

stinct in the second generation occurred. It is interesting that vitamin B₁ in amounts equivalent to 40 times the maintenance requirement seems to have a similar effect in interfering with the capacity of the mother to rear her young and the nursing instinct as does a relative insufficiency of this vitamin. This should in no way discourage the generous use of vitamin B₁ in the diet of human beings, as the difference between the optimal requirement and the amount which may prove toxic after several generations is considerable. The excess amount given to our rats would be equivalent to the administration of between 5,000 and 10,000 I.U. of vitamin B₁ per day for several generations (assuming that the vitamin B₁ requirement of humans is between 100 and 200 units per day).

I am indebted to Mead Johnson for the supply of brewer's yeast for these and other experiments, to Eli Lilly for vitamin B₁ concentrate, and to Winthrop Chemical Company for the generous supply of Betaxin (synthetic vitamin B₁).

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Lysis of *Vibrio comma* by Bacteriophage and by Immune Serum.*

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The very simple idea that a bacteriophage may be a minute particulate living being capable of growing and multiplying within a larger living cell and able there to produce soluble enzymes which may act upon the bacterial structure and may eventually diffuse in solution through the medium has been postulated by d'Herelle and has been supported by ever-increasing experimental evidence assembled by his disciples. Certainly this conception is in accord with what we know about larger organisms such as the coccidia, the malarial plasmodia, and the yeasts. The morphological observations upon the bacteriophage particles have been technically difficult and the results not wholly satisfactory. The study of other filterable viruses has met with similar difficulty.

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Castaneda,¹ by a special staining technic, has been able to demonstrate quite clearly the minute rod-like Mooser bodies that develop on the serous tunic of the guinea pig's testis after inoculation with Mexican typhus fever and the resulting pictures are so definite that one is inclined to accept these minute rods as microbes and as the probable microbic cause of the disease.

In the present study the Austria strain of *Vibrio comma* was subjected to lysis by immune serum in one series of tubes and in a companion series it was subjected to lysis by a bacteriophage, kindly sent to us by Lieutenant Colonel J. Morison, I.M.S., of the Pasteur Institute at Shillong, Assam, India. At intervals of 15 to 30 minutes the bacteria were thrown down by centrifuge and the sediments were stained by Castaneda's technic.

In the microscopic study of the stained preparations from the serum-series a considerable amount of debris, apparently derived in part from the serum, was found associated with the bacterial cells undergoing lysis. The bacterial cells showed variation in intensity of staining but differentially stained granules were not recognized in any of these bacterial cells. In general the lysis by serum seemed to progress without any marked alteration in the size of the bacterial cells, although occasional large cells were seen in the serum-series as also in the controls.

In the bacteriophage-series the macroscopic appearance indicated that lysis was progressing at approximately the same rate as in the serum-series. The microscopic study of the sediments, however, disclosed striking differences. At the end of 90 minutes the bacterial cells exposed to phage were in many instances distinctly swollen and this feature became progressively more exaggerated up to 3½ hours. Along with these enormously enlarged bacterial cells there also occurred very minute bacteria, much smaller than normal and in remarkable contrast to the gigantic cells alongside. Perhaps the most interesting features were found in the changed internal structure of the bacterial cells and especially the intracellular granules which stain a deep blue color with the Castaneda stain. These granules are so minute as to appear as nebulous collections in some parts of the pale red bacterial protoplasm. Sometimes they are assembled in compact clumps a micron in diameter. Their distribution is irregular.

The possibility that these granules represent the particles endowed with the property of reproduction and with the property of secreting the lytic enzymes of the bacteriophagic principle is worthy of careful consideration.

¹ Castaneda, R., *J. Infect. Dis.*, 1930, **47**, 416.