The enzyme was shown to catalyse the oxidation of 3-hydroxypropionaldehyde 3-phosphate in the presence of NAD^+ and arsenate (or phosphate). With phosphate a sixfold decrease in $V_{\max}$ is seen with little change in K_m . Aldehyde substrate inhibition was also observed and this proved to be uncompetitive against NAD^+ . These results suggest that the liberation of products is ordered with NADH being the last product released (Cleland, 1970).

Product inhibition studies showed NADH to be a competitive inhibitor against NAD^+ and non-competitive against the aldehyde and arsenate, thus confirming the order of product release.

3-Phosphoglycerate, inorganic nitrate and α -glycerophosphate were all shown to be effective dead-end inhibitors. The ordered release of products was further confirmed by the observation that 3-phosphoglycerate was an uncompetitive inhibitor no matter what the nature of the variable substrate. The ratio of the apparent inhibition constants [K_i (phosphate)/ K_i (arsenate)] was about 6, correlating with the $V_{\max}$ ratio for the two acyl acceptors. This suggests that with arsenate as the acyl acceptor the rate-limiting step is partly the release of NADH, whereas with phosphate it probably occurs in the quaternary-ternary complex interconversion.

Nitrate was found to be a competitive inhibitor of arsenate but uncompetitive against the aldehyde and NAD^+ . In contrast α -glycerophosphate, although an uncompetitive inhibitor for NAD^+ , was non-competitive towards the aldehyde and arsenate.

The occurrence of uncompetitive effects is characteristic of ordered sequential mechanisms. It is suggested that a compulsory-order mechanism is operating with the order of addition being NAD^+ , aldehyde and then phosphate (or arsenate) followed by the ordered release of products, first acyl phosphate and then NADH. This conclusion is discussed in relation to previous work on the kinetic mechanism of this enzyme (Furfine & Velick, 1965; Keleti & Batke, 1965; Fahien, 1966).

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The Lactate Dehydrogenase System of the Rudd (*Leuciscus erythrophthalmus*)

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The lactate dehydrogenase isoenzyme systems of many vertebrate species have been extensively studied (Latner & Skillen, 1968). Somatic cells of mammals and birds possess five electrophoretically and kinetically distinguishable forms of the enzyme. This corresponds with their possession of two genes coding for monomeric subunits of the tetrameric enzyme. A third type of subunit has been found in mammalian and avian sperm. Fish, however, have been shown to possess from one to 20 isoenzymes, according to species (Markert & Faulhaber, 1965). The explanation of this in molecular terms is more complex, but it is generally thought to be due to polymorphism of individual subunit-producing genes, or to the presence of several distinct genes.

We have studied the properties of the lactate dehydrogenase isoenzyme system in the rudd (*Leuciscus erythrophthalmus*), a freshwater fish and member of the Order Cypriniformes, which includes carp and goldfish. This latter species is the only member of this Order whose lactate dehydrogenase system has been extensively studied. Our investigations concern the chromatographic, electrophoretic and kinetic properties of the system.

Sephadex chromatography of rudd lactate dehydrogenase shows it to be very similar to the mammalian form in size. The rudd system is similar to that of mammals electrophoretically in possessing five major isoenzymes in most tissues. This is a feature uncommon in fish; Markert & Faulhaber (1965) found only three species to be of this type in an extensive survey. Differences exist between rudd and mammalian systems in both the mobilities of their isoenzymes and the tissue-specific predominance of fast-moving and slow-moving forms. Tissue-specific isoenzymes such as the eye form found in most fish, and the sperm form of mammals and birds, do not appear to be present in the rudd. The goldfish, another cyprinid, has a system different from that of the rudd in that many of its tissues possess up to 12 isoenzymes (Hochachka 1965).

Other properties studied in the rudd include specific activities and isoenzyme distribution among the tissues, effects of substrate and product inhibition, temperature, pH and denaturing agents on activity, and variation of the K_m with temperature.

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Immunoenzymology of Purified Urate Oxidase

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Urate oxidase (EC 1.7.3.3) was first obtained in pure form by Mahler, Hübscher & Baum (1953) from the mitochondrial fractions of pig liver. Baudhuin, Beaufay & de Duve (1965) have since shown that the enzyme, when present, occurs in the core of the peroxisomes. It was considered that a method of preparation from the mitochondrial fraction could be developed that first removed the contaminating mitochondrial protein.

Pig liver mitochondrial fraction, which contains the peroxisomes, was prepared by differential centrifugation. The mitochondria were lysed and solubilized by homogenization and treatment with deoxycholate. Removal of the contaminating mitochondrial proteins was then effected by high-speed centrifugation, the peroxisome pellet containing 90% of the urate oxidase. Subsequent treatment, including gel filtration, yielded a pure preparation of the enzyme.

Purified preparations of the enzyme were used to immunize both guinea pigs and rabbits. The antisera obtained were tested for cross-reaction with urate oxidase prepared from other sources. Reactions of complete identity were obtained with the enzyme from a mammalian source (bovine kidney) and partial identity with enzyme extracted from non-mammalian vertebrate sources (*Rhombus maximus* and *Pleuronectes platessa*). The antisera, however, failed to react with various microbial urate oxidases.

The stability of purified urate oxidase from mammalian and microbial sources was studied by heating samples (a) at 50°C for 10min and (b) at 70°C for 30min, and comparing the antigenic integrity with that of unheated controls. Pig liver enzyme and yeast enzyme differed from the fungal enzyme by giving a precipitin line on immunodiffusion after being heated at the lower temperature. Bacterial enzyme differed from all three, giving a positive result after being heated at either temperature.

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Measurement of Glucose Specific Radioactivity in Plasma

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Accurate determination of glucose specific radioactivity is essential for investigations of the kinetics of glucose metabolism in man and experimental animals. Two main procedures have been used previously. First, radioactive glucose has been separated from other labelled compounds present by chromatography. The second type of procedure involves the chemical conversion of glucose into one of a variety of derivatives. These methods frequently have several undesirable features, including several recrystallizations and low recoveries; this is of crucial importance in man, where only tracer quantities of isotope can be administered.

In this communication we report an enzymic method of determining the specific radioactivity of both [1-¹⁴C]glucose and [U-¹⁴C]glucose in plasma. The method involves a single procedure, is reproducible and is specific for glucose. It is based on the use of three coupled enzyme reactions to convert glucose into ribulose 5-phosphate and carbon dioxide.

In initial studies it was found that the total reaction goes virtually to completion spectrophotometrically. The sequential reaction was also demonstrated by radioautography. Subsequently the reaction was carried out in counting vials and radioactive glucose was determined by the disappearance of ¹⁴CO₂ after acidification. It was found that, over a tenfold concentration range of plasma glucose, the reaction always went to over 98% completion. Comparable results were obtained with both [1-¹⁴C]glucose and [U-¹⁴C]glucose. If [1-¹⁴C]glucose is used this results in total loss of all radioactivity due to glucose as ¹⁴CO₂. [U-¹⁴C]Glucose can also be used, resulting in a loss of one-sixth of the radioactivity. Also, the plasma volume can be increased from 0.1 to 1.0ml without loss of sensitivity, and in practice gives excellent recoveries when other ¹⁴C-labelled compounds are present. The entire radioactivity assay is carried out in a scintillation vial and involves only one critical addition, namely the plasma or standard.

The method can be adapted for measuring the