

the azo-albumin anti-azo-albumin reaction were found colorimetrically to contain only about 10% of the colored antigen. In a cross-reaction between azo globulin and anti-azo-albumin the precipitate contained less than 4% of the colored component, despite the fact that the azo-globulin solutions are colored more intensely per unit of nitrogenous substance in solution.

9651 P

Carrion's Disease. I. Behavior of the Etiological Agent Within Cells Growing or Surviving *in vitro*.*

HENRY PINKERTON AND DAVID WEINMAN.

From the Departments of Pathology and Comparative Pathology, Harvard Medical School, Boston.

"Carrion's Disease" commonly includes two very different clinical syndromes—(1) a severe, usually fatal febrile anemia, (Oroya fever) and (2) a remarkable cutaneous eruption of hemangioma-like nodules (*verruca peruana*). Clinical and experimental evidence strongly suggests that these two conditions are successive stages of a single disease, the first (anemic) stage being of variable severity and often inapparent or even absent.

The generally accepted cause of the first condition (Oroya fever) is a small pleomorphic microorganism, first seen by Barton¹ in or on red blood cells and named *Bartonella bacilliformis*.² This organism was grown in Noguchi's leptospira-medium.³ Apparently iden-

* This paper, with the four following papers, summarizes certain observations made by the Harvard 1937 Expedition to Peru, organized by Dr. Richard P. Strong of the Department of Tropical Medicine. Other members were Dr. Henry Pinkerton, Department of Pathology, Dr. Marshall Hertig and Dr. David Weinman, Department of Comparative Pathology, and Mr. Byron L. Bennett, Technical Assistant. The work was made possible by the generous coöperation of Dr. Telémaco S. Battistini, Director of the National Institute of Hygiene and Public Health in Lima, and was carried out in this Institute. Assistance rendered by other Peruvian physicians is also gratefully acknowledged. These investigations were aided in part through grants from the Richard P. Strong Fund (founded by the late Dr. Frederick C. Shattuck), the Proctor Fund for the Study of Chronic Disease, and the William W. Wellington Memorial Research Fund.

¹ Barton, A. L., *Cron. Med. Lima*, 1909, **26**, 7.

² Strong, Tyzzer, Brues, Sellards, and Gastiaturú, *Report of First Expedition to South America*, Harvard University Press, 1915.

³ Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, **43**, 851.

tical organisms were also grown from the cutaneous nodules of *verruca peruana*; the organisms cultivated from both conditions produced angiomatous nodules in the eyebrows of monkeys, in which bartonella-like microorganisms were demonstrated histologically.^{3, 4} Consequently, notwithstanding certain puzzling discrepancies, this cultivable organism has been almost universally accepted as the cause of both these clinical conditions.

The rickettsiæ of typhus and spotted fever form distinctive and constant patterns within mammalian cells grown *in vitro*, patterns that are identical with those found in infected cells of the arthropod vectors of these diseases.^{5, 6} *Bartonella bacilliformis* was found in compact rounded masses in the endothelial cells of lymph nodes, liver, and intestine from fatal cases of Oroya fever.² We wished to determine whether the organisms cultivable from Oroya-fever blood and from the cutaneous nodules of *verruca peruana* would behave alike as intracellular parasites when cultivated in association with suitable mammalian cells *in vitro*.

Mesenchymal cells of guinea pig and rat tissues *in vitro* were found susceptible, although these animals have resisted infection. Tissues from the lung, bone-marrow, spleen, and *tunica vaginalis* were used in the plasma-culture⁷ and in modified Maitland cultures.⁸ Cultures of both types were studied by smears and paraffin sections (Regaud-fixed, Giemsa-stained).

The media were inoculated with one or two drops of material from the following sources: (1) Blood from a severely anemic case of Oroya fever. (2) Cultures from the blood of 7 cases of Oroya fever, showing organisms undoubtedly identical with those described by Noguchi and Battistini, and maintained in leptospira-medium, with bi-weekly transfers, for periods ranging up to 4 months. (3) Similar cultures obtained postmortem from lymph-nodes and bone-marrow of 2 fatal cases of Oroya fever. (4) Similar cultures obtained from blood and from cutaneous nodules from 3 patients with *verruca peruana*, in 2 of whom there had been no apparent fever or anemia. (5) Similar cultures obtained from the blood of monkeys several weeks after inoculation with virus from human Oroya fever and *verruca peruana*.

In tissue-cultures of both types, infected from all of the above 5

⁴ Noguchi, H., *J. Exp. Med.*, 1927, **45**, 175.

⁵ Pinkerton, H., *Parasitology*, 1936, **28** (No. 2), 172.

⁶ Hass, G. M., and Pinkerton, H., *J. Exp. Med.*, 1936, **64** (No. 4), 601.

⁷ Pinkerton, H., *Arch. f. Exp. Zellforsch.*, 1936, **18**, 343.

⁸ Nigg, C., and Landsteiner, K., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 2.

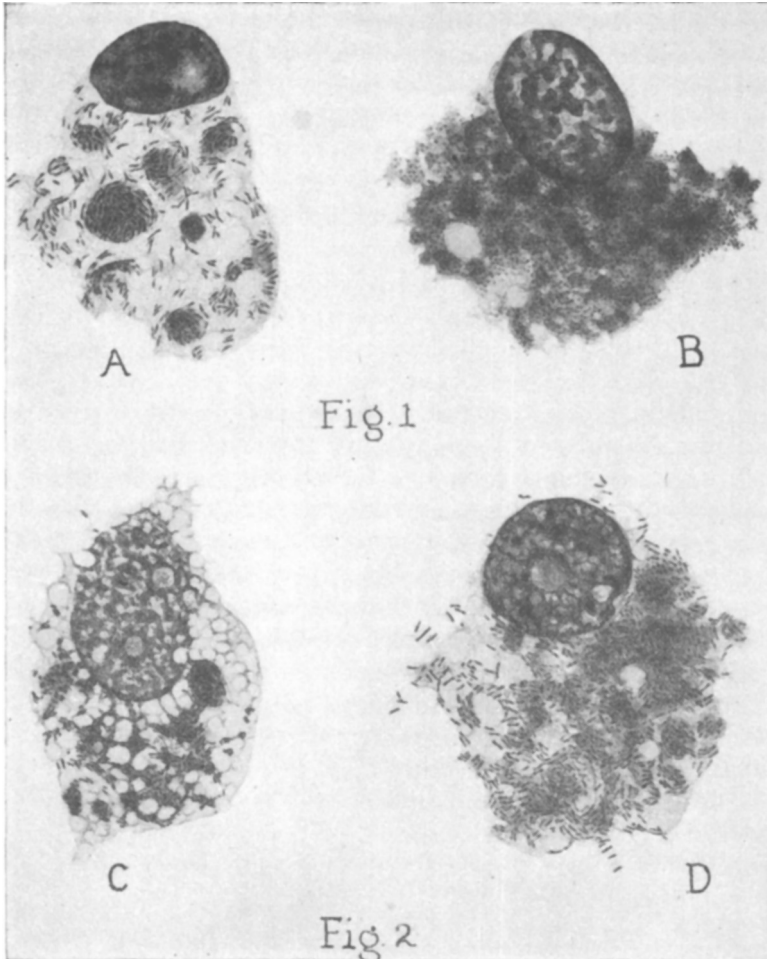


FIG. 1.

Mesenchymal cells from guinea pig's *tunica*, surviving in Maitland medium (Nigg-Landsteiner modification), containing intracytoplasmic clusters of bartonellæ. These cells were infected with cultures of bartonella, obtained in leptospira-medium from the blood of a human case of Oroya fever. Cell A is from a 4-day Maitland culture. Cell B, from an 8-day Maitland culture, shows the granular or globular organisms characteristic of older cultures.

FIG. 2.

Cells infected, under otherwise identical conditions, with cultures of bartonella originating from a human verruga, excised from a patient without fever or anemia. Cell C is from a 5-day culture and cell D is from a 6-day culture.

All cells were drawn from alcohol-fixed Giemsa-stained smear-preparations at a magnification of 1300 diameters.

cytoplasm.† Organisms were absent from controls. In early cultures, small spherical clusters (3-10 μ in diameter) of sharply stained diplobacillary rods were seen in the cells. (Fig. 1. Cell A.) Later, usually by the sixth day, many cells were packed with clusters of less discrete organisms, often coccoid, granular, or amorphous, (Fig. 1. Cell B) accurately reproducing the picture seen in human lymphnode-endothelium from fatal cases of Oroya fever. *This characteristic pattern was found in tissue-cultures infected from all 5 sources.*

The mesenchymal cells in which bartonella multiplied were not accurately identified because fibroblasts, endothelial cells, and histiocytes were not distinguishable by the methods employed. In bone-marrow cultures, organisms were absent from granulocytes but often present in megakaryocytes. Erythrocytes, though well preserved in the cultures, were never invaded.

Cell-nuclei were not invaded. The pattern of cell-infection could be easily distinguished from that of other intracellular parasites which we have studied, although individual organisms, when discrete and sharply stained, could easily be mistaken for typhus rickettsiæ.

Conclusions. Organisms cultivated in leptospira-medium, whether from the blood or tissues of Oroya-fever patients or from cutaneous nodules excised from patients with *verruca peruana*, invariably behaved as facultative intracellular parasites when cultivated *in vitro* with growing or surviving mammalian cells. Organisms cultivated from the blood of monkeys infected with human material from either Oroya fever or *verruca peruana* patients behaved similarly. A characteristic and uniform pattern of intracytoplasmic clustering was obtained with cultures from all of the above sources, this pattern being essentially identical with that seen in naturally infected human cells in both conditions (see Paper II). These observations substantiate the belief that Oroya fever and *verruca peruana* are caused by the same microorganism.

† In their behavior under these conditions, these organisms resemble *B. tularensis* (unpublished observations) and differ from typhus and spotted fever rickettsiæ, which grow only within cells. The resistance of these rickettsiæ to cultivation in cell-free media is probably correlated with this behavior.