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.

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[1] Accepted for publication, July 10, 1945.
[2] G. Knaysi, *Jour. Bact.,* 40: 247, 1940.
[3] A. Imšenecki, *Jour. Bact.,* 49: 1, 1945.

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

[4] S. Bayne-Jones and A. Petrilli, *Jour. Bact.,* 25: 261, 1933.
[1] E. V. Cowdry, Part I, 11th and 12th Repts., Dir. Vet. Educ. and Res., Union of S. Africa, p. 161, 1927.
[2] W. O. Neitz, *Jour. South African Vet. Med.,* 11: 15, 1940.
[3] G. Rake, H. Jones and C. Nigg, *Proc. Soc. Exp. Biol. and Med.,* 49: 449, 1942.
[4] E. V. Cowdry, Part I, 11th and 12th Repts., Dir. Vet. Educ. and Res., Union of S. Africa, p. 181, 1927.

elementary bodies and some larger coccoid forms, which have the morphology and the tinctorial characters of agents of the lymphogranuloma-psittacosis group.

It is true that a careful study of the agent of heart-water fever as observable in intima smears, by individuals accustomed to the agents of the lymphogranuloma-psittacosis group, revealed that certain morphological structures, particularly the ring forms,[5] predominate so among the different morphological forms of the agent as to distinguish it from those of the lymphogranuloma-psittacosis group. Moreover, Cowdry[4] and Jackson[5] both mention bacillary forms which never occur in the agents of the latter group. Nevertheless, the morphological and chemotherapeutic similarities are so great as to suggest to the present authors that further inquiry into a possible relationship should be made.

That relationship among members of the lymphogranuloma-psittacosis group of agents is not limited to morphology and tinctorial characters has been demonstrated by the cross reactions found to occur in the complement fixation test.[6,7,8,9] Sera from 5 cases of heart-water fever in sheep were collected by one of us (R. A.) and tested for complement-fixing activity with an antigen prepared from the agent of lymphogranuloma venereum growing in the yolk sacs of embryonated chicken eggs.

On the occasion of first testing the heart-water fever sera, these were all found to be anticomplementary. A serum from a known case of lymphogranuloma venereum, used as a control in the test, gave fixation at a dilution of 1:160. All other controls were satisfactory. Before retesting, all sera, including that from the known case of lymphogranuloma venereum, were heated at 60° C for an hour on two consecutive days. This procedure rendered all the sera free from anticomplementary action even at a dilution of 1:2 except one which was not anticomplementary at 1:5. When these sera were now tested none of them gave any evidence of fixation of complement even at the highest concentration. The anti-lymphogranuloma serum which had been exposed to the same treatment at 60° C still gave fixation at a dilution of 1:160.

It is clear then that these sera from sheep, that had reacted to heart-water fever 39, 66, 71, 110 and 110 days earlier, respectively, had no antibodies capable of fixing complement in the presence of the agent of lymphogranuloma. This would suggest a lack of antigenic relationship between the agent of heart-water fever and those of the lymphogranuloma-psittacosis group. However, this is not necessarily the case since, as Eddie and Francis have pointed out,[10] the serum of pigeons infected with meningopneumonitis, or at least giving complement fixation with this agent, failed to give cross reaction with lymphogranuloma antigen. Such a species peculiarity could theoretically exist in sheep and account for the results.

It would seem most probable that the agent of heart-water fever, while not distinctly either a Rickettsia or a member of the lymphogranuloma-psittacosis group, is related to both. The relationship of the Rickettsiae and the lymphogranuloma-psittacosis group of agents even in morphology[11] is becoming more and more clearly recognized.

SUMMARY

Sera from sheep which were infected with heart-water fever from 39 to 110 days before the serum was withdrawn failed to fix complement in the presence of lymphogranuloma venereum antigen.

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THE COMPARATIVE ANTIFOULING EFFICACY OF DDT

CONSIDERABLE publicity has resulted from the recent announcement[1] that experimental paints containing DDT (2,2-bis(chlorophenyl)-1,1,1-trichloroethane) showed positive suppression of fouling by Balanus species on panels exposed for from three to six months in Yaquina Bay (Oregon). It is perhaps unfortunate that the average reader automatically associates efficacy against barnacles with a "cure-all" for the fouling of ships' bottoms. This is of course untrue.

The United States Navy, for example, in its Docking Report Manual[2] describes at least eight different phyla and classes of marine flora and fauna known to contribute importantly to the fouling phenomena. Thus:

[5] C. Jackson, 12th Rept., Dir. Vet. Serv. and Anim. Indust., Union of S. Africa, p. 161, 1931.
[6] G. Rake, M. D. Eaton and M. F. Shaffer, *Proc. Soc. Exp. Biol. and Med.*, 48: 528, 1941.
[7] G. Rake, M. F. Shaffer and P. Thygeson, *Proc. Soc. Exp. Biol. and Med.*, 49: 545, 1942.
[8] J. A. Baker, *Jour. Exp. Med.*, 79: 159, 1944.
[9] C. Nigg and M. D. Eaton, *Jour. Exp. Med.*, 79: 497, 1944.
[10] B. Eddie and T. Francis, Jr., *Proc. Soc. Exp. Biol. and Med.*, 50: 291, 1942.
[11] A. M. Begg, F. Fulton and M. van den Ende, *Jour. Path. and Bact.*, 56: 109, 1944.
[1] SCIENCE, 102: 2640, 10, August 3, 1945.
[2] Bureau of Ships, Navy Department, Washington, D. C., "Docking Report Manual," 1942.