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ETIOLOGY OF OROYA FEVER.

IX. BACTERIUM PERUVIANUM, N. SP., A SECONDARY INVADER OF THE LESIONS OF VERRUGA PERUANA.

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PLATE 6.

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In the course of an experimental investigation of Carrion's disease, at the stage when bacteriological and experimental study of the characteristic lesions of verruga peruana was desirable, I was so fortunate as to obtain, through the kindness of Professor Oswaldo Hercelles, of Lima, two subcutaneous nodules, excised under aseptic conditions, from two verruga patients (Cases P 5 and J 45), and forwarded in separate sealed tubes in the ship's refrigerators from Lima to New York. A strain of *Bartonella bacilliformis* was isolated from one of the nodules (Case P 5), as has been reported elsewhere,[1] but another microorganism was also present in the tissue, and was isolated in pure cultures in both instances. This organism is particularly interesting because of its striking morphological similarity to *Bartonella bacilliformis* and its marked difference from this parasite in cultural and pathogenic properties.

Cultural Properties.

The microorganism in question grows rapidly on ordinary bacteriological culture media at 37°C. as well as at 25°C., the latter temperature being the optimum one. Round, discrete, elevated, grayish, translucent colonies measuring 1 to 2 mm. in diameter make their appearance on a plain or blood agar plate within 24 hours. They reach a size of 2 to 4 mm. within several days but seldom become much larger. The colonies are spherical, and the margin smooth; in consistency they are somewhat firmer than colonies of *B. coli* or *B. proteus*, and they show no

[1]Noguchi, H., *J. Exp. Med.*, 1927, xlv, 175.

tendency to spread over the surface of the medium. In 7 to 10 days at room temperature a trace of brownish tint appears in colonies on the surface of agar. On blood agar plates hemolysis takes place and gradually extends to a considerable distance around the colonies. A characteristic pungent odor is noticeable when the plate is uncovered. Broth is diffusely clouded by the growth of the organism, and within a few days a delicate grayish pellicle is formed around the wall of the culture tube at the top of the fluid and a fluffy sediment at the bottom. As the culture grows older the broth becomes markedly mucilaginous. No putrefactive or other odor is produced either in broth or on plain agar. Löffler's serum undergoes slow but complete liquefaction within about 2 weeks at 25°C. No carbohydrates are fermented by the organism, and no hydrogen sulfide is produced on any medium. Cultures remain transplantable for a period of at least 3 months if kept constantly at 25°C. No growth takes place under strictly anaerobic conditions.

Morphology.

Individuals in young colonies on an agar surface are actively motile and measure about 0.3 to 0.4μ in width and 0.6 to 1.2μ in length. In older cultures longer forms, 2 to 4μ, are present. When grown on a blood agar slant or on leptospira medium the organisms are somewhat thinner (0.2 to 0.3μ in width) and longer than the young forms on an agar surface and give the impression of delicate slender rods (Fig. 3). They are less actively motile and may be clumped into masses of many individuals (Fig. 1) which resemble to some extent the characteristic masses of *Bartonella bacilliformis*. Forms grown on broth are of medium size and remain actively motile for many days. In cultures several days old the motile organisms are intermingled with small oval or coccoid forms which may be in pairs or short chains. Except for occasional very long individuals, which may be bent and thickened at one end, the organisms are as a rule straight and have pointed ends. No bifid or branching individuals have so far been noted.

The organism is Gram-negative and stains fairly well with fuchsin or methylene blue. Brief staining in Giemsa's solution gives it a lavender color; with more prolonged staining it becomes reddish. The general aspect of the organism in stained smears (Figs. 1 and 2) resembles that of *Bartonella bacilliformis* in similar preparations, but the individual organisms are more sharply defined than are the individual bartonellas, owing perhaps to a greater affinity for the dyes. No tendency to bipolar staining has been noticed.

One of the striking features of the organism is its characteristic spirally curved flagella (Fig. 4) which are present at one end of the body and may be two, three, or four in number. So far as I am aware, the only other pathogenic organism having flagella of this type is *Bartonella bacilliformis*.

Because of its presence in material derived from Peru, the organism has been named *Bacterium peruvianum.*

Pathogenicity.

When *B. peruvianum* is inoculated intradermally into monkeys (*Macacus rhesus, Cercopithecus callitricus*) or dogs, the sites of inoculation become edematous, congested, and necrotic within a few days, and open ulcers with raised margins finally result; these usually heal within 10 to 14 days. The Strain P 5 was more actively pathogenic than the J 45. Unbroken nodules such as were occasionally produced by the P 5 strain resolved after about 10 days.

Cultures made from the blood of the animals during the height of the local reactions remained sterile in all instances; there was no invasion of the general circulation by the organism. Whether or not the injection of *B. peruvianum* into the blood circulation in large quantities will result in fatal septicemia has not been determined.

B. peruvianum induces an acute fatal septicemia in rabbits, guinea pigs, rats, and mice, when administered intravenously or intratesticularly. A broth culture 48 to 72 hours old was lethal within 24 to 48 hours in a quantity of 0.1 to 1 cc. Autopsy reveals very striking and characteristic changes in the abdominal viscera.

The liver seems to be most affected; it is somewhat enlarged, tense, uniformly peppered with innumerable minute, intensely red spots which make the whole organ appear red. The stomach shows many diffuse and punctiform hemorrhages recognizable through the congested serosa. The entire intestine appears diffusely limpid red. The kidneys are swollen and congested, and the adrenals are much enlarged and deep red. The spleen is not noticeably enlarged but is soft and extremely dark bluish red. The peritoneal cavity contains some limpid pink fluid. No fibrinous exudate was found on the surface of any organ. The abdominal muscles are frequently spotted with hemorrhagic areas of moderate size. The lungs are congested but show no hemorrhages. The heart is flabby, but there is no pericarditis or pleurisy. *B. peruvianum* can be recovered in pure culture from heart's blood or spleen.

When inoculated intratesticularly, the animals succumb within 1 to 3 days and show the same changes as do animals injected intravenously. The scrotum and testicle both show intense edema, congestion, hemorrhages, and necrosis.

There was no intercurrent infection with *B. lepisepticus* or *B. monocytogenes*[2] in any of the animals.

Differentiation of B. peruvianum from Other Pathogenic Microorganisms.

The characteristics of *B. peruvianum* may be summarized as follows: Proper motility by means of multiple unipolar flagella; non-retention of Gram's stain; strict aerobiosis; better growth at 25°C. than at 37°C.; presence of proteolytic and absence of sugar-splitting ferment; mucin production in broth; hemolytic action; and a wide range of characteristic pathogenic properties for rabbits, guinea pigs, rats, mice, dogs, and monkeys. No microorganism having been previously described which corresponds with the foregoing description, the organism in question is regarded as a new species.

Bartonella bacilliformis resembles *B. peruvianum* in morphology, in its inability to ferment carbohydrates, in growing better at 25°C. than at a higher temperature, in not retaining Gram's stain, in being strictly aerobic, and in having one to four unipolar flagella. The two organisms differ widely in other respects, however. *Bartonella bacilliformis* is unable to grow on any fluid medium so far tried, or any medium which does not contain blood elements; it grows very slowly, and the colonies are very minute; it lacks proteolytic or hemolytic activity, it is difficult to disperse into a homogeneous suspension, and above all, it has specific pathogenic properties. Moreover, a potent anti-*bacilliformis* immune serum gives a specific complement fixation and a distinct agglutination reaction with *Bartonella bacilliformis*, but none with *B. peruvianum*.

B. proteus and allied organisms are distinct from *B. peruvianum* in morphological and cultural properties. The flagella of the *proteus* group are peritrichal, and hydrogen sulfide is produced by the organisms in the course of growth.

The *coli-enteritidis-typhoid* group ferments carbohydrates, and does not liquefy Löffler's serum. The flagella of this group are peritrichal.

[2] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, xxix, 407. Pirie, J. H. H., *Pub. South African Inst. Med. Research*, 1927, iii, 185. I am indebted for cultures of this microorganism to Dr. J. C. G. Ledingham, Director of the National Collection of Type Cultures at the Lister Institute.

B. monocytogenes has a single polar flagellum and the property of fermenting a great many carbohydrates.

Bacillus rickettsiformis[3] resembles *Bacterium peruvianum* in some respects, in morphological features, in growing best at low temperatures, and in producing mucin in broth cultures. But the *rickettsiformis* is non-pathogenic and ferments several sugars.

SUMMARY.

A minute, pleomorphic, motile, Gram-negative bacterium has been isolated from two specimens of nodular tissue from human verruga. In films and sections of the original tissues the organism in question is difficult to distinguish from *Bartonella bacilliformis*, with which it was associated, and even in pure culture it has a number of properties in common with that parasite. No sugars are fermented by it, it is an obligate aerobe, the optimum temperature for its growth is 25°C., and it has two to four spiral flagella attached to one end of the body. It is, however, readily cultivated on any ordinary culture medium. Broth cultures contain much mucin, but no hydrogen sulfide is formed. Coagulated serum is liquefied by its growth, and the red corpuscles in a blood agar plate are hemolyzed.

Rabbits, guinea pigs, rats, and mice develop acute, fatal septicemia as a result of intravenous or intratesticular inoculation of young cultures. The liver is characteristically affected and shows a general parenchymatous degeneration and necrosis; the entire gastrointestinal tract is intensely congested, and numerous hemorrhagic areas are present; the spleen, dark and soft, is rarely much enlarged; the kidneys are swollen and congested; the adrenals are much swollen and intensely red; the lungs are sometimes congested but otherwise normal. In the case of intratesticular inoculation the scrotum and testicle both undergo rapid gangrene. In monkeys no septicemia has been observed, but a violent local reaction—swelling, congestion, sometimes necrosis—follows intradermal inoculation.

Since no microorganism corresponding in character with this one has previously been described, it is regarded as a new species, and because of its presence in material obtained from Peru it has been given

[3] Noguchi, H., *J. Exp. Med.*, 1926, xliii, 515.

the name *Bacterium peruvianum.* The significance of the association of *B. peruvianum* with *Bartonella bacilliformis* deserves further investigation; it is not impossible that the two organisms are introduced into the human body by the same blood-sucking insect.

Cultures of *Bacterium peruvianum, n. sp.*, an organism associated with *Bartonella bacilliformis* in verruga tissues.

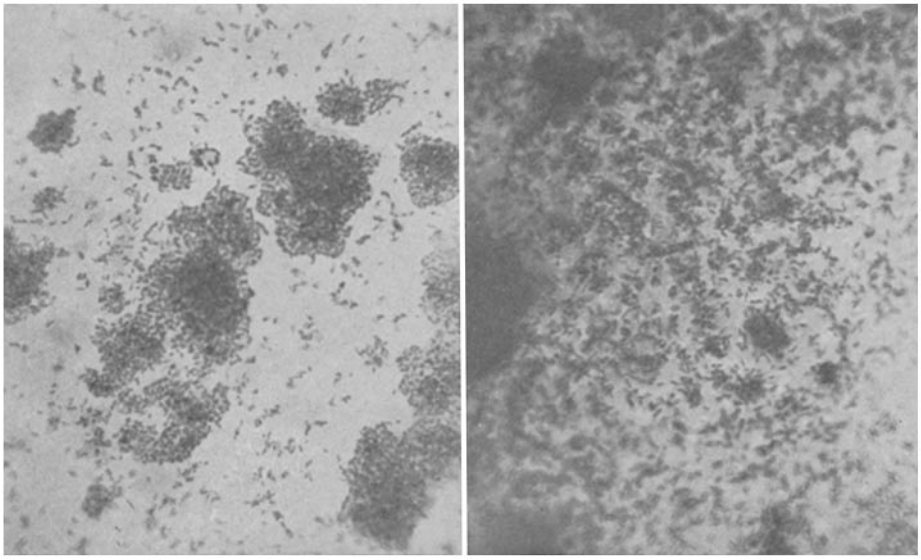

FIG. 1. FIG. 2.

Giemsa's stain. × 1000.

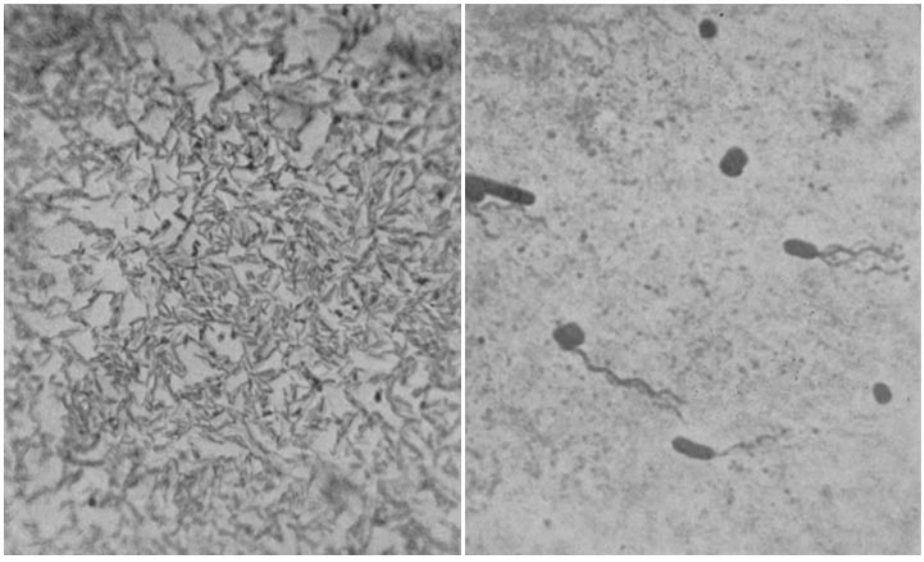

FIG. 3.

Giemsa's stain. × 1000.

FIG. 4.

Zettnow-Fontana flagella stain. × 2000.