

The absence of renin in chronic hypertension suggests another renal mechanism. Such a mechanism might be adduced from the decrease of MAO activity and the increase in DDC activity in the kidney of our hypertensive animals. At least twelve amino acids normally found in the kidney are decarboxylated, forming the corresponding pressor amines. DDC is known to act on dopa with the formation of dopamine. Thus increase in DDC activity suggests the possibility of an increased formation of the corresponding pressor amines<sup>20</sup>. Since all of these amines constitute substrate for MAO which oxidizes them with the formation of ammonia and the corresponding aldehyde with loss of vaso-activity, a decrease in MAO activity leads to the tentative suggestion that a decreased rate of conversion of pressor amines might play a role in continuing elevation of blood pressure. A working hypothesis of the

mechanism involved in the experimental hypertension produced by constriction of the renal artery in the rabbit is represented schematically in Figure 3.

This hypothesis might be in line with some interesting pharmacological work<sup>21</sup> performed in hypertensive patients in whom  $\alpha$ -methyl dopa, a specific inhibitor for dopa decarboxylase, consistently lowered the blood pressure.

**Zusammenfassung.** Bei durch Nierenarterien-Drosselung nach kontralateraler Nephrektomie chronisch hypertensiven Kaninchen wird mit Antirenin regelmässig eine bestimmte hypotensive Wirkung erzeugt. Auffallend niedrige Reninblutwerte, stark herabgesetzte Monoaminoxidase-Aktivität und deutlich gesteigerte Dopadecarboxylase-Aktivität sind charakteristisch. Der Befund zeigt die Bedeutung der Niere in der Erzeugung der Blutdruckamine im Laufe einer chronischen Hypertonie.

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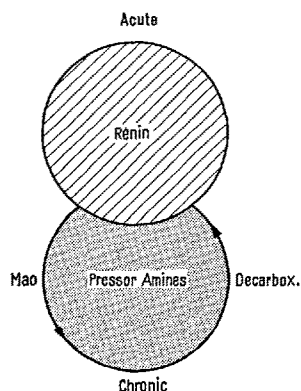


Fig. 3. Scheme of renal hypertensive mechanisms operating during acute (upper circle) and chronic (lower circle) hypertension.

<sup>20</sup> H. A. SCHROEDER and N. S. OLSEN, Amer. chem. Soc. Advances in Chemistry, Series No. 2, Chemical Factors in Hypertension, May 23 (1950).

<sup>21</sup> J. A. OATES, JR., L. GILLESPIE, JR., R. CROUT, and A. SJOERSMA, J. clin. Invest. 39, 1015 (1960).

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## Carbon Dioxide Fixation by the Achloric Alga *Prototheca zopfii*

Whole cells of *Prototheca*, a genus of achloric algae morphologically related to *Chlorella*<sup>1</sup>, are unable to utilize carbon dioxide<sup>2</sup> and the growth of the organism depends on the availability of a source of organic carbon.

Investigations in progress in this laboratory have shown that one species, *P. zopfii*, possesses enzymes of both the glycolytic and the hexose monophosphate pathways<sup>3</sup>. The present report deals with the fixation of radioactive carbon dioxide by cell-free extracts of this species.

Cell-free extracts of glucose-grown cells were prepared by mechanical disruption (full details will be published later) and incubated for 5 min at 37°C in glass-stoppered vials. The reaction was arrested by adding 10 N HCl (1/10 vol) and the vials aerated to remove unfixed carbon dioxide. The precipitated proteins were removed by centrifugation and aliquots of the supernatant solutions were plated onto metal planchets. Radioactivity was measured with a windowless flow counter.

The results (Table) show high carbon dioxide fixation only in the presence of P-enolpyruvate (PEP). Ribulose-1,5-diphosphate (Ru-1,5-P)<sup>4</sup> and pyruvate were not carboxylated to a comparable extent. In the case of Ru-1,5-P, the substrate for the carboxylation is most probably still PEP that may arise from the 3-P-glycerate (3PG) present as a contaminant in the preparation of Ru-1,5-P. Twice as much radioactivity is, indeed, fixed if only 3PG is used as a substrate. A conversion to PEP seems to be taking place also when pyruvate is used; the fixation of carbon dioxide is stimulated by ATP and ADP but not IDP nor by adding reduced di- and triphospho-

pyridine nucleotides, alone or with a hydrogen recycling system such as glucose-6-P and glucose-6-P dehydrogenase.

### Carbon dioxide fixation by cell-free extracts of *Prototheca zopfii*

Addition	Carbon dioxide fixed (c.p.m./ml)
MgCl <sub>2</sub>	93
MnCl <sub>2</sub>	122
PEP + IDP	18675
PEP + MgCl <sub>2</sub>	1185000
PEP + IDP + MgCl <sub>2</sub>	1252000
PEP + IDP + MnCl <sub>2</sub>	1142500
PEP + ADP + MgCl <sub>2</sub>	1112500
Pyruvate + ATP + MnCl <sub>2</sub>	1252
Pyruvate + ADP + MnCl <sub>2</sub>	1322
Pyruvate + IDP + MnCl <sub>2</sub>	542
Ru-1,5-P + MgCl <sub>2</sub>	1572
3PG + MgCl <sub>2</sub>	3227

The basal assay system contained 50  $\mu$ M of Tris buffer (pH 7.9); 0.1  $\mu$ M of Versene (pH 7.9); 0.125  $\mu$ M of NaHC<sup>14</sup>O<sub>3</sub> (specific activity 7.8  $\mu$ C/ $\mu$ M) and 0.1 ml of enzyme preparation (0.49 mg of protein). When present, 10  $\mu$ M of metal activator and 1.25  $\mu$ M of substrate and coenzyme were added.

<sup>1</sup> R. CHODAT, in *Mat. Fl. Crypt. Suisse* 4, 11 (1913).

<sup>2</sup> P. J. CASSELTON, *Nature* 183, 1404 (1959).

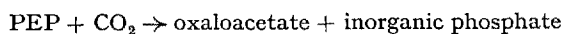
<sup>3</sup> O. CIFERRI, in preparation.

<sup>4</sup> The author is grateful to Dr. M. GIBBS for the generous gift of a sample of ribulose-1,5-diphosphate.

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<sup>5-7</sup> and, more recently, in photosynthetic<sup>8</sup>, chemosynthetic<sup>9</sup> and heterotrophic bacteria<sup>8,10,11</sup>. In the reaction:



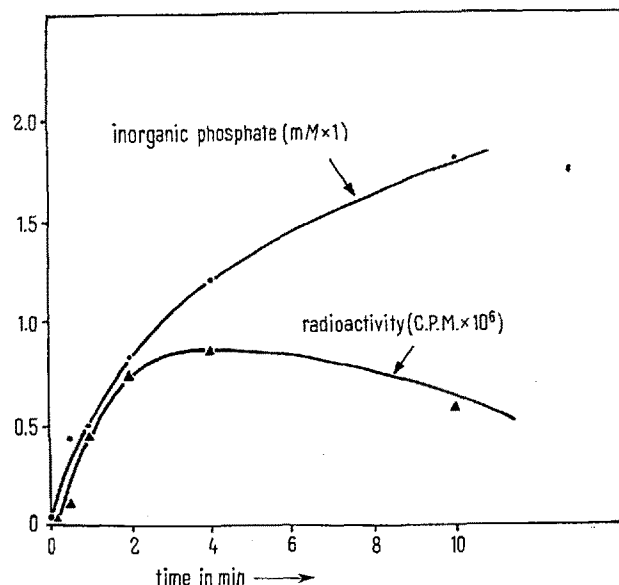
the fixation of carbon dioxide should be accompanied by the release of *o*-phosphate. Indeed, when a reaction mixture containing PEP and radioactive carbon dioxide was assayed at different time intervals, there was a progressive increase in the first minutes of the radioactivity fixed and of the *o*-phosphate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

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<sup>12</sup>, 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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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.

<sup>5</sup> L. L. ROSENBERG, J. B. CAPINDALE, and F. R. WATLEY, *Nature* **181**, 632 (1958).

<sup>6</sup> T. T. TCHEN and B. VENNESLAND, *J. biol. Chem.* **213**, 533 (1955).

<sup>7</sup> P. SALTMAN, G. KUNITAKE, H. SPOLTER, and C. STITTS, *Plant Physiol.* **31**, 464 (1956).

<sup>8</sup> C. L. BAUGH, T. MYODA, D. S. BATES, and C. H. WERKMAN, *Iowa State J. Sci.* **34**, 113 (1959).

<sup>9</sup> I. SUZUKI and C. H. WERKMAN, *Iowa State J. Sci.* **32**, 475 (1958).

<sup>10</sup> T. MYODA and C. H. WERKMAN, *Iowa State J. Sci.* **35**, 73 (1960).

<sup>11</sup> Y. R. QUAYLE and D. B. KEECH, *Biochem. J.* **72**, 691 (1959).

<sup>12</sup> R. C. FULLER and M. GIBBS, *Plant Physiol.* **34**, 324 (1959).

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

Attempts have been made by various earlier workers<sup>1,2</sup> 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 (Fischöeder, 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<sup>3</sup>.

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<sup>4</sup>. The recent researches of PALAY<sup>1</sup>, 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 sudano-

phobe secretory vacuoles, identical with the 'ergastoplasmic sacs' of electron microscopists (see PALAY<sup>1</sup>) 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<sup>1</sup> and is possibly derived from the 'ergastoplasmic membranes' through the addition of more lipoproteins during the growth of the globule. Earlier workers (KANWAR<sup>5</sup>) on the other gland cells have made an attempt to derive this sudanophil

<sup>1</sup> S. L. PALAY, in *Frontiers in Cytology* (Yale University Press, 1958), p. 305.

<sup>2</sup> L. C. U. JUNQUEIRA and G. C. HIRSCH, *Int. Rev. Cytol.* **5**, 323 (1956).

<sup>3</sup> S. S. GURAYA, *Res. Bull. Panjab Univ.* **10**, 305 (1959).

<sup>4</sup> B. M. JORDAN and J. R. BAKER, *Quart. J. micr. Sci.* **96**, 177 (1955).

<sup>5</sup> K. C. KANWAR, *Res. Bull. Panjab Univ.* **10**, 99 (1959).