Biosynthetic Preparation of ^{14}C-labelled 2-Deoxy-D-ribose and Thymidine by Means of *Thiobacillus denitrificans*

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In connection with studies on the metabolism of 2-deoxy-D-ribose and thymidine, it became desirable to perform experiments with labelled material. The possibility of obtaining ^{14}C uniformly labelled DNA as a source for these substances has been considered. It was concluded that the use of an autotrophic organism like *Thiobacillus denitrificans* which can be grown in the dark on a large scale in deep culture under controlled conditions, would offer a simple and satisfactory solution. The strain of *Thiobacillus denitrificans* used was isolated from the fresh water mud of an Oslo lake. The medium and technique used for the large scale cultivation of this organism has been described previously [1]. The organism was grown in the presence of $Na_2{}^{14}C\,O_3$ and utilized 98 % of the total activity present. The cells were harvested from a 50 % ethanol solution and the supernatant contained 20 % of the total activity. The cell residue was treated with 96 % ethanol and, after drying, extracted twice with 10 % NaCl at 100° C. The nucleic acids were precipitated with ethanol, redissolved in 10 % NaCl and reprecipitated. From 17 g of dried cells, 26 mg DNA (as estimated by the Dische test) and 58.4 mg RNA (as estimated by the Euler and Hahn test) were obtained. DNA was separated from RNA by treatment with KOH and acid precipitation. 2-Deoxy-D-ribose and thymidine were isolated from DNA by a method described previously [2]. The mixture of labelled thymidine and 2-deoxy-D-ribose was separated by paper chromatography and isolated by the elution of the appropriate areas of the chromatogram. There was isolated 0.625 mg thymidine (2.58 μM) and 0.347 mg (2.59 μM) of 2-deoxy-D-ribose, with a total activity of 0.93×10^6 c/min and 0.51×10^6 c/min, which corresponds to an activity of 0.36×10^5 c/min/μM carbon and 0.32×10^5 c/min/μM carbon respectively. The total activity of 2-deoxy-D-ribose and thymidine together represented 0.2 % of that taken up by the cell residue.

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Kinetics of Fatty Acid Dehydrogenation

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Four flavoproteins are involved in the first dehydrogenation step of the fatty acid cycle in pig liver [1-3]. Some of the kinetic properties of this system have now been investigated with 2,6-dichlorophenolindophenol as electron acceptor. From these studies the following three step mechanism of reaction appears likely: Electrons are passed from substrate to a primary dehydrogenase; thence to a specific electron transferring flavoprotein; and finally to indophenol. The interaction of the two enzymes concerned with their respective substrates, *i. e.* fatty acyl CoA and reduced primary dehydrogenase, follows Michaelis-Menten behaviour. In the primary dehydrogenation reaction a pronounced product inhibition was observed. The pH optima ranged from 7.6 to 8.4 for the three primary dehydrogenases, when these enzymes were rate limiting. When the transfer step was rate limiting, the optimum was at pH 6.8.

Theoretical considerations reveal that oxidation enzyme systems with tightly bound prosthetic groups, such as the system studied, have special kinetic properties. Michaelis constants and turnover numbers of these enzymes cannot be obtained when overall reactions are measured. The observed apparent Michaelis constants and turnover numbers give the kinetic characteristics of the total substrate-enzyme-acceptor system.

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