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## Organ and Intracellular Location of the Methionine Methyl Group Synthesizing System of the Rat.* (29123)

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Many publications have dealt with the *de novo* biosynthesis of the methionine methyl group by animal systems *in vitro*(1-5), but no work has been reported on the organ and intracellular location of this biosynthetic system. This is the purpose of this communication.

*Procedures.* To determine which organs were the most active in synthesizing methionine methyl groups, 2 rats were used in each of 3 experiments. The brain, pectoralis muscles, kidneys, and liver were removed immediately upon killing. The tissues were rinsed, patted dry, weighed, and homogenized in a Servall "Omni-mixer" at full speed at 0°C for 2 minutes with 4 ml of 0.05 M phosphate buffer (pH 7.2) per g of tissue. The homogenates were then centrifuged at ca. 25,000 $\times$ *g* for 3 hours at 0°C and the supernatants used in the assays.

In the investigations on the intracellular location, the liver was used because of results obtained in the first part of this study, a single rat being used per experiment. After

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removal, the liver was patted dry, weighed, and sliced into 0.25 M sucrose and the slices washed with four 25 ml portions of the sucrose solution. The slices were then homogenized for one minute in a Potter-Elvehjem homogenizer at 0°C and the homogenate fractionated centrifugally according to the procedure of Hogeboom(6). The fraction containing the nuclei and cell debris, and the mitochondrial fraction were each washed once with 10 ml of 0.25 M sucrose and recovered by centrifugation. The various fractions were resuspended in 15 ml of 0.25 M sucrose and assayed for methyl group synthesizing activity.

In the experiment of this series, in which the effects of washing the particulate fractions were to be tested, the fraction containing nuclei and cell debris was washed twice, the mitochondrial fraction, 5 times, and the microsomal fraction, once with 10 ml portions of 0.25 M sucrose. The washed fractions were prepared for assay as described above.

Protein concentration of the various preparations was determined spectrophotometrically according to the procedure of Kalckar(7).

The reaction mixture used in the assay consisted of the following expressed in $\mu$moles per reaction tube: DL-homocysteine, 20; DL-serine, 20; $NADH_2$, 6; Pyr. $PO_4$, 1; FAD, 0.3; FMN, 0.3; $Mg^{++}$, 10; ATP, 20; $FH_4$, 2; $NAD^+$, 6; and glucose, 10.† Two ml of 0.05 M phosphate buffer (pH 7.2) and one ml of the enzyme preparation, completed the reaction mixture. This complete mixture was used, since the requirements of the rat liver system are not yet known. All cofactors were commercial products, except the tetrahydrofolic acid, which was synthesized according to the procedure of Kisliuk(8).

The incubations were carried out at 37°C under a constant stream of $N_2$ for 3 hours. Controls were heated in a boiling water bath at time 0, and all tubes were heated for 5 minutes at the end of the incubation period. The coagulated protein was removed by centrifugation and aliquots of the supernatants were assayed microbiologically with *Leuconostoc mesenteroides* P-60 for methionine content as previously described(5). The difference in methionine content between the controls heated at time 0 and the experimental tubes was considered to be the net methionine synthesized by the system. Each experimental value presented is the average of duplicates.

† Abbreviations used: $NADH_2$ = nicotinamide adenine-dinucleotide, reduced form; $NAD^+$ = nicotinamide adenine-dinucleotide; Pyr. $PO_4$ = pyridoxal phosphate; FAD = flavin adenine-dinucleotide; FMN = flavin mononucleotide; ATP = adenosine triphosphate; $FH_4$ = tetrahydrofolic acid.

TABLE I. Organ Location of the Methionine Methyl Group Synthesizing System.

| Tissue | Net mμmoles methionine synthesized/g tissue/hr Exp 1 | 2 | 3 | Avg ± S.E. |
|---|---|---|---|---|
| Brain | 0 | 0 | — | 0 ± 0 |
| Muscle | 52 | 22 | — | 37 ± 15 |
| Kidney | 916 | 553 | 384 | 618 ± 157 |
| Liver | 2139 | 1714 | 2270 | 2041 ± 168 |

TABLE II. Intracellular Location of the Methionine Methyl Group Synthesizing System.

| Cell fraction | % of total activity found in given fraction Exp 1 | 2 | 3 | Avg ± S.E. | 4* |
|---|---|---|---|---|---|
| Nuclei and cell debris | 13.9 | 20.6 | 19.0 | 17.8 ± 2.02 | 14.2 |
| Mitochondrial | 21.1 | 22.2 | 18.9 | 20.7 ± .97 | 5.6 |
| Microsomal | 8.3 | 4.8 | 9.8 | 7.6 ± 1.47 | 2.1 |
| Supernatant | 56.6 | 52.4 | 52.3 | 53.8 ± 1.41 | 78.1 |

* Results of experiment in which the cell fractions were washed as described in text.

The rats used were all of the Sprague-Dawley strain, 8-12 weeks of age and had been maintained on a commercially available laboratory diet.

*Results and discussion.* The results of the organ studies showed that the majority of the synthetic ability, expressed as mμmoles of methionine synthesized per gram of tissue per hour, resided in the liver with less in the kidney and practically none in the muscle and brain (Table I).

The studies using liver cell fractions (Table II) showed that the majority of the activity, as a percentage of the total activity of the cell, resided in the supernatant fraction, with a considerable portion present in

the mitochondrial fraction. However, extensive washing reduced the total activity of the mitochondrial fraction by 75% indicating that the activity in this fraction may have been due to insufficient washing in the first 3 experiments. The activity of the microsomal fraction was also reduced by washing.

*Summary.* The data indicate that the *de novo* methionine methyl group synthesizing system resides to a large extent in the liver of the rat. Within the liver cells more than 75% of the synthesizing ability was shown to be in the supernatant fraction when the particulate fractions were well washed to remove any adhering cell sap.

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## Infectious and Oncogenic Effect of DNA Extracted from Cells Infected with Polyoma Virus. (29124)

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DNA extracted from cultured mouse cells infected with polyoma virus is infectious *in vitro*. We have previously reported that when this DNA is injected into hamsters it causes the appearance of circulating specific antibodies and tumors(1); the purpose of the present study was to determine with greater accuracy certain conditions affecting manifestation of these specific biological properties.

*Material and methods.* For the viral DNA extraction, use was made of embryonic mouse cells in tissue cultures collected 4 to 6 days after infection with polyoma virus and of uninfected control cultures. These cells were cultured in a medium containing casein hydrolysate with 10% inactivated horse serum. Maintenance medium contained only 2% serum. The polyoma virus strain used came from the laboratory of R. Dulbecco, and was placed at our disposal by K. Habel. For each experiment we used material from 15 to 20 one-liter Roux bottle cultures.

The cells were extracted with phenol, either without prior treatment or after heating to temperatures of 80 to 100°C for periods of 15 to 45 minutes. Details of the phenol treatment have been described(2). Final extracts were centrifuged for 1 hour at 130,000 *g*, and resuspended in 0.5 M NaCl to contain 400 to 600 μg of DNA per ml. They were then injected subcutaneously (0.15 ml) or intracerebrally (0.03 ml) into either newborn golden hamsters, or animals 40 days of age. Other animals were injected with DNA preparations treated with deoxyribonuclease (DNase) for 1 hour at 37°C. Certain animals were given a 6% solution of para-aminosalicylic acid (PAS), since in certain experiments this had been used as the aqueous phase in the extraction of the DNA. Finally, some animals were not injected but left in contact with hamsters inoculated with DNA. The infectious and oncogenic properties of the original virus strain were determined by subcutaneous inoculation of va-

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