ON THE ORIGIN AND FATE OF THE FATTY INCLUSIONS IN A STRAIN OF BACILLUS CEREUS[1]

OF the spore-forming bacteria included in the genus *Bacillus,* there are some which deposit fatty inclusions in their cells. Among these is *Bacillus cereus.* The cells of this species are relatively large (width usually about 1.7 μ), and the fatty inclusions are clearly visible in the living cell without the necessity of staining. Consequently, it should be possible to ascertain the locus, in the living cell, where these fatty inclusions are formed. This we have done with strain C_3 of *Bacillus cereus* frequently used by us in other studies.

The technique of growing bacteria aerobically under optical conditions suitable for microscopic observations has been previously described by the author.[2]

Starting with endospores, it can be easily seen that the progeny consists, during the normal period of culture growth, of cells optically homogeneous except for the cytoplasmic membrane and its extensions which divide the inner protoplasm into compartments; these ultimately become independent cells. It can be easily seen that the inner outline of the cytoplasmic membrane is not a smooth curve but is finely jagged.

Toward the end of the growth phase, the inner surface of the cytoplasmic membrane begins to show protuberances which, under aerobic conditions, soon break off and move into the cytoplasm. Under anaerobic conditions, however, the protuberances remain attached to the cytoplasmic membrane.

At the time of spore formation, each cell usually contains several granules (mostly 3 to 5). Those granules stain deeply with Sudan black B, give the Sharp test for protein, and a positive Feulgen reaction. Those reactions are also given by the cytoplasmic membrane from which they originate, and indicate similarity, if not identity, of chemical composition of the two structures. In view of the fact that we observed no other type of intracellular granules in the organism studied, it seems probable that these inclusions are, at least in some cases, identical with the "nuclei" described by various investigators in the *B. megatherium* – *B. cereus* group. Recently it was also reported by Imšenecki[3] that the fatty granules observed in the aerobic sporeformers may, under certain conditions, stain with basic dyes and have been mistakenly considered nuclei.

The function of these fatty granules is not yet clear to us. Under the conditions of this investigation, they are used up neither by the growing or starving vegetative cell nor during the formation of the spore. Indeed, we have been able to induce spore formation in cells free of any inclusions and, when inclusions were present, we found no evidence that any one is enclosed in the endospore. In this organism, the endospore is formed by a process identical to that described for *Bacillus megatherium* by Bayne-Jones and Petrilli.[4] Furthermore, young spores give homogeneously positive Feulgen reaction, and only mature spores show an internal positive granule or rod which may indicate differentiation, although the possibility of shrinkage of the spore protoplasm upon maturation should also be considered. After the completion of the endospore, the inclusions persist in the sporangium, apparently intact, for several hours, then gradually disintegrate. Often the sporangium disintegrates before some of the granules, and these are liberated with the endospore. If the young sporangium, soon after the completion of the endospore, is transferred together with viable vegetative cells to a fresh medium, the endospore does not germinate and the sporangium and inclusions are preserved, without visible change, until the new culture has again passed the stage of sporulation.

Previous investigators have considered these inclusions to be reserve material. The present investigation seems to indicate that they are the result of a break-up in the cytoplasmic membrane and its extensions, and may represent an abortive tendency of the cell to divide.

The details and records of this work will be published elsewhere.

GEORGES KNAYSI
CORNELL UNIVERSITY

[1] Accepted for publication, July 10, 1945.
[2] G. Knaysi, *Jour. Bact.,* 40: 247, 1940.
[3] A. Imšenecki, *Jour. Bact.,* 49: 1, 1945.
[4] S. Bayne-Jones and A. Petrilli, *Jour. Bact.,* 25: 261, 1933.

THE RELATIONSHIP OF THE AGENT OF HEART-WATER FEVER—RICKETTSIA RUMINANTIUM

ALTHOUGH the agent of heart-water fever has been classified with the Rickettsiae, under the name *Rickettsia ruminantium,*[1] there is reason to question the validity of this classification. It is true that the agent is transmitted normally by ticks, but morphologically it is entirely dissimilar from other Rickettsiae and, moreover, it has proven susceptible to sulfonamide chemotherapy.[2] This latter characteristic would serve to associate it with certain agents of the lymphogranuloma-psittacosis group of agents[3] and morphologically also there are points of resemblance. Thus,[1, 4] in the endothelial cells of the blood vessels appear vesicles filled with characteristic

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[2] W. O. Neitz, *Jour. South African Vet. Med.,* 11: 15, 1940.
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[4] E. V. Cowdry, Part I, 11th and 12th Repts., Dir. Vet. Educ. and Res., Union of S. Africa, p. 181, 1927.