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PRELIMINARY COMMUNICATION

ETHER - O - OXIDASE

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Diethylether has a long metabolic history. Green and Cohen (1) gave labelled ether to a mouse and found that, though much was excreted in the urine unchanged, part came out as CO_2. This metabolic pathway is therefore of much interest. M. Shorthouse and I were led to look at this pathway by a chance observation upon the liver microsomes, collected between 12,000-100,000g from a rat killed with diethylether (2). Traces of ether left in the microsomes caused an extra O_2 uptake. The effect was specific for these microsomes. It was not given by the final supernatant from the 100,000g fraction. It was proved to be due to the formation of an 'acetyl' moitey, by showing that when our 100,000g homogenate was suitably reinforced with synthase, a source of oxaloacetate, CoA and a buffered solution of pH 7.2-7.4, the addition of diethylether in very small amounts formed citrate, as well as taking up O_2 (Fig.1).

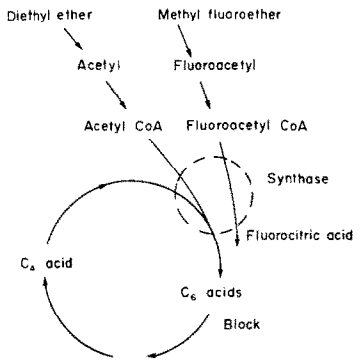

Fig. 1. Diagrammatic representation of the pathway of synthesis of citrate or fluorocitrate from alkyl ethers. Suggested reactions:

$$C_2H_5.O.C_2H_5 \rightarrow CH_2{=}CH.OH + H_2O_2 \rightarrow CH_3.CHO \rightarrow CH_3.COOH \quad (1)$$

$$CH_3.O.CH_2.FCH_2 \rightarrow CH.OH = FCH + H_2O_2 \rightarrow CHO.FCH_2 \rightarrow FCH_2.COOH \quad (2)$$

Figure from J. Physiol. 252 by permission of Editors.

This observation proved that a scission product of the ether, which we can write as $-CH_2.CH_2-$ formed acetyl CoA, and was therefore metabolised via the tricarboxylic acid cycle. This pathway was confirmed further by studying the fate of a specimen of fluorether, kindly supplied by Drs. P.W. Kent and D. Dimitrijevich (3) when incubated with the 100,000g microsomes. Here fluorcitrate was formed by addition of diethylether, with its usual blocking effect on citrate metabolism. Concluding that we were dealing with a special enzyme, we went on to study its properties, and ultimately to attempt a separation from the 100,000 membranes. Naturally we thought that it must be a known enzyme, and were surprised at the start to find no evidence that previous dosing of a rat with phenobarbitone increased the 'etherase' effect. So the enzyme was not inducible. On the advice of Prof. M. Dixon, F.R.S., we called it an ether-O-oxidase. This 100,000g fraction also gave an O_2 uptake with isopropyl ether and with n-butyl ether; in the latter case we detected traces of butyric acid after incubation. But, from this point, the research became a constant matter of surprises. The enzyme factor was clearly not the same as that described by Axelrod (4) for aromatic ethers. It was found also in the same microsomal fraction in the liver of the pig, the guinea pig and the pigeon, appearing to be the general liver factor. Rat heart and kidney did not show it. Most curious of all, we could only find traces of acetaldehyde, and this with much difficulty as the result of enzyme action. The O-oxidase did not require a pteridine for activity and so differed from that of Tietz et al. (5) for plasmalogen. Nor did it respond to -SH reagents. It was not a catalase or peroxidase as neither of these enzymes gave an O_2 uptake with diethylether.

In order to progress further, we embarked on an isolation of the enzyme from the microsomal membranes, using pig liver which we could store as membranes at $-20^{\circ}C$, without loss of activity. After an unsuccessful trial of a large number of modern reagents, (detergents etc.), we succeeded in extracting the ether-O-oxidase with old-fashioned ammonium sulphate, obtaining two active fractions at concentrations of 15-25% (A) and 35-45% (B) ammonium sulphate. Fraction A contained some of the original microsomes. Fraction B contained the ether-O-oxidase in an impure form which we could not purify further. Fraction B contained some 14 proteins as seen in acrylic gel electrophoresis. The enzyme as isolated could be kept at $0^{\circ}C$ for 2-3 days, but lost activity on freezing. It was most active at pH 9.0, losing activity at pH 5.0. With the addition of NADPH, lipid peroxidation or oxidation (6) was unchanged by adding ether to the fraction B, no malondialdehyde being formed. Further more, oxidation of diethylether did not involve cytochrome P450; and it was unchanged by adding a dismutase or by the addition of xanthine. Some enzymes however were present, viz isocitrate and matate dehydrogenases and aconitate-hydratase.

The ether-O-oxidase gave no O_2 uptake with lanosterol or a pure specimen of cholesterol or with 7 -hydroxy cholesterol. So our attempts to prove it was a known enzyme and to find a metabolic function for it failed; we suggested that its normal function was to deal with some unknown plant products; some colour was given to this hypothesis by the fact that the enzyme oxidized the O-methyl aromatic compound guiacol.

More recently, some new work has suggested another tentative hypothesis. Though cholesterol itself gave no O_2 uptake with our ether-O-oxidase, a less pure aged specimen gave an O_2 uptake. I have found that a very small amount of this aged cholesterol (in cyclohexane solution) can lower the interfacial tension (I.T.) between an aqueous buffered phosphate solution (about pH 7.0) and the cyclohexane phase (7), which I attributed to the formation of cholesterol hydroperoxide. In subsequent unpublished observations, I have found that various tocopherol (Vit.E) preparations can eliminate the compound causing the lowered I.T. This leads to the speculative hypothesis that one of the normal functions of the liver ether-O-oxidase is to eliminate possible formation of the hydroperoxide of cholesterol. But it must be emphasized that this will require much more work, when one realizes that in a recent symposium, it was stated that a specimen of cholesterol kept in air can form some 50 oxidation products (8).

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