This method of assay has been applied to blood from various sources, with the results shown in the table.

A. G. SPENCER

Departments of Medicine of the
College of Physicians and Surgeons,
New York,
and
University College Hospital Medical School,
London, W.C.1.

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Direct Oxidation of Glucose-6-Phosphate by Animal Tissues

YEAST[1–4] and red blood cells[5] contain enzyme systems which require coenzyme II and are capable of oxidizing glucose-6-phosphate to 6-phosphogluconic acid. The oxidation can also proceed further[1,3,6], and evidence has been presented that in yeast stepwise C_1- and C_2-oxidation and decarboxylation may proceed via a pentose phosphoric ester; this was considered to be D-ribose-5-phosphate rather than D-arabinose-5-phosphate, which, although it has the configuration of D-glucose, is not attacked by yeast enzymes[6]. Further support for this view has recently been provided by the chromatographic identification of 2-ketogluconate and D-ribose-5-phosphate among the oxidation products of 6-phosphogluconic acid[7].

These oxidative paths in animal tissues have not hitherto received detailed study[3,6,8]; but the preliminary experiments now reported show clearly that similar enzyme systems are, in fact, highly active, sufficiently so to make it possible that they may constitute a main route of carbohydrate oxidation, in some animal tissues.

The tissues were homogenized in 0·01 *M* phosphate of *p*H 7·0 and the inactive precipitates formed by adjusting the supernatant fluid to *p*H 4·6 and 7·0 discarded. Dehydrogenase activity was tested by the rate of reduction of Brilliant Cresyl Blue using Friedemann and Hollander's method[9], and in the dialysed fractions described below by the method of Haas[10]; oxygen uptake was studied as described by Dickens[6]. Active coenzyme II – linked systems for the oxidation of glucose-6-phosphate and 6-phosphogluconic acid are present in extracts of liver, brain and kidney of rat and rabbit and in liver-carcinoma of the rat. These activities are not due to adventitious blood. Extracts of rat and rabbit skeletal muscle and fowl sarcoma No. 1 show relatively feeble activity.

By fractional precipitation of rat liver extracts with ammonium sulphate and subsequent dialysis, partial separation of coenzyme-free dehydrogenases has been effected. The crude fraction, between 30 and 60 per cent saturation with ammonium sulphate, contains glucose dehydrogenase[11] and the dehydrogenases for glucose-6-phosphate and 6-phosphogluconic acid. The percentage saturations for maximum activity of these three dehydrogenases are, respectively, 30–40, 40–50 and 50–60. The last fraction is completely free from glucose-6-phosphate dehydrogenase, showing that these two phosphorylated substrates require distinct dehydrogenases. Oxidation of both glucose-6-phosphate and 6-phosphogluconic acid is specific for coenzyme II and occurs aerobically in presence of a suitable carrier, such as phenazine methosulphate[6], without added flavoprotein. Since glucose dehydrogenase requires coenzyme I and added diaphorase, it could not account for the activity towards glucose-6-phosphate. Moreover, no glucose-6-phosphatase is present in these fractions.

The substrate specificity is high. In the presence of coenzyme II, the 50–60 per cent saturation fraction, though highly active towards 6-phosphogluconic acid, does not oxidize Embden ester, glucose 6-phosphate, gluconic acid, 2-ketogluconic acid, glucose or ribose. The whole system (fraction between 30–60 per cent saturation) vigorously oxidizes glucose-6-phosphate, 6-phosphogluconic acid and D-ribose-5-phosphate (natural or synthetic). It shows slight activity towards D-arabinose-5-phosphate and xylose-5-phosphate, and also towards glycogen and glucose-1-phosphate, the crude system presumably containing some phosphorylase and phosphoglucomutase. Gluconic and 2-ketogluconic acids and ribose are scarcely oxidized.

The Michaelis constants for the glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases are 5×10^{-5} *M* and $1{\cdot}7 \times 10^{-5}$ *M*, respectively. The oxygen taken up by 6-phosphogluconic acid and D-ribose-5-phosphate exceeds the molar ratio of unity corresponding to the formation of ketopentonic acid. The facts that neither the oxidation of glucose-6-phosphate nor that of 6-phosphogluconic acid is inhibited by 0·01 *M* sodium fluoride, and that inorganic phosphate is not essential, show that the Embden–Meyerhof glycolytic pathway is not involved.

The relative importance of this direct oxidative pathway and of the conventionally accepted route via the glycolytic intermediates is being investigated in adult and proliferating animal tissues. The direct oxidative route may have a dual function, in carbohydrate oxidation and in the provision of ribose-5-phosphate for synthesis of nucleotides and nucleic acids.

F. DICKENS
GERTRUDE E. GLOCK

Courtauld Institute of Biochemistry,
Middlesex Hospital Medical School,
London, W.1.
May 5.

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