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The action of the lytic principle on capsulated bacteria.

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Since capsulated bacteria, or bacteria in the capsulated state, are known to be more resistant to the action of harmful agents than are organisms without capsules, the question of the resistance of typically capsulated bacteria to the lytic principle is of interest.

The cultures employed in the experiments to be reported were as follows: (1) A laboratory stock strain of the Friedländer pneumo-bacillus; (2) a strain of the same freshly isolated from the root canal of an infected tooth; (3) a laboratory stock strain of *B. ozenæ;* (4) a laboratory stock strain of *B. rhinoscleromatis.* The culture medium was a beef infusion broth and beef infusion agar, pH = 7.8. From each of these cultures in the presence of sewage-contaminated river water an active lytic agent was isolated by the usual methods of alternate feeding and filtration.

The maximum dilution of the lytic filtrates which gave inhibition of the homologous culture (one loop in broth) was about the same as in the case of the Shiga dysentery lytic principle, namely, 10^{-8}. Beyond this point the titer could not be raised and sometimes it did not attain this degree.

The lytic filtrate developed from the laboratory strain of Friedländer was effective in causing the inhibition and lysis of the strain from the infected tooth, but not against *B. ozenæ* or *B. rhinoscleromatis.* In a similar manner, the lytic filtrate developed against the tooth strain of Friedländer was active against the laboratory strain but not against the others. The lytic filtrates from *B. ozenæ* and *B. rhinoscleromatis* were not reciprocal in action; neither did they influence the Friedländer strains.

In the case of all the lytic filtrates acting, in proper dilution, on their homologous strains on agar surfaces, lytic areas were formed. These were usually smaller than the typical Shiga lytic areas and showed a tendency toward irregular shape rather than round. In addition, and particularly in the case of Friedländer,

there was some overgrowth of the lytic spots by the surrounding culture. For this reason the cultures sometimes came to resemble normal cultures in respect to their freedom from erosions.

Both of the Friedländer strains mentioned above were dissociated into the pure S- and R-components by the usual methods. The S-type much resembled the mother culture while the R-type was quite different. The original cultures were mucoid, both in broth and on agar, and stained preparations from both showed heavy capsules. After dissociation, the S-type showed similar characteristics. The R-type, on the other hand, grew in infusion broth and on agar with no sign of mucoid characteristics and, in stained preparations showed no capsules. In young, agar slant cultures, while the S-type was heavy and opaque, the R-type was thin and translucent. After a few days growth, however, the two types came to resemble each other superficially, but the difference with reference to capsules remained; the S-type showed good capsules while the R-type showed none. Up to the present time the R-type has indicated no tendency to assume capsule formation.

Against the S- and the R-types of both strains of the Friedländer bacilli active lytic filtrates were developed from sewage contaminated water. The lytic agent developed as readily against the capsulated S-type as against the non-capsulated R-type, and both filtrates attained about the same power of inhibition and lysis on the homologous culture. Moreover, the lytic filtrate developed against the S-type produced both inhibition and lysis of the R-type and vice-versa. Both filtrates showed the same action on the original stock cultures of Friedländer and in about the same degree. Comparing the action of the Friedländer filtrates with that of the typical d'Herelle bacteriophage on Shiga cultures the only noteworthy difference observed was a slower action of the Friedländer filtrates.

When a few loops of concentrated Friedländer lytic agent were applied to the surface of agar slants and the slants then streaked with broth culture (either R- or S-type) no growth took place for a day or more. Eventually, however, the customary secondary growth appeared in the form of small colonies of the correlated resistant strain. These colonies (SR- and RR-types) differed from the original Friedländer colonies and also from the S- and the R-colonies. Moreover, the SR-colonies differed from

the RR-colonies. Neither the SR- nor the RR-cultures were susceptible to inhibition or lysis by the original, the S- or the R-lytic filtrates. Thus, by the combined action of dissociation and the lytic principle, the Friedländer cultures were split into at least four sub-types. The biological characteristics of these have not yet been fully studied.

In conclusion it may be said that, at least in the case of the Friedländer bacillus, as also in the instance of *B. ozenæ* and *B. rhisclerom atis,* there is no evidence that capsule formation offers any hindrance to the inhibitive or the lytic action of the bacteriophage. This conclusion confirms the observation of Paul Caublot[1] who has also recorded the existence of a lytic agent for the pneumo-bacillus.

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Studies on the state of the serum calcium.

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In low phosphorus rickets, the animal is unable adequately to utilize its calcium in spite of the fact that the blood contains a normal amount of calcium. In low calcium rickets the total calcium of the blood is low but, as shown by the addition of the fat soluble organic factor, the diet supplies enough calcium to provide for a normal amount in the blood. These facts make it seem of importance to determine the ratio of diffusible and colloidal serum calcium in the blood in the two conditions.

Three litters of rabbits were used, as indicated in Table I. The diffusible serum calcium was separated by negative pressure filtration through a collodion membrane as described by Moritz.[1] The rabbits were fed through a stomach tube twice daily throughout

[1] *Compt. rend. Soc. de Biol.,* 1924, xc, 622.

[1] Moritz, Alan R., The Effect of Ultra-Violet Irradiation on the State of the Serum Calcium. *J. Biol. Chem.,* 1925, lxiv, 81.