The Incorporation of ^{14}C from $NaH^{14}CO_3$ into the Soluble Metabolic Intermediates of Animal Tissues

SIR,—A considerable amount of information is available about the non-photosynthetic fixation of radioactive carbon dioxide by microorganisms (cf. Lynch and Calvin, 1953). However, relatively little is known about the incorporation of $^{14}CO_2$ into animal tissues. Katz and Chaikoff (1955) showed that rat liver slices incorporated between 4–10 per cent of the radioactivity from labelled bicarbonate and that the isotope was distributed amongst the metabolic intermediates as follows: 40–60 per cent in urea; 0–25 per cent in lactate; 10–20 per cent in glucose; 5–15 per cent in alanine; 5–10 per cent in glutamate and smaller quantities in organic acids. We have found that chopped preparations

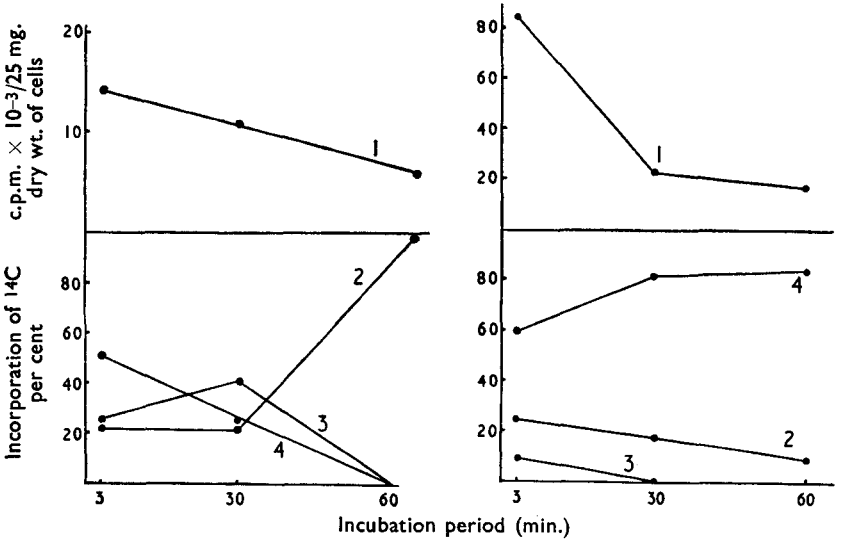

FIG. 1. Incorporaton of ^{14}C from $NaH^{14}CO_3$ into the soluble metabolic intermediates by HeLa cells.

FIG. 2. Incorporation of ^{14}C from $NaH^{14}CO_3$ into the soluble metabolic intermediates by transplantable mouse sarcoma cells.

1. Total ^{14}C. 2. Lactic acid. 3. Tricarboxylic acid. 4. Amino-acids.

of rat liver (McIlwaine and Buddle, 1953) incubated with $NaH^{14}CO_3$ gave an almost identical pattern but that in rat liver homogenates most of the incorporated radioactivity appeared in the amino-acid fraction as follows: 40–50 per cent in glutamate; 10–20 per cent in γ-aminobutyrate; 5–10 per cent in aspartate and only 5–10 per cent in lactate. Suspensions of rat liver mitochondria incorporated over 80 per cent of the ^{14}C into the malate and citrate. Thus, the mitochondria incorporated the radioactivity principally into tricarboxylic acids, presumably via the carboxylation of pyruvate to yield oxaloacetate or malate, whereas in the homogenate the main end products were amino-acids derived by transamination reactions from the tricarboxylic acids. The absence of labelled glucose and urea in the mitochondrial and homogenate experiments suggests that the synthesis of these substances from bicarbonate needs a relatively intact cellular structure as is present in the liver slices and chopped preparations.

Other animal tissue preparations showed different patterns of incorporation. Chopped preparations of rat kidney incorporated 50–60 per cent of the isotope

into amino-acids (glutamic and aspartic acids) and 30–40 per cent into lactate; similar preparations of rat brain showed a 60 per cent incorporation into lactate and 30–40 per cent incorporation into di- and tri-carboxylic acids. Human foetal liver cells (Westwood, MacPherson and Titmuss, 1957) and HeLa cells in culture incorporated most of the isotope into lactate, tricarboxylic acids and amino-acids but the relative proportions of incorporation into these fractions changed with increasing time of incubation. With the HeLa cells, the extent of incorporation of radioactivity in amino-acids was substantially reduced as the incubation proceeded, that into the tricarboxylic acids reached a maximum at 30 min. and then rapidly decreased and lactate remained as the sole labelled end product after 60 min. (Fig. 1).

Transplantable mouse sarcoma 180 cells showed a different behaviour (Fig. 2). The radioactivity in the amino-acids increased with time of incubation whereas the reverse effect was observed to occur with the lactate and tricarboxylic acids.

5 mM salicylate or 0·5 mM 2,4-dinitrophenol reduced by 80–90 per cent the total incorporation of ^{14}C from the labelled bicarbonate into the soluble metabolic intermediates of the chopped tissue preparations except rat brain with salicylate where increases of 20–30 per cent were observed. A similar difference between brain and other isolated rat tissues with respect to the effects of salicylate on the incorporation of ^{14}C from labelled glucose has been reported by Smith and Moses (1960).

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C. Bryant.
A. K. Huggins.
M. J. H. Smith.

Department of Chemical Pathology,
King's College Hospital Medical School,
Denmark Hill, London, S.E.5.
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