Short Communication

The Formation of Propionate from the Side-Chain of Cholesterol in an Infant with an Inborn Error in the Metabolism of Propionate

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Mitropoulos & Myant (1965*a,b*) have shown that the product of cleavage of the cholesterol side chain by isolated liver mitochondria in the presence of the cell sap is propionate or propionyl-CoA. Attempts to show the formation of [^{14}C]propionate from [$^{26}\text{-}^{14}\text{C}$]cholesterol in intact animals have not, however, been successful (K. A. Mitropoulos & N. B. Myant, unpublished work), presumably owing to rapid enzymic oxidation of [^{14}C]propionate formed by the cleavage of the cholesterol side chain. In this communication we report the formation of radioactive propionate from [$^{26}\text{-}^{14}\text{C}$]cholesterol in an infant who died from propionicacidaemia due to an inherited deficiency of propionyl-CoA carboxylase, the enzyme catalysing the first step in the oxidation of propionyl-CoA in animal tissues. Clinical details of this infant and the demonstration of a deficiency of propionyl-CoA carboxylase activity in his liver have been reported elsewhere (Gompertz, Storrs, Bau, Peters & Hughes, 1970).

Methods. On the eighth day of life the infant was given an intravenous injection of [$^{26}\text{-}^{14}\text{C}$]cholesterol (1.3mg with $35\mu\text{Ci}$) emulsified with Tween 20 in 0.9% NaCl. He died from respiratory failure 4 h later. Immediately after death, blood was obtained from the heart and a portion of the liver was removed and homogenized in ice-cold 0.25 M-sucrose. Nuclei, cell debris and mitochondria were removed from the homogenate by centrifugation at 600*g* and 12000*g*. The supernatant was used for the subsequent analysis. A portion of the serum was taken for assay of total radioactivity. The remainder (6.5ml) was acidified and extracted with 20 vol. of chloroform-methanol (2:1, v/v). The filtered extract was separated into two phases by the addition of 0.2 vol. of 0.1 M-NaOH. The upper phase was removed, evaporated to dryness, acidified with H_3PO_4 and steam-distilled. The steam-distillate was neutralized with NaOH, evaporated to dryness under reduced pressure and dissolved in water. A portion was taken for radioassay and the remainder was evaporated to dryness. The residue was dissolved in 1ml of 0.1 M- H_2SO_4 and analysed by column

chromatography (Swim & Utter, 1957). The fractions eluted from the column were titrated with 0.02 M-NaOH and assayed for radioactivity. The same procedure was used for the extraction, analysis and radioassay of the liver homogenate.

Results and discussion. About 2% (9200 d.p.m.) of the total radioactivity in the serum was recovered in the steam-distillate. About 65% of the radioactivity in the steam-distillate (representing 1.26% of the total radioactivity in the serum) was recovered from the column as a well-defined peak corresponding to the titration peak of propionic acid. The amount of propionic acid recovered from the column was equivalent to 4.8 mm in the original serum, compared with 5.06 mm determined by g.l.c. A portion of the eluate corresponding to the titration peak of propionic acid was taken for preparation of the *S*-benzylisothiuronium salt of propionic acid (Vogel, 1951) after the addition of non-radioactive propionic acid. The crystalline salt (m.p. 151°C) was recrystallized from hot water. The specific radioactivity of the crystals was 6.1 d.p.m./mg after the first recrystallization and remained constant thereafter.

Analysis of the liver homogenate gave qualitatively similar results, but the specific radioactivity of the propionic acid obtained from the liver (3.43×10^5 d.p.m./mmol) was higher than that obtained from the serum (2.04×10^5 d.p.m./mmol).

These results show that propionate is formed from the side chain of cholesterol in the intact human being as well as in the mitochondrial preparation described by Mitropoulos & Myant (1965*a*). The daily production of bile acids in a normal adult corresponds to the removal of the three terminal carbon atoms from the side chain of about 1% of the exchangeable cholesterol in the whole body. The rate of production of propionate from [$^{26}\text{-}^{14}\text{C}$]cholesterol observed in the present work was several times higher than this. However, this difference is to be expected, since the injected cholesterol would not have mixed with more than a small proportion of the total exchangeable cholesterol during the

4h for which the infant survived after the injection. During the conversion of cholesterol into steroid hormones in the adrenal cortex, 4-methylvaleraldehyde (isocaproaldehyde) is removed from the side chain. The 4-methylvaleraldehyde arising from [26- ^{14}C]cholesterol would be radioactive and might give rise to radioactive propionate. The possibility that a small proportion of the [^{14}C]propionate formed in the infant's body arose in this way cannot be excluded. However, the specific radioactivity of the [^{14}C]propionate isolated from the liver homogenate was higher than that isolated from the serum. Propionate must therefore have been formed from cholesterol in the liver. Since the enzyme systems required for removing a 4-methylvaleryl unit from the cholesterol side chain are not present in liver (Dean & Whitehouse, 1966), the formation of propionate from cholesterol in this infant's liver presumably took place by cleavage

between C-24 and C-25, as in isolated liver mitochondria in the presence of the cell sap.

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