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Albert Siegel
S. G. Wildman

Department of Botany,

William Ginoza

Atomic Energy Project,
University of California,
Los Angeles.
Aug. 14.

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Enzymic Oxidation of Glyceric Acid

The reduction of hydroxypyruvate to glycerate is brought about by crystalline L-lactic dehydrogenase of muscle[1] with the production of L-glyceric acid[2]. A substrate-specific D-glycerate dehydrogenase, which may or may not be accompanied by lactic dehydrogenase, occurs in extracts of higher plants[2]. Hydroxypyruvic acid phosphate is not able to replace pyruvic acid as a substrate for lactic dehydrogenase[3]. These enzymes require diphosphopyridine nucleotide, and their equilibrium, being far towards the reduced metabolite, is unfavourable for the study of the oxidative production of hydroxypyruvate. The importance of this substance as a substrate for transketolase[4] and transaminase[5] led us to study the action on glyceric acid of preparations containing lactic dehydrogenase from yeast, which is not so inhibited by pyruvate and requires no coenzyme[6-8].

An aqueous maceration extract of dried Delft baker's yeast was fractionated by precipitation with ammonium sulphate (final concentration 0·65 saturated), dialysis against versene (10^{-4} *M* disodium ethylenediamine tetraacetate) and removal of interfering impurities by precipitation at *p*H 5 and adsorption on small quantities of calcium phosphate gel. Lithium hydroxypyruvate, $CH_2OH.CO.COOLi + 1\ H_2O$, was crystallized analytically pure from water, the molecule of water being very firmly bound and possibly constitutive (crystalline salts of this acid have not previously been reported). Hydroxypyruvate (and glycolaldehyde) were assayed, in the presence of glycerate, by a new colour reaction with naphthoresorcinol in 23 *N* sulphuric acid at 100° for 20 min., the absorption of the resulting green solution being read at 660 mμ. The enzymic reduction-rates with methylene blue as hydrogen acceptor showed widely different *p*H optima for lactate (*p*H 5·5) and glycerate (*p*H 6·7). D-Glyceric acid was not attacked, and with DL-glycerate the extent of oxygen uptake and of hydroxypyruvate formation indicated the oxidation of one isomer (L-glycerate) only. The ratio of oxidation-rates at *p*H 6·7 for glycerate/lactate was ½, and the Michaelis constants at 37° were approximately $3{\cdot}5 \times 10^{-4}$ *M* (calculated) for L-lactate and $2{\cdot}5 \times 10^{-3}$ *M* for L-glycerate. DL-3-Phosphoglyceric acid was oxidized at about half the rate of DL-glycerate under these conditions, but some phosphate was split off; D-3-phosphoglycerate was not oxidized. Cytochrome *c*, like methylene blue, is reduced by glycerate in the presence of the enzyme preparation.

It is not yet clear if the same enzyme is responsible for the oxidation of lactate and glycerate in these experiments. The widely differing *p*H optima and the statement of Boeri *et al.*[7] that their highly purified yeast lactic dehydrogenase (cytochrome b_2) is unable to oxidize "any other α-hydroxy acid" would indicate different enzymes; on the other hand, no summation of reaction-rate occurs when lactate and glycerate are both present with our preparations. Further purification of the enzyme, now being undertaken, should decide this point.

Frank Dickens
D. H. Williamson

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

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An Apparent Anomaly in the Distribution of Radioiodinated Serum Albumin when added to Red Cell Suspensions

When 15 ml. of suitably diluted (1 in 500) serum albumin tagged with iodine-131 ('*RISA*', Abbott) is added to an equal volume of washed human red cells with a volume concentration ρ of about 0·4, and when the red cells are thrown down by centrifuging at 7×10^2 *g* for 15 min., the radioactivity of the supernatant fluid is invariably 10–15 per cent greater than that found in a blank system containing 15 ml. of '*RISA*' plus 15 $(1 - \rho)$ ml. of saline, even after a correction is made for the volume of the blank system being smaller than the volume of the system with which it is compared. If the red cells are first rendered spherical by the addition of 1·5 ml. of a distearyl lecithin sol (5 mgm./5 ml.), the supernatant fluid obtained by centrifuging is 20–25 per cent more radioactive than the supernatant fluid from an appropriately prepared blank.

This apparently anomalous result is explained by the examination, with phase optics, of the respective supernatant fluids. While the red cells are thrown down by the centrifuging, the supernatant fluids from the systems to which '*RISA*' has been added contain minute fragments and myelin forms which are not thrown down even at a centrifugal force of 2×10^4 *g*. These fragments and myelin forms apparently take up '*RISA*'. They are not found when only distearyl lecithin is added to the red cells; they appear, usually as tiny spherical fragments, when '*RISA*' is added to the red cells, and usually as much more numerous worm-like myelin forms when '*RISA*' is added to red cells rendered spherical with lecithin. Their association with '*RISA*' accounts for the relatively high radioactivity of the supernatant fluids containing them, and suggests that the effect of serum albumin in reversing the disk–sphere transformation produced by lecithin is related to the loss of myelin forms from the red cell surfaces.