Table 1. Phosphorus : Oxygen Ratios of Different Steps within the Krebs Cycle

(In parenthesis : number of experiments)

	Phosphorus : Oxygen ratio	
	Observed	Probable in intact cell
Pyruvate → Acetyl coenzyme A	2·1 (15)	3·0
*iso*Citrate → Succinate	2·0 (16)*	3·0
Alpha-ketoglutarate → Succinate	1·9 (19)	2·0
Succinate → Fumarate	1·8 (16)	2·0
Malate → Oxalacetate	2·6 (13)	3·0

* Obtained on homogenates

hand, when homogenates were used, the phosphorus : oxygen ratio increased to 2·0 with *iso*citrate (14 experiments) and 2·2 with citrate (10 experiments), which point to a probable phosphorus : oxygen ratio of 3·0 inside the cell. In this respect the embryonic mitochondria behave differently from those of the rat liver ; Copenhaver and Lardy[4] showed an estimated phosphorus : oxygen ratio 2·6 for citrate. Our estimations on the rat liver mitochondria gave an average of 2·1 with citrate as substrate.

Full details of this work will be published elsewhere. It was aided by U.S. Public Health Service Grant *C*–2296 (*C7*).

Wiktor W. Nowinski
William C. Mahaffey

Tissue Metabolism Research Laboratory,
University of Texas Medical Branch,
Galveston, Texas.
Oct. 27.

[1] Hogeboom, G. H., "Methods in Enzymology", edit. by Colowick and Kaplan, 1, 16 (Academic Press, New York, 1955).
[2] Hunter, F. E., "Phosphorus Metabolism", edit. by McElroy and Glass, 1, 297 (1951).
[3] Lowry, O. H., and Lopez, J. A., *J. Biol. Chem.*, **162**, 421 (1946).
[4] Copenhaver, J. H., and Lardy, H. A., *J. Biol. Chem.*, **195**, 225 (1952).

Synthesis of Amylose by Potato *D*-Enzyme

The synthesis of the chain-forming α-1 : 4-bonds of potato starch (amylose + amylopectin) has for long been ascribed to phosphorylase acting on glucose-1-phosphate and a preformed starch-like primer[1]. We now report the synthesis of amylose-like material by another enzyme system from potato. This synthesis makes use of *D*-enzyme, discovered in 1953 by Peat and co-workers[2]. *D*-enzyme catalyses the reversible disproportionation of maltodextrins, the glucose oligosaccharides containing the α-1 : 4-linkage. For example, maltotriose is acted on to give, as the first products of reaction, glucose and maltopentaose, and at equilibrium glucose and a whole series of maltodextrins are present. However, with maltotriose as the initial substrate, none of the synthetic oligosaccharides is of sufficient length to form a coloured complex with iodine. The minimum length for colour formation is at least 12 glucose units[3]. Incubation of *D*-enzyme with a maltodextrin and glucose lowers the average length of the polymer because of transfer of some of its glucose residues to the added glucose. It was realized that a reversal of this procedure, the removal of glucose, should increase the length of the polymers and might ultimately result in the synthesis from a small maltodextrin molecule of an iodine-staining, amylose-like polysaccharide. If, in addition to *D*-enzyme, the potato is equipped with such a glucose-removing enzyme system, then it is presumably capable of synthesizing amylose by a route other than that provided by phosphorylase. A suitable glucose-removing system is hexokinase and adenosine triphosphate, and hexokinase is known to be in the potato[4]. This will convert glucose into glucose-6-phosphate, and *D*-enzyme does not transfer chain segments from maltodextrins to the sugar phosphate as it does to glucose[5]. The system (maltodextrin + *D*-enzyme + hexokinase + adenosine triphosphate) should therefore bring about amylose synthesis. This has been achieved.

We have preferred to use yeast hexokinase in the present experiments because it can be obtained in a better state of purity than the potato enzyme. In the first instance *D*-enzyme was incubated with a mixture of maltodextrins (malto-pentaose, -hexaose and -heptaose) from which some of the products of *D*-enzyme action alone are long enough to stain with iodine[2]. The same system with added hexokinase and adenosine triphosphate produced iodine-staining material at more than twice the rate. The experiment was repeated with maltotetraose, which with *D*-enzyme alone does not give iodine-staining products. Fig. 1 depicts the course of reaction of *D*-enzyme + maltotetraose in the presence and absence of the glucose-phosphorylating system. When glucose was continuously removed as the 6-phosphate there was an abundant production of iodine-staining polysaccharide, and correspondingly adenosine triphosphate disappeared.

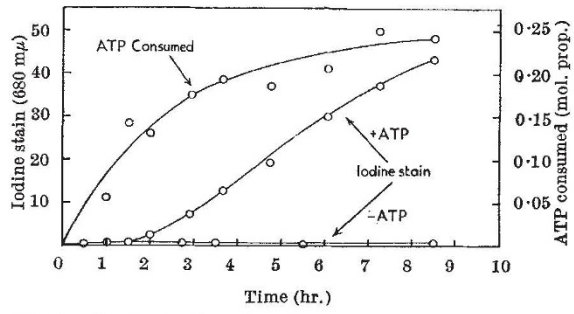

Fig. 1. Synthesis of amylase from maltotetraose. *D*-enzyme was incubated at 25° C. with hexokinase and maltotetraose in the presence and absence of adenosine triphosphate (ATP). The *p*H was maintained at 7·2 by the frequent addition of sodium hydroxide

The suggestion has been made[6] that the potato may use both phosphorylase and *D*-enzyme to synthesize amylose, the product from phosphorylase remaining as amylose and from *D*-enzyme being converted into amylopectin by *Q*-enzyme. Experiments are now in progress to demonstrate the working of such a system *in vitro*.

This work was made possible by grants from the Agricultural Research Council, to which we express our thanks. We also thank Dr. K. Rees for a gift of hexokinase.

Gwen J. Walker
W. J. Whelan

Lister Institute of Preventive Medicine,
London, S.W.1.
Oct. 29.

[1] Hanes, C. S., *Proc. Roy. Soc.*, B, **129**, 174 (1940).
[2] Peat, S., Whelan, W. J., and Rees, W. R., *Nature*, **172**, 158 (1953); *J. Chem. Soc.*, 44 (1956).
[3] Bailey, J. M., and Whelan, W. J. (to be published).
[4] Kotel'nikova, A. V., *Biokhimiya*, **17**, 462 (1952). Saltman, P., *J. Biol. Chem.*, **200**, 145 (1953).
[5] Peat, S., Whelan, W. J., and Jones, G., *J. Chem. Soc.*, 2490 (1957).
[6] Whelan, W. J., "Encyclopedia of Plant Physiology", **6**, 154 (Springer-Verlag, Berlin, 1958).