LETTERS TO THE EDITORS

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Mechanism of Glyceraldehyde Inhibition of Glycolysis

THE effect of low concentrations of monomeric glyceraldehyde in inhibiting lactic acid formation in tissues using glucose but not in those using glycogen has been the subject of much experimentation and discussion. Stickland[1] used dialysed muscle extract, reinforced with yeast hexokinase, and studied both substrates with this system. 0·01–0·001 *M* glyceraldehyde caused 80–90 per cent inhibition with glucose but had little or no effect with glycogen. Stickland found (private communication) by special tests that glyceraldehyde was not inhibiting hexokinase in this system.

After the discovery by Warburg and Christian[2] and by Caputto and Dixon[3] that glyceraldehyde is a substrate for the triosephosphate dehydrogenase of yeast and of muscle, but oxidized much more slowly than glyceraldehydephosphate (at about one three-hundredth of the rate in the case of muscle), it seemed possible that glyceraldehyde might interfere with glycolysis by competing with glyceraldehydephosphate for the enzyme. In the system used by Stickland (and in tissues also) glycolysis depends on repeated rephosphorylation of adenosine diphosphate and adenylic acid formed; with glucose as substrate, the need for this rephosphorylation is twice as great as with glycogen. A factor, such as inhibition of triosephosphate dehydrogenase, interfering with this process will have the effect of diminishing or stopping the initial phosphorylation of glucose. Two lines of work were followed in testing this hypothesis.

(1) Using crystalline triosephosphate dehydrogenase. The activity of the enzyme was tested in a system containing pyrophosphate buffer, arsenate, glyceraldehydephosphate (0·0006 *M*) and co-enzyme I, the rate of formation of reduced co-enzyme being followed in the Beckmann spectrophotometer. With 0·006 *M* glyceraldehyde the inhibition was about 30 per cent. The triosephosphate dehydrogenase was greatly activated by addition of 0·006 *M* cystein. Under these conditions the inhibition by glyceraldehyde was much greater—about 85 per cent by 0·006 *M*. The experiments so far considered were done with DL-glyceraldehyde; when this was compared with D-glyceraldehyde, it was found that the inhibition was to be attributed to the latter component. Increased concentration of glyceraldehydephosphate led to diminished inhibition.

(2) Using the same system as Stickland. (*a*) It was shown that upon addition of 0·0005 *M* iodoacetate (to which triosephosphate dehydrogenase is known to be the most sensitive of the muscle enzymes) a condition was established in which glucose breakdown was greatly inhibited, whereas glycogen breakdown was little affected; (*b*) the inhibition by 0·0025 *M* glyceraldehyde, coming on about 8 min. after its addition, can be postponed for another 12 or 15 min. if extra triosephosphate dehydrogenase (in the form of the pure enzyme) is added to the system; (*c*) the inhibition can be prevented by addition of extra co-enzyme I (final concentration about 0·0003 *M*). The possible significance of this fact and of the prevention of inhibition by addition of traces of pyruvate[4,1] will be considered elsewhere; (*d*) inhibition was greater with D-glyceraldehyde than with an equivalent amount of DL-glyceraldehyde.

When this work was nearing completion, a paper by Rudney[5] appeared, describing the inhibition (60–80 per cent) of hexokinase activity in tissue slices and extracts by 0·004–0·001 *M* DL-glyceraldehyde. This inhibition (like the inhibition of glucose glycolysis in 4½-day chick embryos described by J. Needham and Lehmann[6] and in tumour tissue by Mendel *et al.*[7]) was not caused by D-glyceraldehyde and must be put down to the L-component.

Using rat muscle extract, we have confirmed Rudney's results under the conditions used by him (final concentration of glucose 0·043 per cent). It was found, however, that inhibition became progressively less with increasing glucose concentration, disappearing at about 0·11 per cent glucose with 0·0025 *M* glyceraldehyde. Since the glucose concentration in the system used by Stickland was about 0·32 per cent, the absence of hexokinase inhibition here is readily understood.

It is to be concluded that the inhibitory effect of DL-glyceraldehyde upon glycolysis in tissue slices and extracts depends upon more than one mechanism and will vary with conditions; one factor will be the concentration of glucose and of glyceraldehydephosphate at the hexokinase and triosephosphate dehydrogenase surfaces respectively. It remains a possibility that both D- and L-glyceraldehyde inhibit other enzymes; we found indications that glycerolphosphate dehydrogenase is also inhibited by D-glyceraldehyde.

These results will be described in detail elsewhere.

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D. M. NEEDHAM
L. SIMINOVITCH
S. M. RAPKINE

Biochemical Laboratory,
Cambridge,
and
Institut Pasteur,
Paris.
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Distribution of Protein in the Tissues of Rats Treated with Anterior-Pituitary Growth-Hormone

RATS treated with growth-promoting anterior-pituitary extracts[1] or with purified pituitary growth-hormone[2] gain more body-weight than pair-fed control animals. In experiments with rats, cats and dogs fed a constant amount of food throughout the experiment, Young[3] found that diabetogenic growth-promoting anterior-pituitary extract induced a substantial rise in body-weight even when, as with the