successful and, indeed, inactivated the enzyme responsible for reaction 2.

It is conceivable that carbamylphosphate is synthesized in the elasmobranch liver in some way that differs from that in rat liver, but it is impossible to support such a suggestion on the basis of purely negative results. It is at least equally, or even more, likely that the enzymes responsible for reaction 1 differ somewhat in their properties. It may well be that one or more of these enzymes in the elasmobranch is soluble and removed when the tissue is washed before acetone precipitation. It is possible, too, that the enzyme concerned may not yet have developed a specificity that extends to carbamyl- or to acetyl-glutamate, but might act upon unsubstituted glutamate or, perhaps, glutamine. These possibilities are being explored. Finally, if these possibilities fail to give results, it is not unlikely that more specialized preparations, for example, mitochondrial suspensions, may give results where the acetone powders have so far failed.

The work so far, then, has produced no conclusive evidence that ureogenesis in the elasmobranchs follows any pathways other than those established in the rat, and further work is in progress in the hope of elucidating the initiating reaction.

My thanks are due to Dr. Roger Revelle, director of the Scripps Institution of Oceanography, for his hospitality and facilities while this work was in progress; to the University of California for a visiting professorship; and to Miss Luellen Dale, who gave me the most valuable technical assistance.

Ernest Baldwin

Scripps Institution of Oceanography,
La Jolla, California,
and University College,
London, W.C.1.

[1] Staedeler, G., and Frerichs, F. T., *J. prakt. Chem.*, **73**, 48 (1858).
[2] Smith, H. W., *Biol. Rev.*, **11**, 49 (1936).
[3] Baldwin, E., *Biol. Rev.*, **8**, 74 (1932).
[4] Grisolia, S., in "Methods in Enzymology" (New York), **2**, 350 (1955).
[5] Ratner, S., in "Methods in Enzymology" (New York), **2**, 356 (1955).
[6] Archibald, R. M., *J. Biol. Chem.*, **157**, 507 (1945).
[7] Jones, M. E., Spector, L., and Lipmann, F., *J. Amer. Chem. Soc.*, **77**, 819 (1955).

Intermediates in the Biosynthesis of Porphyrins from Porphobilinogen by *Rhodopseudomonas spheroides*

The formation of porphyrins[1] and of δ-aminolævulic acid[2,3] from glycine by preparations of *Rhodopseudomonas spheroides* has been reported.

Whole-cell suspensions of this organism are not capable of utilizing porphobilinogen for the synthesis of porphyrins[1]. We have found that if the cells are damaged by freezing and thawing, then porphobilinogen is converted, in 50 per cent yield, to coproporphyrin; some uroporphyrin is also formed, but this accounts for less than 2 per cent of the added porphobilinogen. It would appear, therefore, that the inability of whole cells to use porphobilinogen for porphyrin synthesis is due to the failure of that substance to enter the cells.

Acetone-dried cells also convert porphobilinogen to coproporphyrin and are more active than frozen and thawed cells on a dry-weight basis. With low concentrations of acetone-dried cells, considerable amounts of uroporphyrin III are also formed (Table 1).

Cell-free extracts made by grinding with alumina followed by centrifugation at 12,000*g* showed weaker activity for coproporphyrin formation. Coproporphyrin was formed by high concentrations of the extract, but with lower concentrations only uroporphyrin was produced. The uroporphyrin formed under these conditions was a mixture of the I and III series isomers. Coproporphyrin-forming activity in cell-free extracts was lost on storage at −15° C., whereas acetone-dried cells and whole cells were relatively stable. Apparently the enzyme system for converting porphobilinogen to uroporphyrin is more active and more stable than that involved in the formation of coproporphyrin. The coproporphyrin formed by the three systems is mainly type III, but there is also a small amount of type I.

Table 1. Porphyrin Formation by Acetone-dried Cells of *Rhodopseudomonas spheroides*
Acetone-dried cells in 0·4 ml. buffer *p*H 6·5 incubated 4 hr. at 37° with 0·1 ml. (100 μgm.) solution of porphobilinogen

Dried cells (mgm.)	Porphyrins formed (μgm.): Uro	Copro	Total
18	4	30	34
4·5	33	4	37
1·8	24	1	25

When the time-course of the reaction with acetone powders was followed, porphobilinogen was found to disappear very rapidly, and during the initial stages of the reaction uroporphyrin appeared to be the major product; but as the reaction proceeded, the amount of uroporphyrin present decreased while that of coproporphyrin increased (Table 2).

Table 2. Effect of Time of Incubation on Porphyrin Formation by Acetone-dried Cells of *Rhodopseudomonas spheroides*
18 mgm. acetone-dried cells in 0·4 ml. buffer *p*H 6·5 incubated at 37° with 0·1 ml. (80 μgm.) solution of porphobilinogen

Incubation time (min.)	Porphobilinogen used (μgm.)	Porphyrins formed: Uro (μgm.)	Copro (μgm.)	Total (μgm.)
15	31	9	6	15
30	57	16	12	28
60	80	17	24	41
120	80	9	30	39
240	80	5	30	35

Since neither added uroporphyrin I nor III was converted to coproporphyrin by acetone-dried cells, or by frozen and thawed cell preparations, it appeared that the true precursor might be uroporphyrinogen, which, becoming oxidized during isolation, would be included in the estimation of uroporphyrin. The possibility that uroporphyrinogen, or at least a reduced uroporphyrin, was an intermediate was investigated as follows. Acetone-dried cells were incubated with porphobilinogen for 1 hr. (that is, under conditions where maximum uroporphyrin appeared to be formed), and the reaction was arrested by acidifying with 5 per cent hydrochloric acid; the protein-free supernatant, after centrifuging, showed weak absorption due to porphyrin at 548 mμ and in addition an absorption band at 500 mμ. The absorption due to porphyrin increased with time, the change being accelerated by addition of hydrogen peroxide, while simultaneously the absorption at 500 mμ decreased.

When porphyrins are reduced with sodium amalgam, the typical red colour changes to yellow before the colourless porphyrinogen is finally formed. This yellow alkaline solution shows an absorption maximum at 500 mμ, which decreases as oxidation to porphyrin occurs. The intermediate reduction product is yellow in alkaline and pink in acid solution, and this compound can easily be reduced to the

Table 3. COPROPORPHYRIN FORMATION FROM UROPORPHYRINOGENS I AND III

50 mgm. acetone-dried cells in 0·4 ml. buffer *p*H 6·5 with 0·6 ml. porphyrinogen solution *p*H 6·5 containing 10^{-3} *M* sodium dithionite and 10^{-3} *M* cysteine. Controls contained porphyrinogen solution (0·6 ml.) and 0·4 ml. buffer

	Porphyrins formed: Uro (μgm.)	Copro (μgm.)	Total (μgm.)
Uroporphyrinogen I control	116	Nil	116
Uroporphyrinogen I + dried cells	8	62	70
Uroporphyrinogen III control	270	Nil	270
Uroporphyrinogen III + dried cells	19	160	179

colourless porphyrinogen with sodium dithionite. The same yellow compound is formed during re-oxidation of fully reduced porphyrinogens by air; colourless porphyrinogen solutions can be stabilized by the presence of sodium dithionite (10^{-3} *M*).

Uroporphyrinogens I and III prepared in this way and containing in addition 10^{-3} *M* cysteine to protect the enzyme from inhibition by traces of mercury were then incubated with acetone-dried cells of *Rhodopseudomonas spheroides*, whereupon large amounts of coproporphyrin were formed (Table 3). No isomerization occurred in either case; only coproporphyrin I was obtained from uroporphyrinogen I, and only coproporphyrin III from uroporphyrinogen III. The acetone-dried cells therefore contain decarboxylating systems for both the I and III series, and the final type of isomer must be determined in these preparations during the conversion of porphobilinogen to uroporphyrinogen. The isomers were identified by paper chromatography[4] and by the typical crystalline form of the coproporphyrin methyl esters. These results clearly establish uroporphyrinogens as intermediates in the biosynthesis of coproporphyrins by *Rhodopseudomonas spheroides*.

Bogorad[5,6] partially purified an enzyme from spinach which converted porphobilinogen to a colourless precursor of porphyrins. He also reported the formation of very low yields of coproporphyrin on incubating frozen and thawed *Chlorella* with uroporphyrinogens I and III[6]. Cooper[7] has studied a *Rhodopseudomonas* which produces a coproporphyrin precursor with properties akin to a coproporphyrinogen. Neve *et al.*[8] demonstrated the formation of coproporphyrin, and of hæm from uroporphyrinogen III by duck hæmolysates. Townsley and Neilands[9] produced evidence for porphyrinogen formation from δ-aminolævulic acid by cell lysates of *Micrococcus lysodeikticus*. Granick and Mauzerall[10] have shown that coproporphyrinogen III, but not coproporphyrinogen I, is a precursor of protoporphyrin in cell particulate preparations of chicken erythrocytes and of *Euglena*.

When whole cells are frozen in either buffer *p*H 6·5 or water and then afterwards separated centrifugally into a cell residue and a supernatant, an ultrafilterable co-factor for coproporphyrin formation which is stable to heat is removed in the supernatant layer. If the freezing and separation are repeated several times, and an acetone powder made of the cell residue, then this powder can convert porphobilinogen only to uroporphyrin III. Addition of either the supernatant or an acetone precipitate of the supernatant to the acetone powder of the cell residue brings about complete restoration of the ability to form coproporphyrin. The supernatant solution forms only uroporphyrin I from porphobilinogen and no coproporphyrin. Boiling completely destroys the enzyme forming uroporphyrin I. This is the first reported separation of the enzyme systems forming uroporphyrins I and III, although many reports have appeared in which the ability to form uroporphyrin III has been lost after preheating at 50–60° with the simultaneous appearance of uroporphyrin I-forming activity[11].

These results will be fully reported elsewhere.

We are grateful to Prof. C. Rimington for providing porphobilinogen and the pure uroporphyrin and coproporphyrin isomers and for his continued interest in this work.

D. S. HOARE
H. HEATH

Department of Chemical Pathology,
University College Hospital Medical School,
London, W.C.1.

[1] Lascelles, J., *Biochem. J.*, **62**, 78 (1956).
[2] Kikuchi, G., Shemin, D., and Bachmann, B. J., *Biochim. Biophys. Acta*, **28**, 219 (1958).
[3] Gibson, K., *Biochim. Biophys. Acta*, **28**, 451 (1958).
[4] Falk, J. E., Dresel, E. I. B., Benson, A., and Knight, B. C., *Biochem. J.*, **63**, 87 (1956).
[5] Bogorad, L., *Science*, **121**, 878 (1955).
[6] Bogorad, L., *Plant Physiol.*, **30**, xiv (1956).
[7] Cooper, R., *Biochem. J.*, **63**, 25*P* (1956).
[8] Neve, R. A., Labbe, R. F., and Aldrich, R. A., *J. Amer. Chem. Soc.*, **78**, 691 (1956).
[9] Townsley, P. N., and Neilands, J. B., *J. Biol. Chem.*, **224**, 695 (1957).
[10] Granick, S., and Mauzerall, D., *Fed. Proc.*, **17**, 233 (1958).
[11] Bogorad, L., and Granick, S., *Proc. U.S. Nat. Acad. Sci.*, **39**, 1176 (1953). Boolj, J. L., and Rimington, C., *Biochem. J.*, **65**, 4*P* (1957). Lockwood, W. H., and Rimington, C., *Biochem. J.*, **67**, 8*P* (1957).

Glucose, Trehalose and Glycogen in *Porrocaecum decipiens* Larvæ

RECENT proof that trehalose, formerly regarded as a plant sugar, is an important constituent of a nematode, *Ascaris lumbricoides*[1], and various insects[2–4] prompted the examination of several parasitic and free-living invertebrates for this non-reducing disaccharide. Qualitative tests suggested that mature larvæ (30–45 mm.) of *Porrocaecum decipiens* (Nematoda) contained unusually large amounts. More extensive analyses were therefore performed on 108 gm. of larvæ collected from codfish muscle. Glucose and trehalose were extracted from these with several changes of cold 70 per cent ethanol. The ethanolic extracts were concentrated and washed with ether, after which trehalose was isolated[1]. The yield was 1·39 per cent of fresh tissue weight, or 6·0 per cent of the dry weight.

Two other carbohydrates were present. Glucose, determined by means of the specific glucose oxidase reaction[5], comprised only 0·6 per cent of the dry weight. Glycogen (55 per cent) was identified by its characteristic stability in alkali, insolubility in 50 per cent ethanol, and quantitative yield of glucose following acid hydrolysis. Thus, more than 60 per cent of *Porrocaecum* solids consisted of glucose residues. Lipids, as expected, were correspondingly low (3·7 per cent).

Porrocaecum larvæ live in the flesh of cod and other fishes, where they can presumably survive until presented with an opportunity to complete their development in the stomach of various seals. As the larvæ examined were in the third or fourth stage and were sheathed with the remains of the second- or third-stage cuticle, it is improbable that they were able to feed actively. It follows that the relatively enormous carbohydrate deposits are formed