THE EFFECT OF SOME OF THE CHEMICAL CONSTITUENTS OF TUBERCLE BACILLI ON THE PROTOPLASM OF AMŒBA DUBIA.*

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PLATE 5.

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The successful isolation of various chemical constituents from tubercle bacilli by Johnson and Coghill (1) and Anderson (2, 3) has afforded an opportunity of using these substances in biological experiments. Recently Sabin and Doan (4) have tested the chemical fractions on rabbits and found distinctly different reactions to these components on the part of the clasmatocytes and monocytes.

The action of the derivatives from the tubercle bacilli on *Amœba dubia* was studied with the micrurgical technique, the same procedure being used that has been described previously (5). Although there is no evidence that fresh water amebæ are associated with tubercle bacilli, the use of such material was considered desirable because the ameba represents a simple cell upon which many data have been gathered in this laboratory with respect to its reaction to various chemicals.

Immersion Experiments.

Protein.—Amebæ were immersed in suspensions of proteins 304, 304-A (from unautoclaved, defatted organisms), 304-B (from autoclaved, defatted organisms), 308, and Dr. Florence B. Seibert's culture medium protein.[1] These suspensions were brought with NaOH to a hydrogen ion concentration varying from pH 7 to pH 8.2, depending

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upon their maximum solubility. As controls, suspensions of serum albumin were used. Amebæ, placed in such suspensions, and kept in the ice box, showed no change in motility, in ability to produce pseudopodia, or in longevity, compared to amebæ kept under normal cultural conditions for at least 5 days. This innocuous effect of the proteins is true even though some denaturation occurs. If the containers are permitted to remain at room temperature and the proteins become more quickly and completely denatured the amebæ lose their pseudopodia, their plasmalemmæ become stiff, and they become very sluggish.

Phosphatides.—Amebæ were immersed in emulsions of the phosphatides A-3 and A-4. The resulting hydrogen ion concentration varied between pH 5.5 and 6. Amebæ immersed in emulsions of A-3 died in 1 day in concentrations of 0.01 per cent, in 2 days in 0.003 per cent, and were not able to exist in a normal state for 5 days until a dilution of 0.0015 per cent was reached. In A-4 amebæ died in 3 days in a 0.006 per cent emulsion and were living and well for at least 5 days in a 0.003 per cent emulsion.

The manner of death of amebæ in the phosphatide emulsions is characteristic of that due to surface-dispersing action, such as is caused by soaps (6). A normal ameba (Fig. 1) is characterized by a distinct plasmalemma, varying degrees of pseudopodial formation, and, as judged by the rate of the movement of the granules, an actively flowing endoplasm and a more quiescent ectoplasm. When amebæ are placed in emulsions of phosphatide derived from tubercle bacilli, the plasmalemma becomes sluggish and unable to flow even though the cytoplasm is in active motion. The cell, therefore, forms no pseudopodia and becomes round. Gradually the plasmalemma loses its form, breaks in places, and disappears as though it were dissolved, leaving the naked protoplasm as solidified debris (Fig. 2).

With emulsions of lecithin and of lecithin and cholesterol as controls no evidence of toxicity was seen when amebæ were immersed in as strong a mixture as 0.06 per cent, which is the strongest emulsion compatible with visibility of the amebæ in such a thick suspension.

Fatty Acid.—The fatty acid derived from the phosphatide is immiscible with the aqueous medium containing amebæ. If, however, amebæ are brought to an adjacent drop of fatty acid with the micro

needle, the plasmalemma of the cell which is in contact with the fatty acid quickly disappears.

If the ameba is pushed back into the water the plasmalemma is reformed. In one case an ameba was snared rapidly out of the aqueous medium into the fatty acid droplet and the plasmalemma disappeared instantly. When this acid is dissolved in 95 per cent alcohol, it gives an indication of having a pH between 2.8 and 3.0.

Polysaccharide.—Amebæ immersed in a 1 per cent aqueous solution of the polysaccharide A-8 (pH 7 with NaOH) derived from tubercle bacilli show no toxic effects even after 4 days. The growth of fungi makes longer observations impossible.

Injection Experiments.

Protein.—Injections of moderately large amounts ($\frac{1}{3}$ of an ameba full) of suspensions of 304 and 308 render the amebæ sluggish for about 5 minutes. Smaller amounts ($\frac{1}{4}$ of an ameba full) have only slight effect and larger injections ($\frac{1}{3}$–$\frac{1}{2}$ of an ameba full) usually cause quiescence and death. Injections of serum albumin and of Dr. Seibert's culture medium protein were relatively without effect upon the amebæ. Fig. 3 shows an ameba injected with protein 304. All the granules stand out distinctly, indicating almost complete quiescence, as contrasted with the blur seen in the normal ameba represented by Fig. 1, indicating rapid movement during the time of photographic exposure.

Phosphatides.—Injections of phosphatide emulsions have no other effect than that seen if the solvent alone is injected.

Fatty Acid.—This substance is not readily miscible with protoplasm. When injected it forms a discrete sphere in the cytoplasm. Usually the ameba relegates the droplet to the rear and appears to be in the process of pinching it off with the surrounding protoplasm. Before this can be accomplished, however, the plasmalemma in the vicinity of the drop disappears. If a sufficiently large drop of fatty acid is injected, the plasmalemma of the entire ameba can be made to dissolve. If the nucleus approaches the drop, it becomes hyaline. In one case the nucleus flowed into the fatty acid droplet and became hyaline.

Polysaccharide.—Injection of as strong a solution of polysaccharide

A-8 as 1 per cent in large amounts had no more effect upon the ameba than injection of water.

DISCUSSION.

The action of the chemical constituents derived from tubercle bacilli upon amebæ may give little indication of the exact physiological responses of other cells to these bodies. But the general effect of these bacterial substances on any particular part of the ameba gives a suggestion of the probable chemical reaction involved. It is interesting to note that the phosphatide and fatty acid act upon the surface of the cell and the protein affects the interior. The polysaccharide has no apparent action on the normal ameba.

One function of the apparently non-toxic polysaccharide, as far as its chemical significance is concerned, may be to make the fatty acid miscible with protoplasm when these two substances are combined as a phosphatide. What other functions, either chemical or immunological, the polysaccharide may have is beyond the scope of these experiments.

It is of interest to consider the possible relation this work may have to the action of the whole organism on the cell. If the phosphatide fraction is in the waxy outer coat of the organism and this is miscible with the plasma membrane of the cell, it is easy to conceive how the bacillus can penetrate into the cell. If the outer covering of the organism is digested once the bacterium is engulfed, the protein may be free to act on the internal protoplasm.

In these experiments there is no direct evidence that the substances used have some specific effect by virtue of their derivation from the tubercle bacillus. As a matter of fact, except for their slower rate of action, the phosphatide and the fatty acid act as all plasma membrane solvents such as soaps (6), CO_2, or lactates (7). There is some evidence that the proteins 304 and 308 are more toxic upon the internal cytoplasm than serum albumin or culture medium protein. But Dr. Seibert (8) finds that her protein gives a 5+ skin reaction as contrasted to a 3+ with 304. This question of specificity of action, therefore, needs further investigation. These experiments aim merely to indicate the general chemical reactions of the derivatives used upon a simple cell.

CONCLUSIONS.

1. Protein fractions derived from tubercle bacilli are toxic to the interior of *Amœba dubia* but have no action on the plasmalemma.

2. Phosphatide fractions dissolve the plasmalemma but have no effect on the internal cytoplasm.

3. The fatty acid fraction has a marked solvent action on the plasmalemma when brought in contact with the surface of the cell. When injected it may slowly penetrate the cytoplasm to dissolve the contiguous plasmalemma.

4. The polysaccharide fraction has no effect upon the surface membrane or upon the internal cytoplasm of *Amœba dubia.*

BIBLIOGRAPHY.

1. Johnson, T. B., and Coghill, R. D., The chemical analysis of tubercle bacillus, *Tr. 22nd Ann. Meeting, Nat. Tuberc. Assn.*, 1926, 277.
2. Anderson, R. J., The separation of lipoid fractions from tubercle bacilli, *J. Biol. Chem.*, 1927, lxxiv, 525.
3. Anderson, R. J., A study of the phosphatide fraction of tubercle bacilli, *J. Biol. Chem.*, 1927, lxxiv, 537.
4. Sabin, F. R., and Doan, C. A., The biological reactions in rabbits to the protein and phosphatide fractions from the chemical analysis of human tubercle bacilli, *J. Exp. Med.*, 1927, xlvi, 645.
5. Chambers, R., and Reznikoff, P., Micrurgical studies in cell physiology. I. The action of the chlorides of Na, K, Ca, and Mg on the protoplasm of *Amœba proteus*, *J. Gen. Physiol.*, 1927, viii, 369.
6. Reznikoff, P., Micrurgical studies of soaps, glycerine, dextrose and ethylene glycol on *Amœba proteus*, *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 380.
7. Reznikoff, P., and Chambers, R., Micrurgical studies in cell physiology. III. The action of CO_2 and some salts of Na, Ca, and K on the protoplasm of *Amœba dubia*, *J. Gen. Physiol.*, 1927, x, 731.
8. Seibert, F. B., personal communication.

EXPLANATION OF PLATE 5.

FIG. 1. A normal ameba. Blur in center indicates rapidity of motion of endoplasm; ectoplasm moving more slowly; distinct plasmalemma.

FIG. 2. Effect of immersion of amebæ in phosphatide emulsion. Lower ameba rounded, plasmalemma quiescent and beginning to disappear, internal cytoplasm active. Upper ameba, plasmalemma dissolved, dead debris left.

FIG. 3. Effect of injection of protein fraction into ameba. Quiescence of internal cytoplasm.

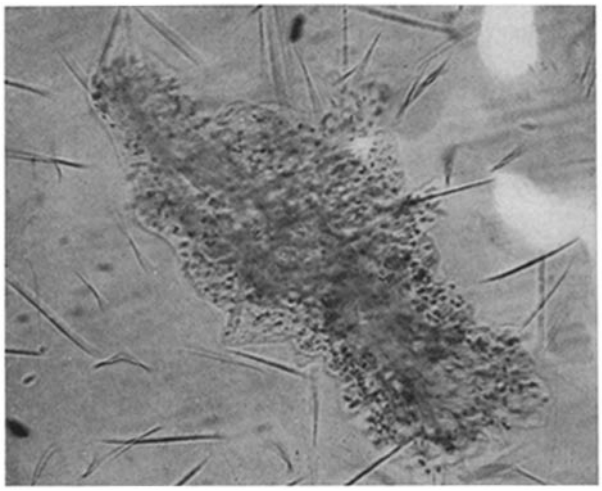

FIG. 1.

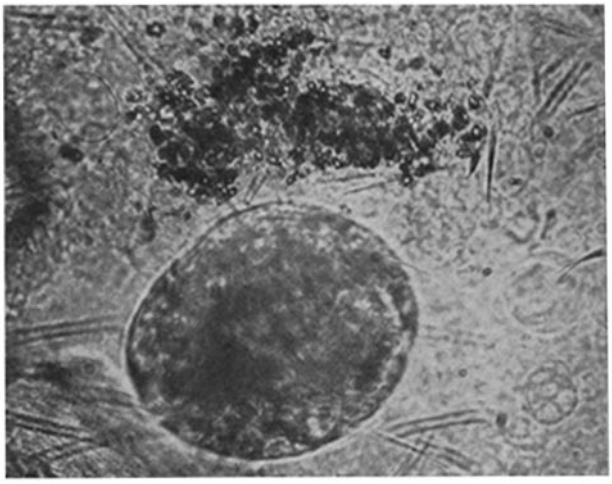

FIG. 2.

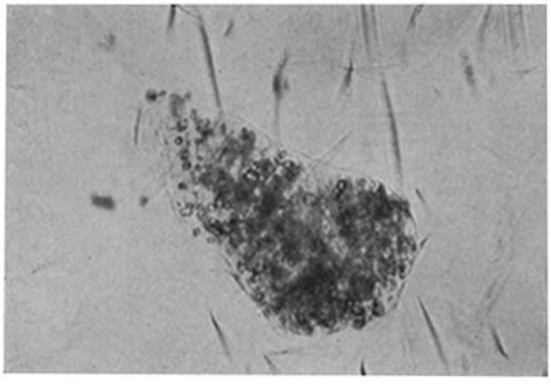

FIG. 3.

(Reznikoff: Chemical constituents of tubercle bacilli.)