acids into protein and indicate that the functional form in the animal is similar to that found by BARKER *et al.* for the microorganism *Clostridium tetanomorphum.*

It was also reported earlier from this laboratory[4] that vitamin B_{12} may be involved in the activation of amino acid; however, recent studies carried out using hydroxylamine as the trapping agent show no lowering of hydroxamate formation in the deficient-liver preparations.

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Mono- and dimethylethanolamine isolated from rat-liver phospholipids

It is well known that ethanolamine and a series of "single carbon unit donors" serve as precursors of choline in the living animal. It is presumed that mono- and dimethylethanolamine are intermediates in this biosynthesis. This idea is supported by the isolation of these compounds from the phospholipids in mutant strains of *Neurospora crassa*[1], but so far there has been no report on the isolation of these compounds from animal tissue.

We have recently studied choline synthesis in the living rat, using [*Me*-^{14}C]-methionine as the precursor.

In short-time experiments we have been able to isolate small amounts of radioactive mono- and dimethylethanolamine from rat-liver phospholipids (Fig. 1). The identification of these compounds has been verified by the following procedures: (1) They are eluted off a Dowex 50 column exactly in the positions of mono- and dimethylethanolamine (Fig. 1). (2) They move identically with these compounds in a paper chromatographic system which separates ethanolamine, mono- and dimethylethanolamine and choline (50 g phenol, 50 ml *n*-butanol, 3 ml 80 % formic acid, 10 ml water). The mixture was shaken with solid KCl and the paper pretreated with 1 *N* KCl. The KCl prevents tailing on the paper. R_F values found were: ethanolamine, 0.13; methylethanolamine, 0.38; dimethylethanolamine, 0.63; choline, 0.75. (3) The compounds are converted to choline when treated with alkaline methyl iodide. The radioactive compounds were eluted from the paper, the appropriate carrier mono- or dimethylethanolamine, alkali and alcoholic methyl iodide were added and the mixtures were shaken at 45° for 2 h. The choline formed was precipitated as the reineckate and subsequently recrystallized three times from acetone with *n*-propanol. The choline reineckate from the methylethanolamine showed a specific activity of 11, 9 and 10 counts/min/mg after one, two and three recrystallizations, respectively; the choline reineckate from the dimethylethanolamine 67, 66 and 68 counts/min/mg.

As seen from Fig. 1, the radioactive mono- and dimethylethanolamine were found in the phospholipids of the rats killed 20 and 40 min after the injection of radioactive methionine, but not in the animal killed after 80 min. This shows that the turnover rate of these methylated compounds indeed is very high compared to what is known about the turnover of other phospholipid bases.

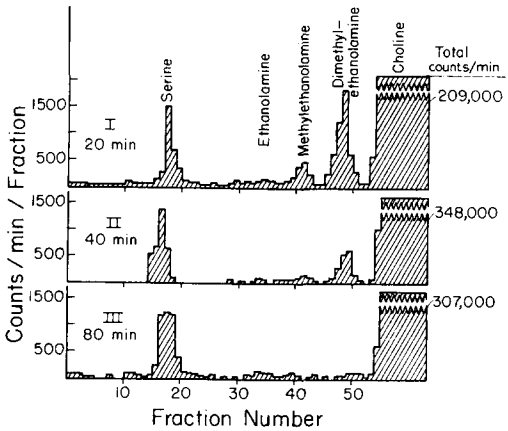

Fig. 1. Distribution of radioactivity in column chromatograms of phospholipid bases. Three rats were each injected intraperitoneally with about 6 μmoles [*Me*-^{14}C]methionine (about $1.2 \cdot 10^6$ counts/min) in 1 ml 0.9% NaCl and killed after 20, 40 and 80 min (I, II and III). The livers were immediately dissected out, washed in cold water and homogenized in 90 ml 67% ethanol and centrifuged. The residues were washed three more times with 67% ethanol. The combined supernatant and washings were concentrated and the extracts were then shaken with two portions of choroform (50 and 30 ml). The chloroform-ethanol layer was used in several portions for extraction of the lipids from the liver residues[2]. The chloroform-ethanol extracts were evaporated to dryness and the lipids extracted from the residue with petroleum ether. The petroleum ether extracts were washed with a little water. The phospholipids were subsequently precipitated twice from petroleum ether with 8–10 vol. acetone at —10°. The crude phospholipids were then refluxed with 3.8% HCl for 16–20 h, filtered, and the filtrate evaporated to dryness. The residues were chromatographed on Dowex 50-H^+ (200–400 mesh) columns (1 × 50 cm) and eluted with 1.5 *N* HCl[3], flow rate 0.4 ml/min. Fractions of approximately 5.5 ml were collected.

In addition to following the appearance of [*Me*-^{14}C]methionine in the phospholipids, we also followed the incorporation of radioactivity into cytidinediphosphocholine. The maximum activity in this compound was reached 1–2 h later than that in the phospholipid choline, which reached its maximum after about 40 min.

These results, we think, indicate that the methyl acceptor in the synthesis of choline is the fully formed cephalin molecule, and not the free ethanolamine, phosphoethanolamine or cytidinediphosphoethanolamine, which are the known precursors in cephalin synthesis.

A detailed report on these findings will be published shortly.

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