 hours there was a rise of temperature (Chart 1) which lasted for 2 days, but blood taken at this time yielded no growth. On Apr. 14 the temperature rose again and remained high for 6 days. Blood taken during the fever (Apr 16) and inoculated into leptospira medium yielded, in a 1:10 dilution, pure growth of a microorganism indistinguishable from *Bartonella bacilliformis*. The sites of intradermal inoculations on the eyebrow showed some induration at this time, but they never increased in size and receded within a few weeks. The lymph glands in the inguinal and axillary regions became markedly enlarged about Apr. 16. Blood cultures made on two successive occasions, May 27 and June 20, 1926, both gave pure cultures of the *Bartonella*-like organisms.

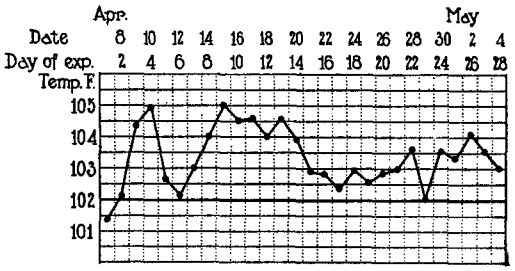


CHART 1. *M. rhesus* 33.

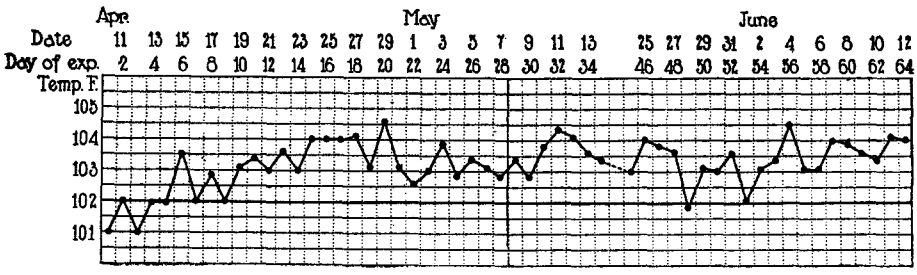


CHART 2. *M. rhesus* 34.

M. rhesus 34, inoculated Apr. 10, 1926, with the same material and in the same way as *M. rhesus* 33. A moderate rise of temperature occurred 14 days from the time of inoculation (Chart 2), and the animal remained febrile for 4 days. Blood cultures made on Apr. 28, 1926, yielded a pure growth of the same organism as had been isolated from *M. rhesus* 33. At this time the lymph glands had become markedly enlarged, but no local reactions were noticeable. Blood taken on May 12 and on May 27 was culturally positive in a 1:10 dilution. At the beginning of June a large subcutaneous nodule at the middle portion of the tail was noticed

(Fig. 10), and on June 18 it was excised.* It showed the typical histology of verruga tissue (Fig. 11) and yielded a pure culture of the same organism as had been obtained from the blood. Cultivation of the blood was again successful on June 21, this time with a dilution of 1:100.

The foregoing experiments demonstrate the infectivity of the verruga nodule which had been kept at refrigerator temperature for at least 2 weeks, and the association of infective power (as evidenced by febrile reaction and the production of a metastatic local verruga lesion) with a definite microorganism recoverable by culture both from the blood of the animals and from the local lesion. Blood counts revealed no anemia in either animal, and in neither instance did the blood films contain a sufficient number of organisms to be detected microscopically.

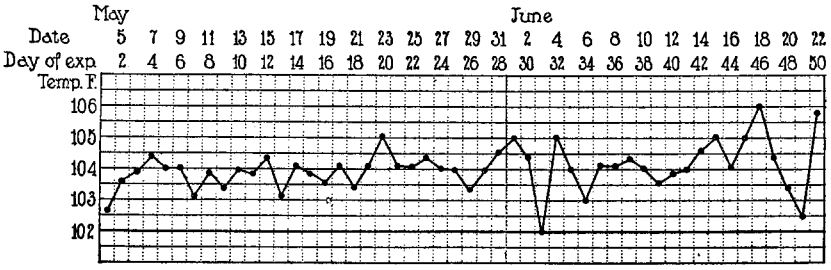


CHART 3. *M. rhesus* 41.

The pathogenicity of the culture obtained from the blood of *M. rhesus* 33 on April 16 was tested by inoculation of *M. rhesus* 41. As the protocol shows, the culture gave rise not only to a local reaction but to a systemic infection of pronounced severity.

M. rhesus 41 (Fig. 1) inoculated May 4, 1926, with culture derived from the blood of *M. rhesus* 33 and grown for 18 days on leptospira medium. The material was inoculated on the right eyebrow and abdomen intradermally and on the left eyebrow and abdomen by scarification. An irregular remittent febrile reaction (Chart 3) began to be manifest 72 hours from the time of inoculation and continued during the 2 months of observation. Induration became noticeable at the site of intradermal inoculation on the right eyebrow after 14 days. The lesion had the gross appearance of a typical verruga nodule, and histological examination of the

* All operations were carried out under ether anesthesia.

excised tissue showed the characteristic granulomatous structure (Figs. 4, 12) and the presence of bacilliform organisms in the endothelial cells (Fig. 13). A suspension of the nodular tissue yielded pure cultures of the organisms in a 1:10 dilution, as did also blood withdrawn on May 21. On May 27 the blood titer by culture was 1:10,000, and on June 3, 1:100,000. The nodules on the eyebrow rapidly increased in size, becoming scarlet-red and protruding (Fig. 1). Blood films made on June 3 showed numerous bacilliform bodies in the red corpuscles, the organisms being present in the largest numbers so far observed in *rhesus* monkeys (Figs. 2, 14). Small nodules developed at the sites of intradermal inoculation on the abdomen 40 days after inoculation; at that time the nodules on the eyebrow measured about 10 × 12 mm.

Blood counts made by Dr. J. H. Bauer showed a gradual diminution of red corpuscles and hemoglobin and an unusually high leucocytosis:

	Erythrocytes per c.mm.	Hemoglobin	Leucocytes per c.mm.
		per cent	
May 4 (day of inoculation).....	5,088,000	80	
" 14 (10 days after inoculation).....	4,632,000	70	
June 3 (30 " " ").....	4,760,000	55	17,600
" 15 (42 " " ").....	4,800,000	40	23,000
" 23 (50 " " ").....	3,392,000	30	43,000
" 25 (52 " " ").....	3,460,000	25	40,000
" 28 (55 " " ").....	3,552,000	25	37,800

The reduction in hemoglobin, it will be noted, was relatively greater than that in the number of red cells, a phenomenon so far not observed by us in animals infected with the strain of *Bartonella bacilliformis* from Oroya fever.

Blood smears stained on June 25 revealed a peculiar appearance of the intracorpuscular parasites, which were still present in rather large numbers, but stained reddish rather than violet, appeared thinner, and in some instances were fragmented. The general features of the organisms suggested that they were undergoing degeneration (Figs. 3, 15). Blood taken at this time yielded cultures in dilution of 1:10, but not in 1:100. A week later no intracorpuscular parasites could be demonstrated, the animal was more active, and the nodules had decreased somewhat in size. Conditions seemed to indicate the development of a state of immunity. No cultures could be obtained from the blood after June 25. On Aug. 11 there was still anemia (red blood cells 3,362,000, hemoglobin 50 per cent), but on Sept. 14 the number of red cells had increased to 4,336,000.

The nodule excised on May 19 and the blood withdrawn 2 days later were inoculated into two *rhesus* monkeys. As the protocols show, both materials gave rise to local lesions and also to systemic infection.

M. rhesus 42, inoculated May 19, 1926, with a saline suspension of the nodule from the eyebrow of *M. rhesus* 41, on the left eyebrow and left abdominal wall by intradermal injection and on the right eyebrow and right abdominal wall by scarification. This animal had fever for only a few days (Chart 4) during the 45 days of observation, but cultures made with the blood on June 2 and 14, 1926, gave pure growth of the bacilliform organisms in dilutions of 1:10 and 1:1,000 respectively.

Small reddish indurated areas along the lines of scarification on both eyebrow and abdomen became noticeable on June 1 (Fig. 6), and 10 days later the scarified areas appeared as linear rows of eruptions (Figs. 7, 8). Within another 10 days the adjacent lesions had become confluent (Fig. 9) but the transverse lines were still well separated. The intradermal inoculations in this instance, contrary to the usual case, failed to induce any lesions.

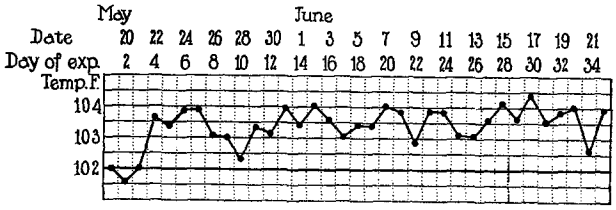


CHART 4. *M. rhesus* 42.

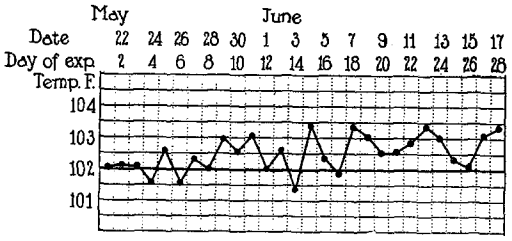


CHART 5. *M. rhesus* 43.

The lesions had all healed by Aug. 6, 1926.

M. rhesus 43, inoculated May 21, 1926, under ether anesthesia, with the citrate blood withdrawn on the same day from *M. rhesus* 33. Intradermal inoculations were made on both eyebrows and on both sides of the abdomen. Blood was also applied to scarified areas on the eyebrows and abdomen, and 0.5 cc. was injected intravenously.

The animal showed no febrile reaction at any time during the 45 days of observation (Chart 5). Blood cultures made on June 26 were negative. Small nodules, 2 × 2 mm., appeared at each of the two sites of intradermal inoculation within about 24 days. They showed no change during the ensuing 10 days but

subsequently gained in size rather rapidly. They were situated subcutaneously, were perfectly round, and had a semitransparent, bluish, pearl-like color. On July 1 there were noticed about the periphery the minute red streaks which *Bartonella* lesions of this type show just before vascularization. One of the nodules excised at this time (July 1) yielded cultures in a dilution of 1:100,000. By Aug. 6, 1926, the lesions had all healed.

The microorganism obtained in culture from a monkey inoculated with human verruga tissue (Case P. 5) was capable, therefore, of inducing in *rhesus* monkeys a local and systemic infection the manifestations of which were in all respects similar to those obtained by inoculation of the strain of *Bartonella bacilliformis* from Oroya fever, *i.e.*, a prolonged course of irregular, remittent fever, during which the intracorpuscular parasites are present in the blood, and a skin lesion characterized by extensive proliferation of endothelial cells and by the presence of the parasites in the cytoplasm of these cells. The infection in the second passage animal (Monkey 41) was of the severe type; there was absolute anemia and hyperleucocytosis, and the local lesions were very large. The organisms were present in large numbers at one period of the disease, but only degenerated forms were found at a later stage. The blood from which the culture was obtained, however, induced only a mild, slowly developing infection, with no fever.

The local inoculation of nodular tissue from Monkey 41 into Monkey 42 (third passage) induced a severe local reaction and a febrile reaction of brief duration, during which the blood culture titer was as high as 1:1,000. As stated previously, direct microscopic detection of the parasites in the blood was unsuccessful in the case of mild infections. The cultural procedure was by far the most delicate means of diagnosis.

Cultural and Morphological Characters of the Microorganisms from Verruga.

The character of the lesions of *M. rhesus* 41 and the microscopic findings in the blood of this animal appear to leave no doubt that the organism injected was identical in pathogenic properties with the strain of *Bartonella bacilliformis* previously obtained from Oroya fever, a conclusion which is further substantiated by cultural and morphological study.

The first culture was obtained on the so called leptospira medium, which was inoculated with various dilutions of citrated blood of *M. rhesus* 33 and kept at 25°C. Growth became recognizable macroscopically within a week, the uppermost layer of the medium showing some grayish, minute particles; these gradually increased in number during subsequent weeks (Fig. 19). Under the dark-field microscope these particles were seen to consist of numerous tightly packed masses of irregular minute bacillary bodies. Individual organisms were occasionally seen, but the chief characteristic of the organisms is their constant tendency to form firm aggregates difficult to disperse (Fig. 21). Free flagella are seen in the agar mass.

On the surface of blood agar round raised colonies of variable size, the smallest almost microscopic (Fig. 20), the largest having the appearance of fine granules, are formed within about 6 to 8 days and after a few days reach 1 mm. or more in diameter, but seldom 2 mm. A light grayish color is noticeable when the light falls obliquely on the culture. The colonies are firm and are peeled off the agar readily by a platinum loop. It is difficult to break them up in fluid to form a uniform suspension. Under the dark-field microscope masses of agglomerated organisms are seen to predominate (Fig. 22); a few single or paired individuals may be found, and these are usually motile. Locomotion is in one direction only, but rotatory movement is also seen.

The organisms are Gram-negative, pleomorphic, and stain unevenly with Giemsa's solution. When stained a long time they appear reddish and ill defined in contour; they stain much less intensely than most bacteria. Special staining reveals two or more flagella attached to one end of the organism (Fig. 24), the length of the normal flagella varying from 2 to 5 μ. Abnormal detached flagella may reach a length of 20 to 30 μ.

Individuals in young cultures are more uniform in size and have a definite contour. Short rods, 0.3 to 0.4 μ wide and 0.45 to 1.5 μ in length, predominate. In older cultures the individuals are much more irregular in size and form, and numerous granular elements measuring less than 0.2 to 0.5 μ are found in masses; these stain intensely and have very indefinite outlines, the appearance simulating that of degenerative changes. Exceptionally long rods 2.5 to 3 μ may be found. There is no polar staining.

The optimum temperature for growth is 25–28°C.; at 37°C. growth ceases within 4 to 5 days. The optimum reaction of the medium lies between pH 7.8 and pH 8.

For the cultivation of this organism the addition of animal blood or serum and hemoglobin to solid or semisolid media is essential. No satisfactory fluid medium has so far been found. The organism is an obligate aerobe. The cotton plugs of culture tubes may be impregnated with paraffin, but sealing them hermetically with sealing wax prevents growth.

Like the strain of *Bartonella bacilliformis* from Oroya fever, the organism is non-spore-forming and hemophilic. It is provided with

TABLE I.

Anti-Oroya serum	Antigens	
	Oroya	Verruga
	0.5 cc.	0.5 cc.
cc.		
0.1	++++	++++
0.01	++++	++++
0.001	++	−
0.0001	−	−
0	−	−

++++ = complete fixation.
++ = 50 per cent hemolysis.
− = complete hemolysis.

several unipolar flagella of the characteristic bacterial type. A distinct cell wall, such as is seen in bacteria, can be recognized in young, actively motile forms.

The organism does not ferment any carbohydrate so far tested. Red corpuscles contained in the culture medium are not hemolyzed in the course of its growth. No putrefactive process seems to occur in the culture. Slight growth may be obtained on Loeffler's serum medium, but no liquefaction is observed.

Serological Identification of the Verruga Strain.

The morphological, cultural, and pathogenic properties of the verruga strain resemble so much those of the strain of *Bartonella*

bacilliformis obtained from a case of Oroya fever that the identity of the two organisms appeared extremely probable. Preliminary serological tests confirmed this conclusion.

A rabbit was repeatedly inoculated intravenously with saline suspensions of cultures of *Bartonella bacilliformis* during a period of 4 months, 1 to 2 cc. being injected at intervals of 4 to 5 days, and the serum obtained from the animal was tested for complement fixation with its homologous strain and also with the strain from verruga. Saline suspensions of colonies removed from the surface of blood slant cultures after 14 days growth at 25°C., and washed twice with saline solution, served as antigens. The suspensions were rather granular in appearance, owing to the difficulty of breaking up the colonies. To 0.5 cc. of the antigen (this quantity did not bind complement) were added the immune serum in quantities of 0.1, 0.01, 0.001, or 0.0001 cc., and 0.04 cc. guinea pig serum as complement. Two hemolytic units of anti-sheep amboceptor were used. Controls with normal rabbit serum were made in each instance. The total volume in each case was made up to 1.2 cc. with saline solution. The results are shown in Table I.

The results of the complement fixation tests show that there is an undeniably close serological relationship between the two strains, although the immune serum reacted more strongly with the Oroya than with the verruga strain.

SUMMARY AND CONCLUSIONS.

A saline suspension of a subcutaneous nodule excised from a verruga patient, and kept in the refrigerator for 14 days, on inoculation into two young *Macacus rhesus* monkeys (Nos. 33 and 34) induced irregular febrile reactions and enlargement of the lymph glands, and in one instance a subcutaneous nodule arose, independently of direct inoculation, on the tail. A microorganism has been isolated from the blood of both animals, and from the experimental nodule, which in pathogenic properties and in cultural and morphological characteristics is indistinguishable from the strain of *Bartonella bacilliformis* isolated from a case of Oroya fever.

The spontaneous skin lesion of Monkey 34 and the subcutaneous nodules induced by intradermal inoculation of cultures of the microorganism were histologically typical of experimental verruga lesions in monkeys and identical with the skin lesions induced in monkeys by *Bartonella bacilliformis*. The organism, like *Bartonella bacilliformis*, is an intracellular parasite, being found in the cytoplasm of the prolif-

erating endothelial cells of the lesions and in the erythrocytes of the blood.

The same variations in the manifestations of disease which have been noted in experimental infection with *Bartonella bacilliformis* were observed in the experimental verruga infection. In the second passage (Monkey 41) the infection induced by local inoculation of cultures was severe both locally and constitutionally and was accompanied by marked anemia. The organisms were found in the red cells in large numbers. In the third passage the systemic infection was less severe, but the local lesions were more striking.

Detection of the parasites in the blood is far more certain by the cultural method than by microscopic examination, the latter procedure being successful only in rather severe infections. The result of blood culture is therefore the decisive method in the final diagnosis of the disease.

Preliminary serological study shows that the organism isolated in the present instance from the skin lesion of a verruga patient and that previously obtained from the blood of a case of Oroya fever belong to the same serological group.

The data obtained justify the conclusion that verruga peruana is caused by *Bartonella bacilliformis*. They also definitely establish the fact that the inoculation of blood or sanguineous exudate from lesions of verruga peruana is capable of inducing in susceptible individuals a severe febrile systemic infection, such as that to which Carrion succumbed. The designation "Carrion's disease"[6] is therefore the appropriate one for both forms of the infection.

Bartonella bacilliformis may be regarded as a bacterium, since it has the essential features of that group of microorganisms.

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. *M. rhesus* 41, showing three verruga nodules on the right eyebrow 30 days after intradermal inoculation. The two nodules on the inner side had arisen at the site of removal of the initial nodule 16 days previously. Natural size.

FIG. 2. Verruga organisms in the red corpuscles of *M. rhesus* 41, 30 days after inoculation. × 1,500.

FIG. 3. Verruga organisms in the red corpuscles of the same monkey 52 days

after inoculation. They took a more reddish stain at this time and were less definite in outline, probably because they were in process of degeneration. Anemia was also most marked at this time. Subsequent attempts to find the parasite in the blood were unsuccessful. × 1,500.

FIG. 4. Verruga organisms in the nodule of *M. rhesus* 41, removed 14 days after inoculation. Giemsa's stain after fixation in Regaud's fluid. × 1,500.

FIG. 5. Verruga organisms in the nodule of a patient suffering with verruga peruana. Courtesy of Professor R. P. Strong. Giemsa's stain, after fixation in Zenker's fluid. × 1,500.

FIG. 6. Early lesions of experimental verruga in *M. rhesus* 42, 16 days after inoculation of a scarified area on the right eyebrow with a suspension of the nodule from *M. rhesus* 41. Natural size.

FIG. 7. Same lesions 23 days after inoculation.

FIG. 8. Early lesions on the scarified skin of the abdomen of the same monkey 16 days after inoculation. Natural size.

FIG. 9. Same lesions 23 days after inoculation.

FIG. 10. Subcutaneous nodule which arose spontaneously on the tail of *M. rhesus* 34, 59 days after the animal had been inoculated with a suspension of a nodule of the verruga patient P. 5. Natural size. For histological appearance of the nodule see Fig. 11.

PLATE 3.

FIG. 11. Histological appearance of the subcutaneous nodule on the tail of *M. rhesus* 34, 69 days after inoculation. (The gross appearance of the nodule is shown in Fig. 10.) Giemsa's stain after fixation in Regaud's fluid. × 152.

FIG. 12. Histological appearance of the nodule on the eyebrow of *M. rhesus* 41, removed 14 days after inoculation. Giemsa's stain after Regaud's fixation. × 152.

FIG. 13. Verruga organisms in the nodule of *M. rhesus* 41. Same section as shown in Fig. 12. × 1,000.

FIG. 14. Verruga organisms in the red blood corpuscles of *M. rhesus* 41. Eight different fields have been placed side by side. In some of them are seen red corpuscles containing one or more organisms. Blood films made 30 days after inoculation. Giemsa's stain. × 1,000.

FIG. 15. Blood of the same monkey 52 days after inoculation. The organisms appear somewhat degenerated but quite numerous. Giemsa's stain. × 1,000.

FIG. 16. *Bartonella bacilliformis* in the blood of an Oroya fever patient, for comparison (Patient S. A. 15, case from which *B. bacilliformis* was cultivated). Giemsa's stain. × 1,000.

PLATE 4.

FIG. 17. Verruga nodule from Case P. 5, fixed in Regaud's fluid and stained with Giemsa's solution, 14 days after removal from patient. The characteristic verrucous structure is still recognizable, although many cells have undergone degenera-

tion. The dark masses of irregular size are aggregates of minute bacilliform organisms. × 152.

FIG. 18. The same section as that shown in Fig. 17 but under a higher magnification. × 1,000.

FIG. 19. Appearance of colonies of the verruga organisms in the upper portion of a tube of leptospira medium. 28 days old at 25°C. Natural size.

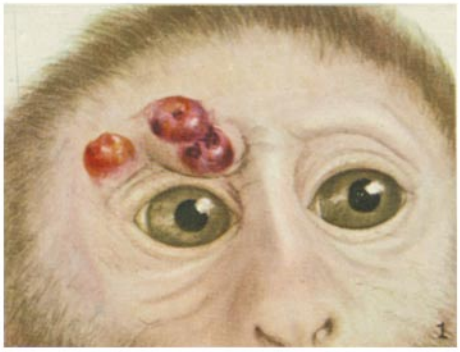
FIG. 20. Appearance of colonies of the verruga organisms on the surface of horse blood agar slant, 12 days old at 25°. Natural size.

FIG. 21. Dark-field view of the culture shown in Fig. 19. × 1,000.

FIG. 22. Dark-field view of the culture shown in Fig. 20. × 1,000.

FIG. 23. The verruga organisms from a blood agar slant culture, 11 days old at 25°C. Giemsa's stain. × 1,000.

FIG. 24. Flagella of the verruga organisms. Zettnow's stain. × 2,000.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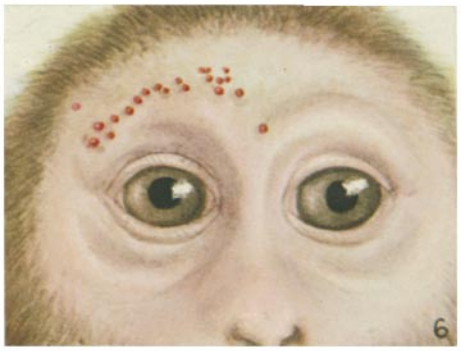
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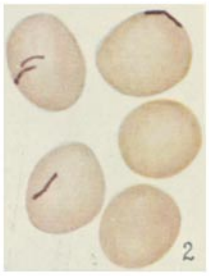
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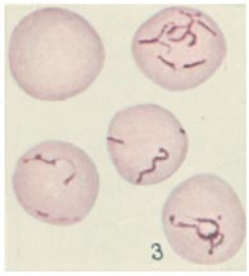
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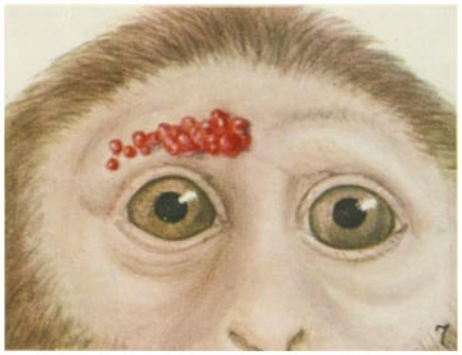
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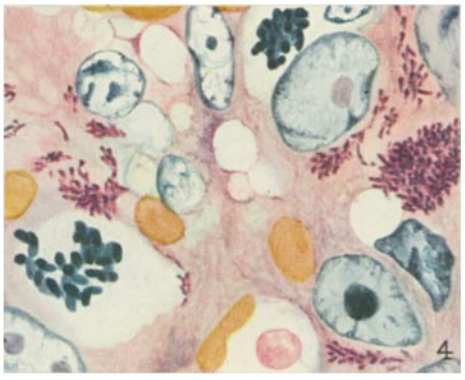
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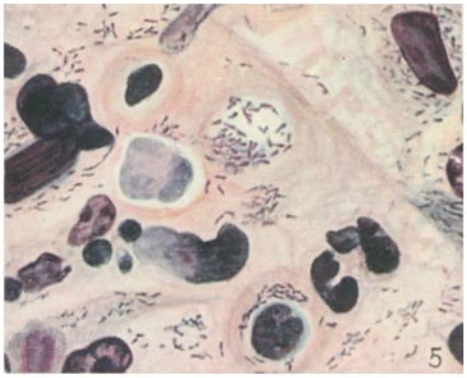
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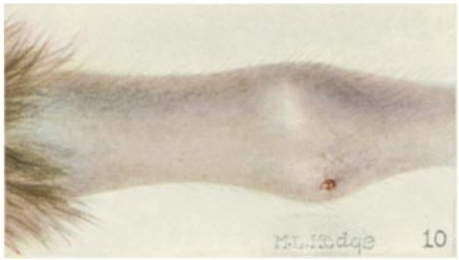
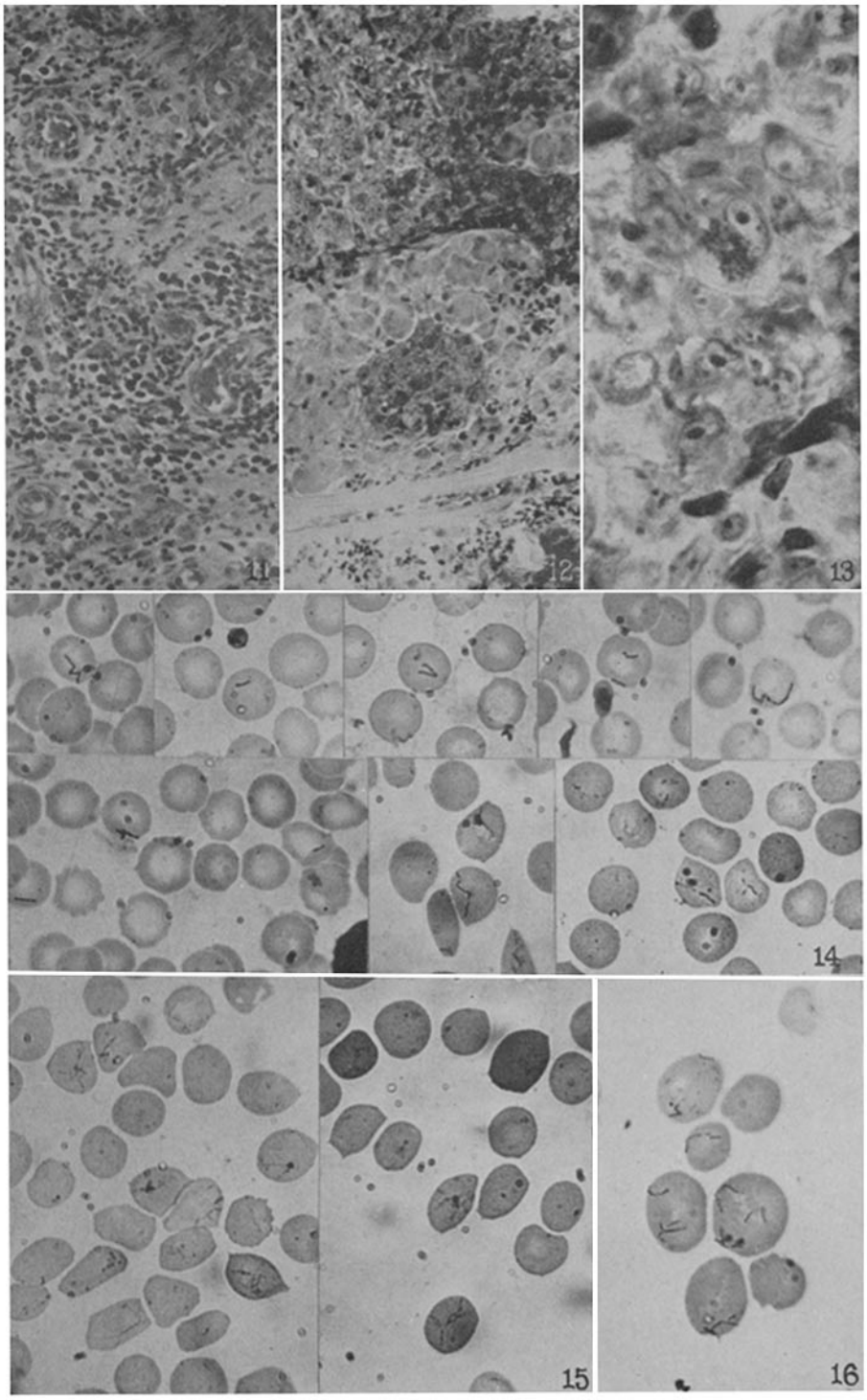
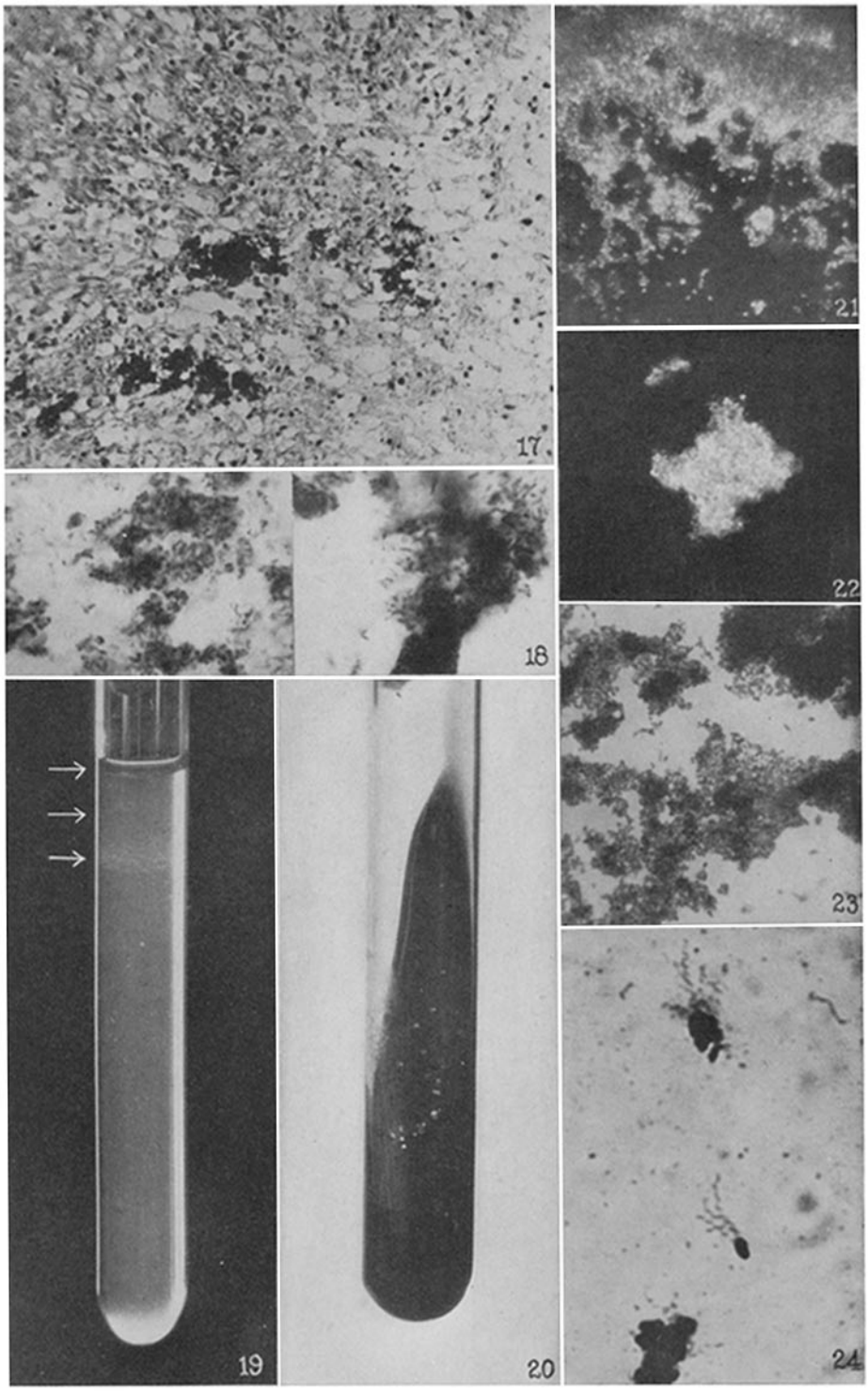

M.L.Hodge
10



(Noguchi: Etiology of verruga peruana.)



(Noguchi: Etiology of verruga peruana.)