

sheath or shell from the discrete lipid bodies (so-called Golgi bodies) by attributing the origin of the secretory material in their central core. In the present material the lipid bodies (so-called Golgi bodies) and mitochondria do not play any visible role in the process of secretion and continue to exist as such among the secretory globules which, however, distort the shape of the lipid spheres whose participation in the secretion can easily be eliminated by the fact that they have not been observed, at the time of the appearance of the secretory vacuoles, in *Fasciola*. The mitochondria seem to provide energy for the secretion, as they have been observed to lie in the vicinity of the secretory vacuoles. When the cytoplasm is fully packed up with the mature secretory globules, no basiphilia can be distinguished. The visible changes in the basiphilia (ergastoplasm), suggest its direct role in the synthesis of vitelline gland secretion in trematodes.

When the secretory globules become fully mature, then some physico-chemical changes begin to occur in them as evident from (1) the sloughing-off of their lipid sheath, (2) the loss of their affinity for haematein and (3) their disintegration into small pieces. Such changes in them make their first appearance near the nucleus and then proceed towards the periphery until the whole of the globules are reduced to very small particles. This also indicates that the 'surface membrane' or the lipid sheath simply acts as a protector to the globule. When such changes are going on, the basiphilia (ergastoplasm) begins to reappear in the form of patches among the disintegrating globules. Only at this stage a dictyosome, having

a complete or incomplete sheath of phospholipids and a sudanophobe sphere, appears in *Fasciola*. The patches of the basiphilia, with the complete disintegration of the globules, move near the nuclear membrane to form a compact mass (rich in RNA and lipoproteins). This indicates that the nucleus possibly plays some important role, at this stage, in some reorganization or rejuvenation of the basiphilia for the next secretory cycle. Similar suggestions have also been put forth by various earlier workers (see references by WEISS<sup>6</sup>). Very soon the dictyosome of *Fasciola* disappears, while the lipid bodies of *Paramphistomum* continue to exist. The significance of the appearance of dictyosome in *Fasciola* for such a short period could not be determined.

In conclusion, the ergastoplasm (RNA) is directly involved in the synthesis of vitelline gland secretion, rich in proteins, in trematodes.

**Résumé.** Les corps lipides (corps paranucléaires) et les mitochondries ne participent pas visiblement à la sécrétion de la glande vitelline des Trématodes qui contient des protides et provient de l'ergastoplasme.

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<sup>6</sup> J. M. WEISS, J. exp. Med. 98, 607 (1953).

<sup>7</sup> My thanks are due to Dr. S. KHERA for identifying the trematodes.

## The Nature and Synthetic Capacity of Fragile Cells of *Bacillus megatherium* Partly Deprived of Cell Walls

In the protoplasts of *B. megatherium*, proteins and nucleic acids are synthesized normally<sup>1</sup> and the protein-forming capacity is retained even by the ghosts<sup>2</sup> derived from the protoplasts. For the study of the synthesis of the peptidic component of the cell-wall, the authors have tried to use a system not only capable of forming the cell wall, but also being as amenable to fractionation as are the protoplasts. In making such a subcellular preparation, advantage was taken of the fact that *B. megatherium* strain KM formed protoplasts in a C/G medium with 0.5 M phosphate buffer pH 7<sup>3</sup> much more slowly than on a medium stabilized with saccharose<sup>4</sup>. The course of formation of protoplasts in both stabilized mediums was therefore studied in detail. During a precultivation period, the cell walls were labelled with diaminopimelic acid (DAP)-2-<sup>14</sup>C<sup>5</sup> which enabled us to determine the solubilization rate effected by 0.2 mg of lysozyme per ml (Figure 1a). In the phosphate medium, about 50% of the cell-wall material still remained intact, after an incubation at 30°C for 1 h, whereas in the saccharose medium, about 85–90% of the <sup>14</sup>C-DAP was in solution after a period of only 5 min. The remaining 10–15% of radioactivity bound in the protoplasts was mostly due to <sup>14</sup>C lysine formed by the decarboxylation of DAP during the precultivation. The same interpretation of the solubilization of the cell wall is given by microscopic observation. In the saccharose medium, about one half of the cells are transformed to protoplasts in the course of 5 min and after 15 min the number of cells falls below 1%. On the other hand, in the 0.5 M phosphate medium only a gradual decrease of gram-positivity took place during the incubation with lysozyme,

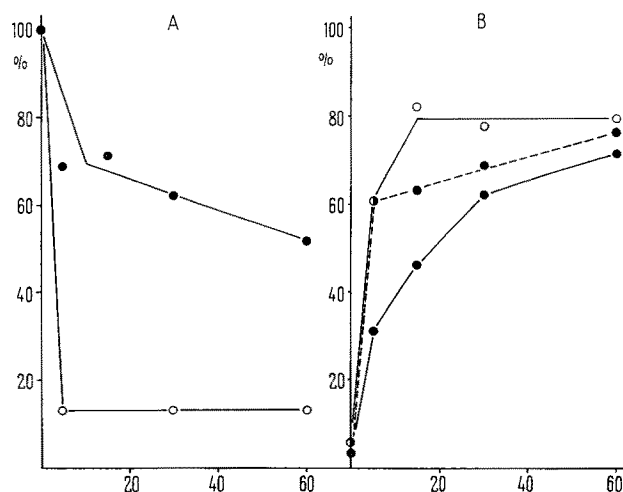


Fig. 1a. The releasing of <sup>14</sup>C-DAP from cells of *B. megatherium* by the action of lysozyme. ○ Incubation in C/G medium with 10% of saccharose, ● Incubation in C/G with 0.5 M phosphate. 1b. Solubilization of proteins from protoplasts and fragile cells by osmotic shock. ○ protoplasts, ● fragile cells; Full line: the solubilization measured at intervals of 10 min after the osmotic shock; Dashed line: the solubilization was measured after freezing to -15°C and thawing.

<sup>1</sup> K. McQUILLEN, Biochim. biophys. Acta 17, 382 (1955).

<sup>2</sup> J. A. V. BUTLER, A. R. CRATHORN, and G. D. HUNTER, Biochem. J. 69, 544 (1958).

<sup>3</sup> M. McQUILLEN, Biochim. biophys. Acta 18, 458 (1955).

<sup>4</sup> C. WEIBULL, J. Bacteriol. 66, 688 (1953).

<sup>5</sup> S. P. L. SÖRENSEN and A. C. ANDERSEN, Hoppe Seyler's Z. 56, 253 (1908).

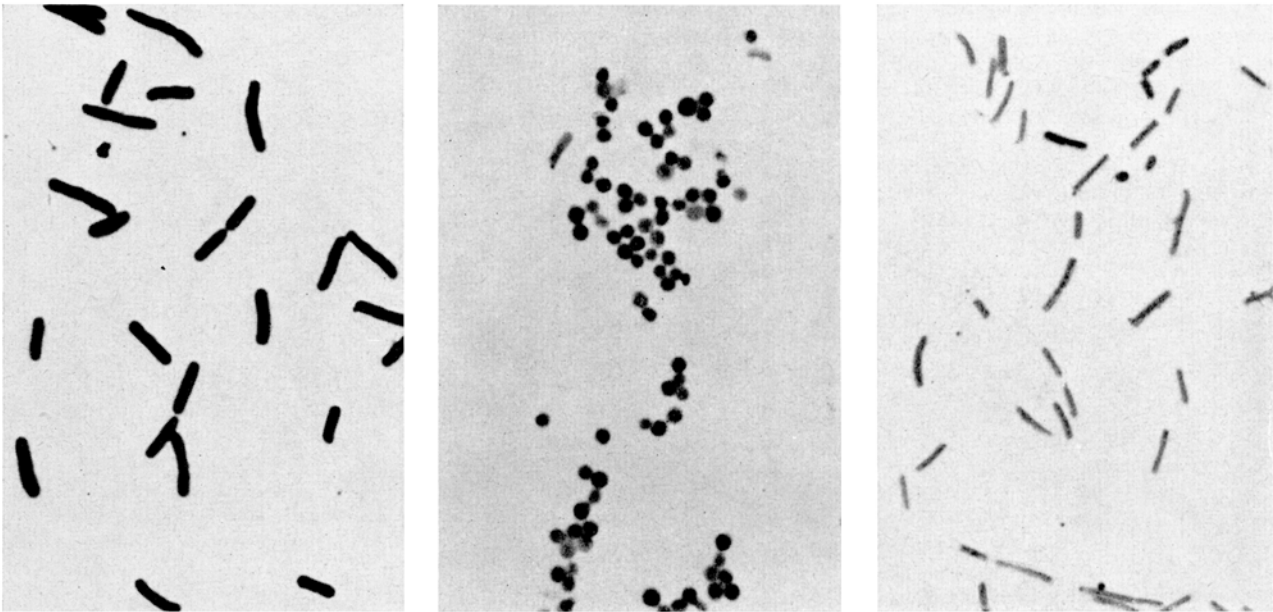


Fig. 2. Samples were fixed with 4% formaline and stained according to Gram. (A) Prior to the addition of lysozyme. (B) After an incubation of 15 min in saccharose. (C) After 60 min of incubation in phosphate.

and the amount of protoplasts after 1 h did not exceed 20–30% (Figure 2). Cells treated with lysozyme, however, very soon become sensitive to osmotic shock and this sensitivity was increased by freezing. In the course of the osmotic shock, the cytoplasm is solubilized into the medium (Figure 1b). The release of proteins into the fraction which does not undergo sedimentation at 10 000 g was determined colorimetrically<sup>6</sup>. The fragile cells from the phosphate medium retain to a limited extent the capacity of forming colonies on 2% peptone with 10% of saccharose. The capacity to form cell walls was studied on fragile cells formed after a 30 min action of lysozyme and on ghosts separated by centrifugation after the breaking up of these cells by an osmotic shock. The fragile cells, ghosts derived from them, and protoplasts, were incubated for 1 h in a C/G medium containing 0.25  $\mu$ C <sup>14</sup>C-DAP/ml, and the material insoluble in hot TCA was degraded with

trypsin<sup>7</sup>. The radioactivity in the trypsin-resistant fraction was measured by means of a methane 2  $\pi$  Friessecke-Hoepfner counter. According to the Table, it is evident that the incorporation into the material corresponding to the cell wall occurred only in fragile cells and in ghosts derived from these cells.

*Zusammenfassung.* Kurzfristige Einwirkung von Lysozym in Phosphatpufferlösung auf *Bazillus megatherium* ergibt Zellen mit bis zur Hälfte reduzierter Zellwand. Solche «fragile Zellen» sind gegen osmotische Schocks empfindlich und lassen sich in Formen überführen, die ausser der cytoplasmatischen Membran nur Zellwandreste besitzen. «Fragile Zellen» (auch von ihnen hergeleitete sedimentäre Formen) behalten die Fähigkeit der Zellwandsynthese. Sie sind fähig, in Medien, die 10% Saccharose enthalten, in eingeschränktem Masse sich zu normalen Zellen zu regenerieren.

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The incorporation of <sup>14</sup> C-DAP into the cell walls			
	cpm/mg Protoplasts	Fragile cells	Ghosts
Without chloramphenicol	51	877	1236
+ chloramphenicol 100 $\mu$ g/ml	36	850	—

<sup>6</sup> H. O. LOWRY, N. J. ROSENBOUGH, A. L. FARR, and R. J. RANDALL, *J. biol. Chem.* **193** 265 (1951).

<sup>7</sup> J. T. PARK and R. HANCOCK, *J. gen. Microbiol.* **22**, 249 (1960).

A propos du mode d’action de l’iproniazide,  
inhibiteur de la mono-amino-oxydase

Afin de mieux connaître les relations existant entre l’adrénaline et les inhibiteurs de l’amino-oxydase, nous avons étudié la glycogénolyse des coupes de foie, dont SUTHERLAND<sup>1</sup> a montré qu’elle est stimulée quantitativement par l’adrénaline. Nous avons ainsi observé<sup>2</sup> une augmentation de la sensibilité à l’adrénaline des coupes d’animaux traités à l’iproniazide. Cet effet est indirect et

ne dépend pas d’une inhibition par cette substance de la désamination oxydative de l’adrénaline.

Ces résultats nous ont amené à étudier dans les mêmes conditions l’action du facteur hyperglycémiant du pancréas sur le système des phosphorylases. On sait en effet que le glucagon stimule la glycogénolyse au même titre que l’adrénaline mais que son mode d’action est probablement différent.

<sup>1</sup> E. W. SUTHERLAND, *Ann. New York Acad. Sci.* **54**, 693 (1952).  
<sup>2</sup> F. MEYER, *C. R. Acad. Sci.* **252**, 2616 (1961).