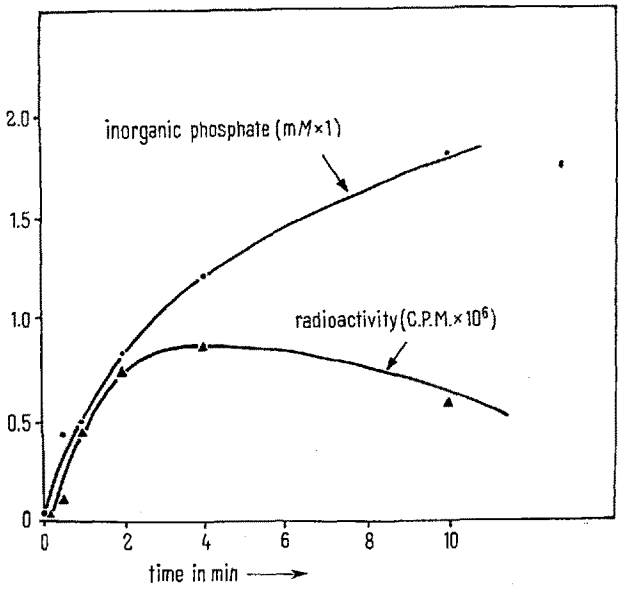
Two dimensional paper chromatography of the reaction mixtures from the experiments containing PEP, Ru-1, 5-P and pyruvate plus ATP, revealed in all cases radioactivity in the spots corresponding to malic, aspartic, fumaric, citric, and, succinic acid. Such results are consistent with the carboxylation of PEP to oxaloacetic acid, conversion of oxaloacetic acid to other organic acids by enzymes of the citric acid cycle and to aspartic acid by transamination.

The carboxylation of PEP is greatly stimulated by $MgCl_2$ and $MnCl_2$ but appears to be independent from ADP or IDP. The enzyme is similar to that demonstrated in plants[5-7] and, more recently, in photosynthetic[8], chemosynthetic[9] and heterotrophic bacteria[8,10,11]. In the reaction:

$$PEP + CO_2 \rightarrow \text{oxaloacetate} + \text{inorganic phosphate}$$

the fixation of carbon dioxide should be accompanied by the release of *o*-phosphate. Indeed, when a reaction mixture containing PEP and radioactive carbon dioxide was assayed at different time intervals, there was a progressive increase in the first minutes of the radioactivity fixed and of the *o*-phosphate released (Figure).




Carbon dioxide fixation and *o*-phosphate release from PEP. Conditions as in the Table. At the time intervals indicated, aliquots were withdrawn and pipetted in a solution of 10 *N* HCl. After centrifugation, the supernatants were assayed for radioactivity and inorganic phosphate.

In view of the presence of Ru-1, 5-P carboxylase in organisms devoid of chlorophyll, e.g. *Escherichia coli*, and in photosynthetic organisms artificially bleached or heterotrophically grown[12], its absence in a microorganism very closely related to the green forms seems of a certain interest. In *P. zopfii*, carboxylation of PEP is the only mechanism so far known for the fixation of carbon dioxide.

*Riassunto.* Estratti cellulari dell'alga aclorica *Prototheca zopfii* fissano anidride carbonica esclusivamente sul fosfoenolpiruvato. La reazione è stimolata da $MgCl_2$ e $MnCl_2$ ma non da IDP o ADP.

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## Cytochemical Evidence for the Origin of Vitelline Gland Secretion in the Ergastoplasm in Trematodes

Attempts have been made by various earlier workers[1,2] to derive the secretory granules from the cytoplasmic inclusions: mitochondria, Golgi bodies, ergastoplasm or microsomes and nucleus. The present cytochemical investigations on the vitelline glands of the trematodes: (a) *Fasciola indica Varma*, 1953; (b) *Paramphistomum* (Explanatum) *bathycotyle* (Fischoeder, 1901) collected from cattle, were undertaken to elucidate the participation of the cell organelles in the secretion. To the best of my information, no previous publication in the present line has been reported. For the cytochemical techniques used, reference may be made to GURAYA[3].

Before the commencement of the secretion, the cytoplasm of the vitelline gland cells shows lipid spheres (of phospholipid nature), rod-shaped mitochondria (made up of lipoproteins) and abundance of basiphilia, interpreted as due to RNA from its positive reaction with methyl green pyronin technique[4]. The recent researches of PALAY[1], both cytochemical and electron microscopic, have clearly shown that the cytoplasmic basiphilia (RNA) comprises the ergastoplasm in gland cells. As the secretory activity in the vitelline gland cells starts, some sudanophobe secretory vacuoles, identical with the 'ergastoplasmic sacs' of electron microscopists (see PALAY[1]) make their appearance among the basiphilia throughout the cytoplasm, and simultaneously the sudanophobe secretory material, which stains intensively with acid haematein even after pyridine extraction controls, begins to be deposited in their interior. The staining of the secretory material with acid haematein has been interpreted as due to its protein contents. Each secretory vacuole is bordered by a sudanophil membrane, seems to consist of lipoproteins, which, as the secretory globule grows, becomes thickened and forms a shell around the fully developed secretory globule. It is identical with the 'surface membrane' of the electron microscopists[1] and is possibly derived from the 'ergastoplasmic membranes' through the addition of more lipoproteins during the growth of the globule. Earlier workers (KANWAR[5]) on the other gland cells have made an attempt to derive this sudanophil

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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[6]). 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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[7] 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 synthetized normally[1] and the protein-forming capacity is retained even by the ghosts[2] 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[3] much more slowly than on a medium stabilized with saccharose[4]. 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-$^{14}$C[5] 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 $^{14}$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 $^{14}$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,

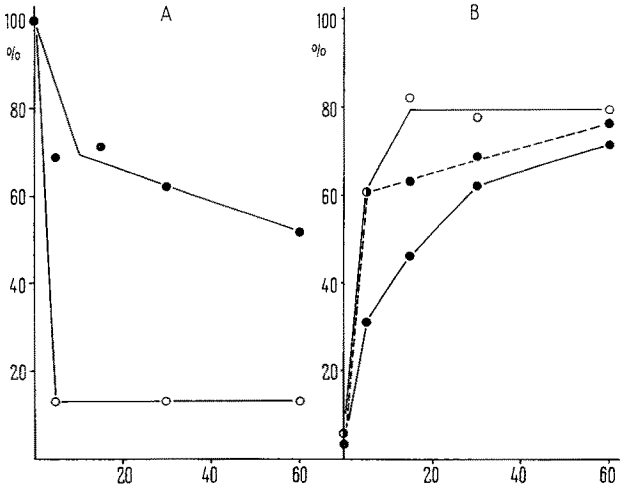


Fig. 1a. The releasing of $^{14}$C-DAP from cells of *B. megatherium* by the action of lysozyme. ○ Incubation in C/G medium with 10% of saccharose, ● Incubation in C/G with 0.5 *M* phosphate. 1b. Solubilization of proteins from protoplasts and fragile cells by osmotic shock. ○ protoplasts, ● fragile 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.

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