of the preparation, a field showing abundant spores. Suppose there are five kinds of spores in the mount; assign a letter to each kind. Move the slide from left to right so that the spores appear to be slowly travelling along between the two lines. Call off to an assistant the proper letter for each spore passing by the vertical line within the parallel lines. Let the assistant record the letters on plotting paper conveniently divided off into 50 or 100 squares. This method will enable a record to be made almost as fast as one can talk. Of course, simply counting the letters will give the percentage of each type of spore. This procedure will obviously eliminate some of the experimental error common in "field counts," and is much faster since there is no pause to answer the question, "Have I counted that one before?" The writer is using the method in studying a species of Fusarium.

FRANK P. McWHORTER
VIRGINIA TRUCK EXPERIMENT STATION,
NORFOLK, VIRGINIA

## CULTURE MEDIUM FOR THE CILIATE LACRYMARIA

LACRYMARIA has been observed by numerous biologists who noted its form and structures, "its phenomenal power of elongation, its wonderful elasticity and its great freedom of movement,"[1] but no one has as yet made a thorough study of this remarkable organism. This is no doubt due to its scarcity.

Mast, who has had wide experience in collecting protozoa, says ('11, p. 230): "Lacrymaria is relatively scarce in nature. It is occasionally found in cultures containing decaying aquatic plants but never in great numbers. One rarely finds more than two or three specimens in a drop of solution." And no one has heretofore succeeded in cultivating it in the laboratory. Professor Mast called my attention to this and suggested the following experiments:

Various concentrations of (1) timothy hay, (2) wheat, (2) beef extract and (4) malted milk in distilled water were prepared in two sets, one of which was boiled and the other not. All were seeded with Lacrymaria and examined from time to time for several weeks.

The Lacrymaria died out, without any apparent increase in numbers, in all the cultures except those containing malted milk, 1–5 mgr to 100 cc water. The best growth was obtained in cultures containing 3 mgr malted milk to 100 cc water. In some of these the Lacrymaria became very abundant and continued to thrive for more than six weeks without adding anything to the cultures.

These cultures contained Halteria and another similar organism which was not identified and numerous bacteria. The Lacrymaria were observed to capture Halteria, but they appeared to feed mostly on the other organisms.

Y. IBARA

ZOOLOGICAL LABORATORY,
THE JOHNS HOPKINS UNIVERSITY

[1] Mast, 1911, "Habits and Reactions of the Ciliate Lacrymaria," *Journ. Animal Behavior*, Vol. 1, pp. 229–243.

# SPECIAL ARTICLES

## A PRELIMINARY REPORT ON THE CULTIVATION OF THE MICROBE OF OROYA FEVER

OROYA fever, or Carrion's disease, is a highly fatal infection endemic in certain regions of Peru. Its most striking clinical feature is a rapidly progressing severe anemia, associated with febrile reactions. In the red blood cells of patients suffering from the disease Barton found, in 1905, peculiar bacilliform elements, the specificity of which has been confirmed by Barton's subsequent observations and those of later investigators. Strong, Tyzzer, Sellards, Brues and Gastiaburu concluded that these bodies are of protozoan nature and proposed for them the name of *Bartonella bacilliformis*. Their cultivation, however, had not been achieved, and the problem offered opportunity for the trial of procedures recently developed for the cultivation of certain spirochetes, flagellates and rickettsia-like microorganisms.

In the summer of 1925 one of us (B.) went to Lima and secured the material for study. Through the generous permission of Dr. Olaechea, of the Dos de Mayo Hospital, Lima, blood was withdrawn into citrate solution from a case of oroya fever and brought to the Rockefeller Institute, where the cultural and experimental work has been carried out.

Of the various media employed, including those which had been found suitable for the cultivation of anaerobic treponemata, as well as aerobic media used for the cultivation of the leptospiras, flagellates and rickettsia-like microorganisms, only the aerobic media, solid or semisolid, containing blood or serum yielded growth of *Bartonella bacilliformis*. The initial cultures were obtained both on leptospira medium and on blood agar slants (20 to 30 per cent. of defibrinated horse blood) containing certain carbohydrates. The organism grew in pure condition on the first attempt, and pure cultures have been repeatedly obtained from the original citrated blood. Growth occurred at 37° C. and also at 28° C. within 48 to 72 hours. Subcultures were readily obtained on similar media, and the strain has been maintained in the laboratory since the beginning of October, 1925.

The only change produced in the leptospira medium is a very delicate grayish, often minutely granular, appearance of the surface layer. The zone in which growth occurs gradually becomes evident as a faint gray haze, which, within 10 to 14 days, extends as far as a centimeter below the surface and resembles the appearance of growth of the leptospiras on this medium. The colonies on the surface of blood slants are almost microscopic during the first few days and appear translucent, slightly raised and discrete. They may attain a diameter of 0.2 to 0.5 mm., while still remaining discrete and practically colorless, reaching their maximum size in about three days at 37° C. and seven days at 28° C.

*Bartonella bacilliformis* is a motile organism but this characteristic is lost as the cultures grow older. In form it is minute and pleomorphic, ranging from round, oval and lanceolate to rod shapes. There is a marked tendency for the individuals to clump together in masses of hundreds and perhaps thousands. The organism is Gram negative and stains reddish violet with Giemsa's solution. It varies in width from less than 0.2 to 0.5 μ and in length from 0.3 to 2.5 μ.

Inoculation of cultures of *Bartonella bacilliformis* into young rhesus monkeys induced intermittent fever lasting many weeks. Typical endoglobular forms of *Bartonella bacilliformis* have been demonstrated in the red blood corpuscles of these animals. Intradermic inoculation of the culture into the eyebrows gave rise to highly vascular nodules, resembling the nodules of experimental verruga induced in monkeys by previous investigators. *Bartonella bacilliformis* can readily be recovered in pure culture from the blood, lymph glands, spleen and nodules, and passages from animal to animal are easily carried on.

A detailed report of the foregoing experiments will appear in a forthcoming number of the *Journal of Experimental Medicine.*

HIDEYO NOGUCHI,
TELEMACO S. BATTISTINI
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

## SEASONAL AND REGIONAL VARIATIONS IN CURLY-TOP OF SUGAR BEETS

OBSERVATIONS made during the past season (1925) in various sugar-beet growing areas in the states west of the Rocky Mountains revealed striking contrasts between different localities in the amount of curly-top occurring. In southern California there was more curly-top last year than has occurred before in the eight years during which the fields have been observed. In the Salinas Valley and also in the sugar-beet areas of the Sacramento Valley (even including the delta region which has previously been relatively free from curly-top damage), the damage was comparable to that resulting in the years of worst outbreaks.

By contrast with conditions in California the condition of the crop in the intermountain region of the northwest was the most favorable which has been seen in a number of years. In the Yakima Valley of Washington in 1924 approximately only 25 per cent. of a normal crop was harvested from a large acreage. The losses from curly-top in this valley have been so serious and frequent in occurrence that this past year beet-growing was practically abandoned; three fields, comprising only about twenty acres, were planted this past season. In southern Idaho, where in 1924 somewhat over ten thousand acres were ruined by curly-top, the crop in 1925 was in excellent condition. In 1924 enormous numbers of beet leafhoppers, which are the only known natural agents transmitting the virus of curly-top, invaded the beet fields of this area in the latter part of May and early June. In 1925 there had been no general flight of the insects into the cultivated areas, at least up to July 1. In Utah there was this year relatively little curly-top damage, whereas the only previously recorded outbreak of curly-top in Utah which can be compared in seriousness with that of 1924 occurred in 1905.

The observations which have been given when considered in connection with the climatic conditions which prevailed during the winters of 1923–24 and 1924–25 strongly support the idea that the abundance or scarcity of beet leafhoppers and presumably also the amount of curly-top disease in the sugar beet fields is determined by the climatic conditions of a given area rather than that the severe outbreaks occur simultaneously throughout the range of the insect at periodic intervals.

In the northwest area the winter of 1923–24 was relatively mild and the precipitation was decidedly below normal. Undoubtedly this allowed a large proportion of the leafhoppers to survive the winter and forced them to leave the drying vegetation of the deserts early in the spring. The winter of 1924–25 was severe so that probably a relatively small proportion of the insects survived. The precipitation was relatively high and continued late into the spring so that favorable conditions in the natural breeding grounds delayed the movement of the insects into the cultivated areas.

In the lower half of California, on the other hand, which includes the principal natural breeding areas of the insect in the state, both the winters referred to were mild and with subnormal rainfall. The seasons of 1924 and 1925 were both characterized by outbreaks of the leafhopper and curly-top, though the