

9652 P

Carrion's Disease. II. Comparative Morphology of the Etiological Agent in Oroya Fever and *Verruga Peruana*.

HENRY PINKERTON AND DAVID WEINMAN.

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

The material for this study included human tissues removed postmortem from 3 fatal cases of Oroya fever, and cutaneous nodules excised from 7 cases of *verruca peruana*, 2 of which were apparently afebrile and non-anemic. Cutaneous nodules, produced in monkeys with material from both Oroya fever and *verruca peruana* were also excised for comparative histological study. Impression-smears and paraffin sections were prepared from all of the above types of tissue. Air dried impression smears were stained by the Giemsa method with or without previous fixation in absolute methyl alcohol. For the demonstration of bartonellæ in sections, a technic involving the combination of Regaud-fixation and Giemsa-staining was found to be invaluable. This combination was used for the demonstration of *Rickettsia ruminantium* by Cowdry¹ and for the demonstration of bartonellæ in verrugas by Noguchi and Battistini.² The technic used in this work was that evolved by one of us³ for staining typhus and spotted fever rickettsiæ.

Oroya Fever. In 2 of the 3 fatal cases of Oroya fever* in which postmortems were performed, the endothelial cells lining blood and lymph channels of many organs were distended with clusters of organisms, as previously described⁴ (Fig. 1, Cell A). In many cells these organisms were discrete and rod-shaped but frequently they were represented by granular or amorphous spheroidal masses, recognizable only by their characteristic staining reaction. This picture was found in lymph nodes, liver, (Kupffer cells), bone marrow, spleen, kidney, adrenal, pancreas, thyroid, and testicle. Infected and distended cells were present in all these organs in such numbers that they were prominent on low-power examination. Oc-

¹ Cowdry, E. V., *J. Exp. Med.*, 1925, **42**, 253.

² Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, **43**, 851.

³ Pinkerton, H., *J. Exp. Med.*, 1931, **54**, 181.

* The third case was complicated by a secondary infection and relatively few bartonellæ were found in the erythrocytes at the time of death.

⁴ Strong, Tyzzer, Brues, Sellards, and Gastiaboru, *Report of First Expedition to South America*, Harvard University Press, 1915.

casional infected cells were found in the heart and lungs. The distribution of infected cells suggested that they were largely members of the reticulo-endothelial system. In the Kupffer cells of the liver, less than 20% of the infected cells showed the characteristic clustering of organisms, the remainder being uniformly filled with bartonellæ. In all other organs, more than 90% of the infected cells showed the characteristic clustering.

We are inclined to interpret the various appearances of the organisms, ranging from discrete bacilli to granular and even homogeneous masses as pleomorphism rather than as stages in a progressive life-cycle. Even in leptospira-medium, the organisms go through similar changes.

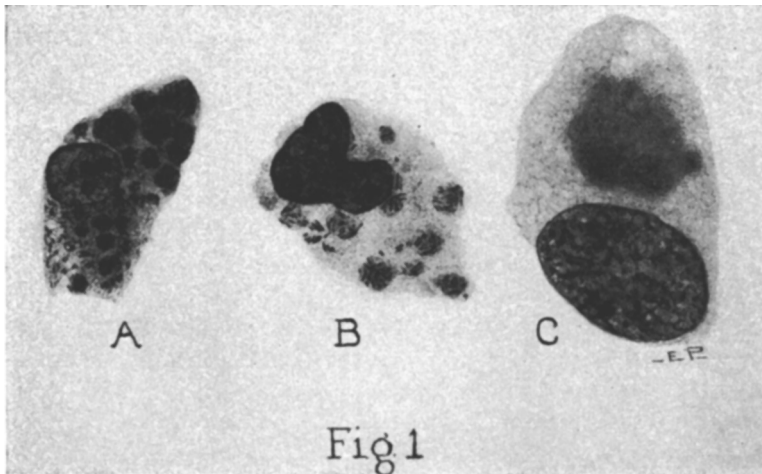


FIG. 1.

Cell A. Kupffer-cell in liver from a fatal case of human Oroya fever, showing intracytoplasmic clusters of bartonellæ. From a Regaud-fixed, Giemsa-stained paraffin section. $\times 1300$.

Cell B. Endothelial cell from a human cutaneous nodule excised from a patient with afebrile and non-anemic *verruca peruana*. Note similarity of intracytoplasmic bartonellæ, both morphologically and in their distribution to those seen in Oroya fever (Cell A). From an alcohol-fixed Giemsa-stained smear preparation. $\times 1300$.

Cell C. Endothelial cell from the same preparation as Cell B, showing the more homogeneous, bright-red-staining type of inclusion. Intermediate stages were found between this picture and that shown in cell B. $\times 1300$.

Verruga peruana. In 10 cutaneous nodules (representing 7 different patients) bartonella-filled cells were invariably present, often in enormous numbers. Distention of cells was much less common than in the organs of Oroya-fever cases. Clustering of organisms in cells was rarely as pronounced as in Oroya-fever organs, but was often definite. (Fig. 1, Cell B.) In smear-preparations, or-

ganisms appeared to be entirely intracellular in position. In paraffin sections, no definite evidence of extracellular multiplication was seen, although the presence of bartonellæ in elongated cytoplasmic processes of spindle-cells made it difficult to determine this point. In several verrugas, particularly in those containing fewer cells definitely filled with bartonellæ, red-staining "inclusions" occupying from 10 to 75% of the cytoplasm were seen (Fig. 1, Cell C). These structures, sharply demarcated from the uninvolved blue-staining cytoplasm, were finely granular and most often approximately spherical. They are undoubtedly the structures described by Mayer, Rocha Lima, and Werner,⁵ and considered by them as similar to "chlamydozoa". Intermediate forms between these structures and definite clusters of bartonellæ were seen. Whether they represent clusters of degenerating organisms or a stage in a life-cycle has not been ascertained. They have, in many instances, considerable resemblance to clusters of the psittacosis organisms as described by Bedson and Bland⁶ and by Bland and Canti.⁷

Cutaneous Nodules from Monkeys. No differences were observed between the cutaneous nodules (verrugas) produced in the eyebrows of monkeys by the injection of emulsified human Oroya-fever lymphnodes and those produced by injecting emulsified cutaneous nodules from cases of *verruca peruana*. Such lesions invariably contained numerous bartonella-filled cells identical with those seen in human verrugas and frequently also contained red-staining "inclusions" of the type described above.

Conclusions. Bartonella-filled cells were found in large numbers in nearly all organs of human Oroya fever cases, in cutaneous nodules from afebrile and non-anemic cases of *verruca peruana*, and in cutaneous nodules produced in monkeys by inoculation with human material from both of the above sources. The morphological identity of the organisms, and the essential identity of the patterns produced by them within cells in all of the above tissues furnish additional evidence that Oroya fever and *verruca peruana* have a common etiological agent, *Bartonella bacilliformis*.

⁵ Mayer, M., Rocha Lima, H., and Werner, H., *Munch. Med. Woch.*, 1913, **60**, 739.

⁶ Bedson, S. P., and Bland, J. O. W., *Brit. J. Exp. Path.*, 1932, **13**, 461.

⁷ Bland, J. O. W., and Canti, R. G., *J. Path. and Bact.*, 1935, **40**, 231.