blood cultures. Three of these had no past history of Carrion's disease; 2 had previously suffered from *verruga peruana*. It is possible that asymptomatic cases may constitute a reservoir of virus which is important in the natural transmission of the disease.

Peruvian guinea pigs were found to be spontaneously infected with an unidentified bartonella. This infection has not been found in guinea pigs from other regions than Peru, but was transmitted to splenectomized previously uninfected stock guinea pigs obtained in Boston. This guinea pig bartonella is being further studied, in an attempt to identify it more accurately.

9655 P

Carrion's Disease. V. Studies on Phlebotomus as the Possible Vector.

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Shannon[1] collected Phlebotomus sandflies in the verruga zone of the Rimac Valley, Peru, and shipped them under refrigeration to New York where they were injected into monkeys.[2] Four of 10 batches, including chiefly *Phlebotomus noguchii* and *P. verrucarum* produced inapparent infection with *Bartonella bacilliformis* as evidenced by blood culture. Microscopic examination of these sandflies was not reported. These experiments demonstrated the harboring but not the actual transmission, of bartonella by Phlebotomus, since no living sandflies were used.

During March to June, 1937, the writer collected sandflies in Verrugas Canyon where much of Shannon's field work was done. Houses yielded almost entirely *P. verrucarum;* material from excavations included both *P. noguchii* and *P. verrucarum.* They were collected and transported in 10x75 mm. cotton-stoppered test tubes, inverted in moist pots. Moist plaster-lined pots were used for rearing.[3] Larvae were fed on moist guinea pig's feces. A second method was devised, permitting more ready examination of larvae

1 Shannon, R. C., *Am. J. Hyg.*, 1929, **10**, 78.

2 Noguchi, H., Shannon, R. C., Tilden, E. B., and Tyler, J. P., *J. Exp. Med.*, 1929, **49**, 993.

3 Young, C. W., and Hertig, M., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 611.

and more suitable for rearing progeny of individual females. Gravid females, kept at high humidity in their original tubes, laid eggs chiefly on cotton stoppers. The egg-bearing portion was then transferred to a small test tube, completely lined with plaster of Paris, except for a long, slit-like window. This tube was then stoppered with cotton and inverted in a moist pot.

Laboratory-reared adults were not available for experimental work, since both species required about 3 months from adult to adult at 20-23°C. Wild sandflies of both species, but chiefly *P. verrucarum,* were therefore used exclusively in the following experiments.

Examination of Wild Sandflies. Giemsa-stained smears of 15 wild sandflies revealed no bartonella-like organisms. Preliminary examination of Regaud-Giemsa sections has shown minute extracellular cocco-bacillary organisms forming an irregular layer over the entire surface of the mid-intestinal epithelium of one wild female *(P. verrucarum).* Intestines of 26 wild female sandflies yielded no growth in leptospira medium in 23 cases; contaminants in 3.

Sandflies Fed on Patients. An organism morphologically consistent with *Bartonella bacilliformis* appeared in the intestinal contents of about 75% of 90 wild sandflies after feeding on 4 patients showing many bartonellæ in blood films. Sandflies fed on these same patients after the bartonellæ had disappeared from the blood stream, or on other patients with negative blood films at the time of feeding were invariably negative. These organisms were found in large numbers in the brown fecal drops passed 4-5 days after feeding; they were practically absent from clear or whitish fecal drops. They were absent from the feces of wild sandflies fed in nature. In sections of sandflies they appeared in large numbers, occasionally in dense spheroid colonies and usually in the lumen of the mid-intestine. They were never intracellular in any organ. In one instance they were found around the proventricular fold, extending through the short oesophagus to its opening into the pharynx, but they were not found elsewhere in the pharynx. These organisms failed to grow in leptospira medium and their pathogenicity has not been tested; their identity, therefore, remains uncertain.

Transmission Experiments. A series of wild sandflies fed on patients were given opportunity to refeed on 2 rhesus monkeys. Sandflies fed in the laboratory refeed with reluctance. One monkey was bitten by only 2 flies out of 49; the other by 5 out of 50. One monkey died within 4 weeks under circumstances which pre-

vented examination. The other yielded negative blood cultures up to 14 weeks.

Over a period of several weeks, recently captured sandflies were applied to the belly and eyebrows of 2 monkeys. One was bitten by 70 out of 242, and the other by 84 out of 335. Blood cultures up to 4 months were negative.

Two monkeys were inoculated intraäbdominally and endermally on the eyebrow, with suspensions of 10 batches totalling 340 wild females and 5 males. One monkey died within 20 days and was negative. The other received an additional suspension of 70 females and yielded only negative blood cultures up to 4 months.

Summary. Sandflies *(P. verrucarum* and *P. noguchii)* fed on patients showing many bartonellæ in blood films became infected with a bartonella-like organism not as yet identified. A similar infection was found in only one of the wild sandflies examined.

Transmission experiments with sandflies and rhesus monkeys resulted negatively, but were too limited to be conclusive.

9656

A Color Test for Pentoses.

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One drop (0.05 cc.) of a pentose solution (l-arabinose, d-arabinose, xylose or ribose) containing 0.05 mg. or more of the sugar is placed in a test tube. 0.5 cc. of benzidine solution (1 gm. benzidine in 25 cc. glacial acetic acid. This solution keeps for 4 days) is added and the mixture is brought to vigorous boiling. The test tube is then put in cold water. Within a few seconds a very stable cherry red color develops. Glucose, fructose and galactose give yellow to brown colors with the benzidine solution. Even large amounts of hexoses, however, do not interfere with the test. There is only a slight delay in the formation of the red color (0.1 mg. of arabinose and one mg. of glucose). As little as 10 gamma of pentose may be detected. For instance urine specimens containing 0.1 mg. of arabinose per cc. gave a distinct red color when 0.1 cc. of urine was added to 0.5 cc. of benzidine solution. Normal and abnormal urinary constituents do not interfere with the test. Too large amounts of proteins may be removed from pathological urines by adding