Ten species of mosquitoes were found in Huchow, but only 5 species have been used for the experimental purposes because the remaining, namely, *Culex pallidothorax, C. vichnui, C. mimeticus, C. (Lutzia) vorax,* and *C. (Lutzia) fuscana* were either uncommon or did not suck human blood. All mosquitoes, except *M. (Mansonioides) uniformis,* were bred from larvae or pupae in the laboratory. The results of these experiments are given in Table I.

From the table it will be seen that *Anopheles hyrcanus* var. *sinensis* is an excellent intermediate host for *Microfilaria malayi.* Under the room temperature of 29-32°C. (July to August) the microfilariae developed in this species of mosquito quite normally and they reached maturity from the 6th day after the infective meal. Labial infection has been found very frequently beginning from the 6th day. Out of 30 mosquitoes in which mature microfilariae were found, 14 harbored microfilariae in the labium.

Microfilariae reached maturity also in *M. (Mansonioides) uniformis.* But this species of mosquito is probably not as good an intermediate host as *A. hyrcanus* var. *sinensis* since only a small number of the ingested microfilariae completed development. Majority of the microfilariae stopped development from the 5th day and became granular and degenerated.

The other 3 species of mosquitoes, namely, *Culex pipiens, Aedes (Stegomyia) albopictus* and *Armigeres obturbans* are not intermediate hosts of *Microfilaria malayi* since the microfilaria died and disappeared either in the stomach or after they reached the thorax of the mosquito.

7738 P

On the Nature of the Specific Reacting Substance of B. proteus X19 in the Weil-Felix Reaction.

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Through methods commonly employed for the recovery of specific soluble substances (polysaccharides) it is possible to obtain from the X 19 strains of the proteus bacillus specific reactive substances which flocculate with antiproteus and typhus serum, (Lim and Kurotchkin,[1] White,[2] and Castaneda[3]). Castaneda[3] has also shown

[1] Lim, C. E., and Kurotchkin, T. J., *Nat. Med. J. China,* 1929, **15**, 6.

[2] White, P. B., *Brit. J. Exp. Path.,* 1933, **14**, 145.

[3] Castaneda, M. Ruiz, *J. Exp. Med.,* 1934, **60**, 119.

that suspensions of Mexican Rickettsia similarly treated will yield a specific reacting substance which gives the same precipitation reactions with typhus and antiproteus sera as do the polysaccharides extracted from *B. proteus X19*. It appears, then, that there is in *B. proteus X19* and typhus *Rickettsia* a common antigenic complex which is responsible for the Weil-Felix reaction. This paper corroborates the above reports and emphasizes White's findings in regard to the dual antigenic nature of the polysaccharidal substance obtainable from the proteus bacillus.

Thirty-four samples of human typhus sera have been tested against extracts of *B. proteus X19* (0-504 strain). All of these were purposely selected from late or convalescent cases. All of them agglutinated the test organism at titers above 1 to 640 at the time the flocculation tests were carried out.

Fourteen of these sera were tested against *B. proteus* extracts made by White's method (hot NaOH extraction with alcohol precipitation) and also against extracts as prepared by Castaneda according to the method of Heidelberger and Avery[4] which includes extraction in cold antiformin, glacial acetic acid precipitation, precipitation of the supernatant fluid with cold alcohol followed by treatment with ammonium sulfate.

Twenty other samples of serum were tested both with White's extract and extracts made according to Castaneda's second method, that of alcohol precipitation of concentrates of 8-day broth cultures of *B. proteus X19*.

Antigen dilutions from 1 to 10 up to 1 to 10,240 were mixed with equal volumes of the typhus serum diluted 1 to 2. The dilutions were incubated 2 hours at 55°C., followed by 18 hours in an ice box at 13°C.

In all tests flocculation was observed at antigen dilutions ranging from 1 to 2560 as high, in 2 cases, as 1 to 20,560. This demonstrates that extraction methods employed in obtaining polysaccharidal substances from other organisms will remove from *B. proteus X19* a specific soluble substance which flocculates with human typhus sera. Control tests using serum from typhoid, pneumonia and one tularemia case did not result in visible flocculation under the same conditions.

In testing the 3 extracts against antiproteus serum (agglutinating titer 1 to 10,240) we found, as did White, that antiproteus serum did not flocculate with the extract prepared by treating the proteus

[4] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

bacilli with hot alkali. Flocculation did occur with the extracts prepared according to Castaneda's methods.

In a smaller series of tests, White[2] reported essentially the same results. He, therefore, feels that the somatic complex of *B. proteus X19* presents 2 distinct serological factors: one labile in hot alkali and responsible for O agglutination in its own antiserum without having any part in the Weil-Felix reaction; and another stable in hot alkali and responsible for the Weil-Felix reaction.

As to the chemical nature of the reacting substance, Castaneda and White report that this substance gives a powerful Molisch reaction, withstands boiling in alcohol or water and a negative biuret reaction. The extracts we prepared from the 0-504 strain of *B. proteus X19* reacted in the same way.

In using the alkali extract, we observed that it is necessary to adjust the H ion concentration within the limits of pH 7.2 and pH 7.4. Extracts which are too acid or too alkaline are liable to spontaneous flocculation at the incubation temperatures we employed.

We had difficulty in controlling the potency of hot alkali extracts which, although subjected to the same treatment, did not yield as much reactive substance as did similar preparations. We are unable to offer any explanation for this. One-day cultures do not yield as much reactive substance as 3 to 4-day cultures at 37°C.

Our work does not permit us to draw conclusions as to time in the disease when the reacting antibody may appear in the patient's serum. For this study we purposely selected sera which had a high reactive quality as indicated by their agglutinating titer. From the few tests we made with serum taken shortly after the onset of the disease, it would appear that significant flocculating titer parallels that of agglutination.

7739 C

Morphological and Tinctorial Behavior of B. Leprae During its Adaptation to an In Vitro Habitat.

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Much has been written concerning the variations in morphology and amphoteric staining property with respect to acid-fastness for the supposedly cultivated Hansen bacillus of leprosy during that