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The *in vivo* formation and turnover of *S*-adenosylmethionine from methionine in the liver of normal rats, of animals fed dimethylnitrosamine, and of partially hepatectomised animals

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S-ADENOSYL-L-methionine (SAM) was first shown by Cantoni[1] to be the form in which methionine acts as a methyl donor. The metabolic importance of SAM is illustrated by the fact that it is the active intermediate in more than 40 transmethylation reactions. Thus it is involved in methylation of diverse compounds of low molecular weight,[2] and also of macromolecules including myosin,[3,4] nuclear protein,[5] transfer RNA[6] and DNA.[7] The metabolism of these compounds *in vivo* is often studied by giving methionine labelled in the methyl group to intact animals, and later the extent of labelling of SAM and of the compound under investigation are determined. This approach assumes knowledge of the rate of synthesis of SAM from methionine, the specific radioactivity of SAM which is attained, and also of the time period for which the specific activity is high in the tissue under consideration. However, the rapid rate of turnover of SAM[8] has not always been appreciated; the specific activity of SAM reaches a peak within approx. 10 min of injection of methionine and then rapidly decreases. Therefore measurements made at 1 hr[9] are difficult to interpret.

During investigations of biochemical changes involved in carcinogenesis, the methylation of transfer RNA[10] and of DNA [11] was studied after injection of [^{14}C]methionine into animals fed a diet containing dimethylnitrosamine. The results suggested that t-RNA became more highly methylated in precancerous liver, but that the methylation of DNA increased only in proportion to the increased rate of synthesis. To determine how far these changes might be explained not by altered methylation of nucleic acids but by altered metabolism of the methyl donor, SAM, it was necessary to study the turnover of SAM during carcinogenesis. This was of special interest as there is in fact evidence for deranged l-carbon metabolism during carcinogenesis. Thus there is evidence for an increased concentration of SAM in leukaemic white cells[12] and in a neuroblastoma,[13] and for a decreased level of SAM synthetase, ATP: L-methionine adenosyl transferase EC 2.4.2.13, in a mouse hepatoma[14] and in Novikoff hepatoma.[15] The turnover of SAM was therefore studied in the liver of rats fed the carcinogen dimethylnitrosamine, under conditions used in the previous study of methylation of t-RNA and DNA.[10,11]

There is little information concerning the formation of SAM in dividing cells. This is of interest as replicating cells are a suitable system for studying how closely methylation of DNA, t-RNA and histones is geared to the synthesis of these macromolecules. The specific activity of SAM was therefore studied in regenerating liver at different times after administration of [^{14}C]methionine.

MATERIALS AND METHODS

L-[Me^{14}C]methionine, 56·8 mCi/m-mole, was purchased from The Radiochemical Centre, Amersham, Bucks.

Female rats, 190–210 g body wt, were used.

The animals treated with labelled methionine were either normal rats, or animals which had been partially hepatectomised by the method of Higgins and Anderson.[16] Female rats were fed a diet containing 50 ppm dimethylnitrosamine from the time they were 100 g body wt, for a period of 18 weeks, by which time the body wt was approximately 200 g. Under this feeding regime rats now begin to die with liver tumors after 23 weeks instead of at 26 weeks as recorded previously.[17] At 18 weeks the gross anatomy of the liver is abnormal. Very often the two parts of the median lobe are of unequal size, one having

enlarged and the other having decreased in size, and the left lateral lobe tends to increase in size while the caudate and right lobes become smaller.

Animals were given a single trace dose of labelled methionine by i.p. injection, at first 100 μCi/animal, and in later experiments 50 μCi/animal. Preliminary experiments showed that when 100 μCi [^{14}C]methionine was given, the specific activity of SAM was double that reached when 50 μCi were used. At the appropriate time the animal was killed by cervical dislocation and the liver was rapidly removed. SAM was isolated by a modification of the method used previously.[18] An $HClO_4$ extract of liver was chromatographed on a column of Dowex-50 × 8H$^+$ 12 × 1 cm, using a 1N/4N HCl exponential gradient. The E_{257} was measured on each fraction of the effluent. The peak eluting after adenine contained only SAM and *S*-adenosyl-homocysteine. This was evaporated to dryness by rotary evaporation at 30–40°. The residue was redissolved in 1N HCl and the column chromatography repeated. Adenosyl-homocysteine is more acid labile than is SAM,[19] and preliminary experiments showed that it decomposed quantitatively during rotary evaporation to give products which eluted before SAM on the second chromatography column. A pure peak of SAM was thus obtained, preliminary experiments showing a recovery of 88 per cent.

From each 10 ml fraction of the SAM peak, 5 ml were evaporated to dryness in a stream of warm air and the radioactivity was determined after solubilisation in hyamine solution (1 ml) and addition of scintillator (10 ml) composed of 2,5 diphenoxazole (0·6 per cent in toluene). The remaining 5 ml of each 10 ml fraction were pooled, evaporated to dryness, and examined by paper chromatography in ethanol–acetic acid–water(65 : 1 : 34). The paper chromatogram was examined under u.v. light, then cut crosswise into thin strips which were eluted with 1N HCl and the radioactivity in the eluates determined after evaporation to dryness as described above.

RESULTS AND DISCUSSION

The specific radioactivity of SAM was calculated from the E_{257} and the total dpm of the peak of SAM eluted from the second successive chromatography column. Paper chromatography confirmed that SAM was the only radioactive compound present. The results for SAM isolated from normal rat liver of animals killed at different times after injection of [^{14}C]methionine are shown in Fig. 1. The specific activity reaches a maximum at approximately 15 min after injection and then decreases rapidly. A similar result for normal animals has been found previously.[8] Labelling of macromolecules by methylation after injection of methionine has therefore almost finished within 1 hr of treatment. There seems to be no justification for the

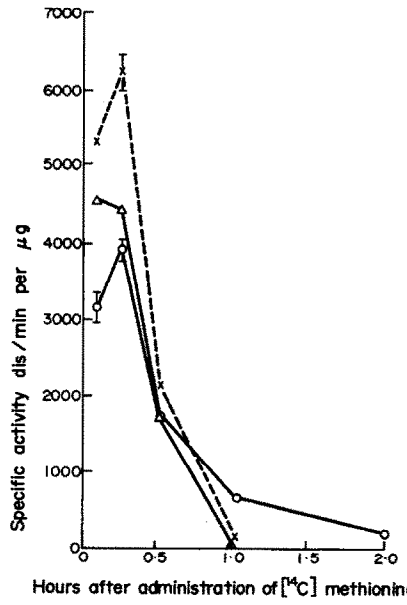

FIG. 1. Specific radioactivity of *S*-adenosylmethionine of liver after administration of [^{14}C]methionine to normal (○) precancerous (△) and partially hepatectomised (×) rats. In most experiments, the dose of [^{14}C]methionine was 100 μCi/rat. Where 50 μCi were used, the specific activities were doubled to allow comparison of results. Each point represents an experiment on a single animal. Duplicate experiments were done at certain times as shown.

statement that maximum concentration of labelled SAM occurs 1–3 hr after administration of methionine.[20]

The average concentration of SAM in normal rat liver calculated from these experiments was 30 ± 3 μg/g wet wt. This agrees fairly well with values found previously using different methods of estimation, which have been 31,[21] 33,[22] 24[23] and 21[24] μg/g.

The turnover of SAM in livers of animals fed a diet containing dimethylnitrosamine for 18 weeks is similar to the normal rate (Fig.1). The average concentration of SAM from a total of six experiments on precancerous liver was 34 ± 4 μg/g fresh wt, i.e. it was not significantly different from normal liver. It therefore seems unlikely that the increased labelling of the methylated bases of t-RNA which was found to occur during carcinogenesis,[10] in which the increased labelling of the methylated guanine residues approached 100 per cent, could be explained by an increase in the extent or duration of labelling of SAM.

In animals studied 24 hr after partial hepatectomy there is also a similar rate of turnover of SAM and the average concentration of SAM found in these experiments was 33 ± 6 μg/g fresh wt of liver i.e. it did not differ significantly from normal. The higher specific activity at short time periods after injection may be due to the fact that after excision of 2/3 of the liver more labelled methionine is available to the remnant per unit liver weight.

Thus in normal liver there is a rapid turnover of SAM, the peak specific activity being reached at approximately 15 min after injection of [^{14}C]methionine. When the metabolic activity of liver is increased by cell replication as in the precancerous condition produced by feeding dimethylnitrosamine, there is no significant alteration in the the rate or extent of labelling of SAM. It is the specific activity of SAM which is relevant in interpretation of measurements of methylation of RNA and DNA in normal and precancerous animals. Alterations in, for example, the pool size of methionine, or in the blood supply to the premalignant liver, would affect the level of macromolecular methylation only indirectly via an effect on the synthesis of SAM. Therefore, as a change in the labelling of SAM is considered not to be responsible for the increased labelling of t-RNA which had been found to occur in precancerous liver after injection of [^{14}C]methionine, this increase in labelling is thought to represent an actual increase in the extent of methylation of t-RNA.

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