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Formation of Large Bodies in a Member of the Genus *Bacillus*

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Cultures of a spore-forming *Bacillus* of the brevis-polymyxa group were examined on thin blocks of serum agar. A Bausch & Lomb phase contrast microscope attachment was used on a Leitz "Panphot" microscope with a 97× objective and a 15× ocular. Time-sequence pictures were taken with Kodak Microfile film. "Large bodies" (of the Dienes and Klieneberger-Nobel type) were often found in great numbers in cultures that had completed logarithmic growth. Such large body formation continued for one or two days afterward. Fig. 1 shows pictures of the same

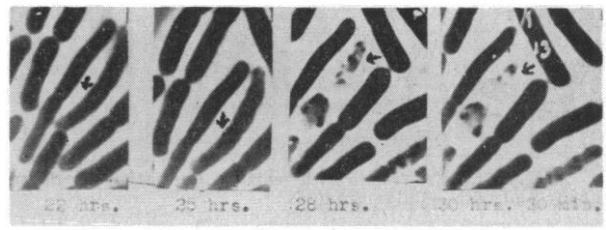

FIG. 1. Formation of two large bodies from a single rod.

cell taken at the indicated time intervals. It demonstrates the direct transformation of a rod into two separate large bodies, one of them rodlike in shape and the other ovoid. Both elements show clearing of the cellular material and abundant granules. These granules were highly motile, and their movement appeared to follow a definite pattern, like a chain that is pulled from one end. This movement was observed for periods of time up to 48 hr, after which it gradually ceased. The granules were also found outside the cells, swimming freely on the agar, singly or in chains. Their fate could not be determined by direct observations. In Fig. 2, the cell labeled *1* shows a central swelling that is followed by swelling of the rest of the cell, with the formation of a round, ovoid-shaped large body. Cell 2 also has undergone a change into an element that is similar to, but smaller than, the preceding. Cell 3 developed into a rod-shaped element of similar structure. In Fig. 3, the culture was transferred from a 24-hr culture that showed an abundance of large bodies. Over a period of 30 min the cell undergoes a clearing and shows granules; granules are also seen outside and adjacent to the cell.

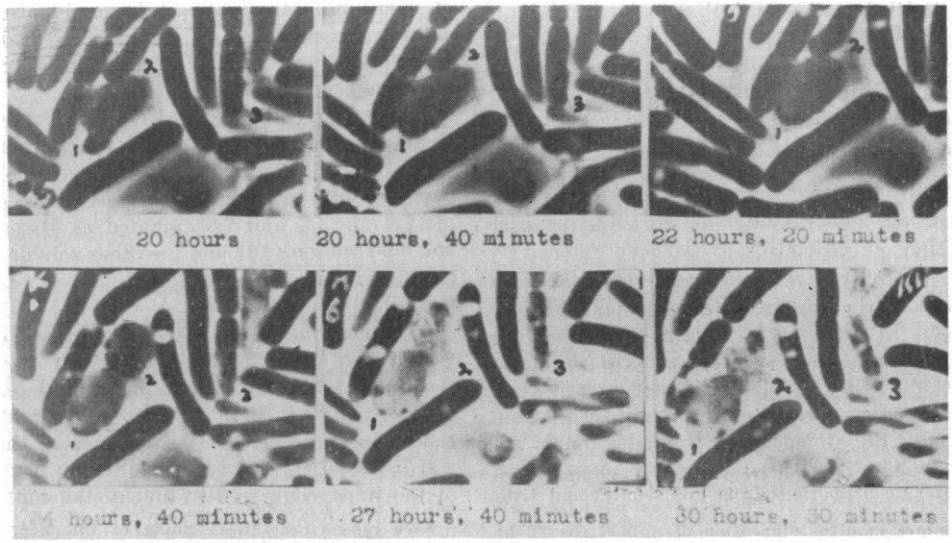

FIG. 2. Formation of balloonlike large bodies from rods (*1* and *2*) and of rodlike elements (*3*).

The formation of large bodies was observed to occur

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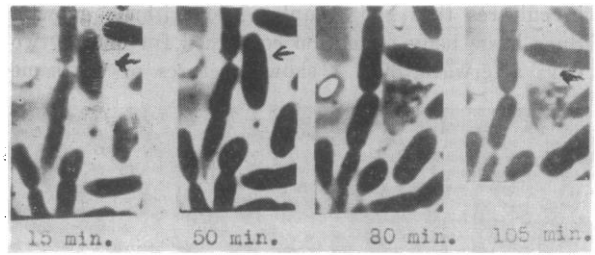

FIG. 3. Transfer from a 24-hr culture. Formation of a large body and extracellular granules.

in this fashion in a great number of cultures. No evidence of fusion was found, as described for *Proteus* (*1*). The development of these elements was not determined, since they remained unaltered for periods up to 3 weeks, except for budding in a few instances. No cell-wall stains were made to determine whether fusion between different cells of the same rod took place. It appears that in this organism the formation of large bodies follows a pattern of direct transformation from rods to either balloon-shaped or rodlike structures. They are more like the ones described for *Azotobacter agile* (*2*) than for other gram-negative bacilli. No evidence of the events described by Klieneberger-Nobel (*3*) was found here.

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Prevention of Ulcers in the Shay Rat by Ox Bile[1]

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As part of our general plan of work on the cure and prevention of gastric ulcers we have extensively studied (*1*) the preventive action of certain salts of organic acids on the formation of stomach and esophageal ulcers in the Shay (*2*) rat.[2] We came to a clear-cut conclusion that certain salts, particularly potassium acid acetate, display a marked curative and preventive action when given per os, in a dose of about 75 mg. Usually the stomachs of treated animals are coated with a mucuslike lining, and the action is independent of the alkalinity of the salts, excellent results being obtained with material given at pH 4.5.

In our second communication (*3*) we studied the role of hematuria and pigmented urines in the causation of ulcers. The relationship of the two phenomena, and the finding that practically no ulcers were observed in animals having hematuria or pigmented urine, suggested a possible role of bile in ulcer prevention. Madden, Ramsburg, and Hundley (*4*) reported that ligation of the esophagus, ureters, or the bile duct resulted in gastric ulcer prevention. Our inquiries among several internists as to whether icterus is associated with ulcer remissions yielded no answer.

Our experiments with bile were started with fresh ox bile and later continued with a USP bile powder preparation and with some isolated fractions and purified bile acids obtained from fresh bile. The experimental methods and the formulation of the "ulcer index" in this study were identical with those of our previous communications.[3]

The bile fractions from fresh bile were prepared as follows. Bile, normally alkaline, was neutralized to pH 7.0 with dilute sulfuric acid, and evaporated *in vacuo* to dryness. The residue was dissolved in alcohol, and a small amount of insoluble material (mostly mineral) was filtered off. Benzene was added to the filtrate. After it was allowed to stand at $-10°$ C overnight, an insoluble material (Bile-3) was filtered off. The filtrate (Bile-4) was evaporated and dried *in vacuo*. Bile-4 was extracted with ethyl acetate, and the soluble fraction precipitated with ether (Bile-11). Concentrated sulfuric acid was added to the ether soluble. A precipitate formed (Bile-12), which was separated off by centrifugation. In view of the possible preventive action of some mineral organic acid combinations, the ash content of our fractions was estimated and found to be below 8%, a quantity entirely insufficient to exert any beneficial effect.

The commercial bile powder was exhausted in a large Soxhlet, allowing a cold extraction with ethyl acetate. The extract, sterol material (13) free from bile acids was tested, the extracted powder was dissolved in methyl alcohol and fractionated as Ba-salts, one insoluble fraction (14) and the other soluble (15). These salts were decomposed quantitatively with sulfuric acid.

The material to be tested was given by mouth in two divided doses totaling 50–150 mg, the first one at the time of ligation, the second 3 hr later. The total dose was given in ⅛ ml volume, the amount of wastage thus being minimized. The presence of the bile material in the stomach was evidenced by heavy milkiness of the juice, compared to that of the controls.

[1] Work supported by a grant from the U. S. Vitamin Corp. and by numerous contributions from this country and Latin America.

[2] The procedure involves starvation of animal for 72 hr, then ligation of pylorus under ether anesthesia. The abdominal wall is clipped and coated with collodion to prevent licking of blood. Animals are sacrificed 8 hr after ligation and stomachs removed. Stomach contents are collected, and the washed stomach is stretched upon a white surface for examination. Some animals die from perforated ulcers before 8 hr.

[3] We repeat here the formula of the ulcer index: Each ulcer animal is graded from 0 to 4, according to the extent of ulcerations, perforations, and time of survival, 4 being maximum ulceration. The individual ratings for each group of animals are added, multiplied by 100, and divided by the number of rats in the group, so that: $\frac{\text{Ulcer} \times 100}{\text{No. of rats}} = \text{ulcer index}$. Maximum, therefore, is 400.