



Fig. 2. Formate- ^{14}C uptake by human bone marrow cells *in vitro* in 24-hr. culture with $0.75 \mu\text{c./ml.}$ formate- ^{14}C medium. Smear alcohol fixed, not acid hydrolysed. Stained autoradiograph, $\times 2,000$. Note the localization of the isotope in the chromosomes

uptake by the cells. The difference in the specific activities (1.04 mc./mM for formate- ^{14}C , and 1.56 mc./mM for adenine- ^{14}C) was compensated for in the exposure times of the autoradiographs, namely, ten days for adenine- ^{14}C slides and seventeen days for formate- ^{14}C slides. The grain counts on the acid-hydrolysed slides indicated a similar amount of carbon-14 uptake in both cases, the average maximum uptake being of the order of 1.2×10^7 atoms of deoxyribonucleic acid- ^{14}C .

However, the comparison of the non-hydrolysed and acid-hydrolysed preparations from the formate- ^{14}C cultures indicated scarcely any change in the average maximum grain counts after hydrolysis (see Fig. 1). The failure of acid hydrolysis to remove appreciable amounts of carbon-14 from the cells, together with the predominantly nuclear (and chromosomal) localization of the isotope on the non-hydrolysed preparations (Fig. 2), indicated that, as opposed to adenine- ^{14}C , most of the formate- ^{14}C taken up by human bone marrow cells *in vitro* is incorporated into deoxyribonucleic acid, and only a very small fraction is incorporated into other compounds (for example, ribonucleic acid).

These findings are at variance with those obtained in rat liver with formate- ^{14}C ^{4,5}; but the cause of the difference may lie in the different rates of synthesis of deoxyribonucleic acid in bone marrow and resting liver. There is evidence that in chick bone marrow slices more than 70 per cent of the formate- ^{14}C in deoxyribonucleic acid is found in the methyl group of thymine⁶, and preliminary experiments in our laboratory also indicate that probably most of the formate- ^{14}C administered to human bone marrow cells *in vitro* is incorporated into thymine in deoxyribonucleic acid.

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Cocarcboxylase Activity of Thiamine Phosphoric Esters

AFTER the chemical synthesis of thiamine phosphoric esters was achieved¹⁻⁴, reports were published on their cocarcboxylase activity⁵⁻⁷. Investigations have shown that monophosphothiamine has no cocarcboxylase activity, but different values have been found for the activity of triphosphothiamine. The discrepancies may be accounted for by the facts that: (a) chemical synthesis provides mixtures of three esters (mono-, di- and tri-phosphoric) which are very difficult to separate; (b) diphosphothiamine is very active; and therefore quantities as small as $0.1 \gamma/\text{ml.}$ occurring as impurities with the other esters invalidate the results; (c) washed yeast, which has usually been employed as apoenzyme, makes it difficult to reproduce results.

We have separated the esters by paper chromatography⁷, employing Whatman No. 1 paper and *n*-propanol-water- $1 M$ acetate buffer, pH 5 ($70:20:10$), as the solvent.

The position of each ester was established by developing duplicate spots in parallel and spraying one of the series. Fragments of strips not sprayed were placed in Warburg flasks and compared with known amounts of chromatographically pure diphosphothiamine under identical experimental conditions.

As apoenzyme we have used purified apocarcboxylase according to Green *et al.*⁸. Each flask contained: an amount of apoenzyme which in the presence of excess (80γ) of diphosphothiamine produces carbon dioxide at $4.0 \times 10^3 \mu\text{l./hr.}$; 0.27 mgm. of magnesium ions and 0.19 mgm. of manganese ions; in the side bulb, 0.4 ml. of $1.09 M$ sodium pyruvate; $0.04 M$ citrate buffer, pH 6.0, making up the volume to 3.0 ml. ; temperature, 30°C. ; atmosphere, air; shaking, $160-170$ oscillations/min. The rate of evolution of carbon dioxide was determined by a graphical method⁹. The manometers were read every 2 min. after allowing 15 min. for the establishment of equilibrium. The quantities of each ester were established by colorimetric determination of phosphorus¹⁰.

The following results were obtained: (1) monophosphothiamine at concentrations of $0.025-0.13 \mu\text{mol./ml.}$ did not manifest any cocarcboxylase activity; (2) diphosphothiamine at a concentration of $0.04 \mu\text{mol./ml.}$ liberated carbon dioxide at the rate of $2.3 \times 10^3 \mu\text{l./hr.}$; (3) triphosphothiamine at concentrations of $0.005-0.020 \mu\text{mol./ml.}$ did not manifest any cocarcboxylase activity.

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