Table IV summarizes data on the absorption of methionine analogs. Substitution of the α-amino group on methionine by an hydroxyl caused a reduction in transport velocity. In isolated tissue studies, WILSON[11] found no active absorption of lactic acid and others have reported β-alanine to be poorly transported[12]. The minimal absorption of the hydroxy as well as the N-acetylated derivative may have been due to diffusion, though apparent transfer of the latter compound may result from enzymatic conversion to methionine. The absorption of DL-ethionine appeared to be faster than DL-methionine but quantitative comparison was impossible because of high variability. In vitro work showed L-ethionine to have about the same affinity for the methionine transport system as L-methionine, while D-ethionine had much less affinity than D-methionine[1].

*Zusammenfassung.* Die Darmresorption von Methionin und seine Analoge bei Hühnern wurde in vivo untersucht. Das Verhältnis der mittleren Resorption von L- und D-Methionin stimmt befriedigend überein mit aus In-vitro-Versuchen erhaltenen Aussagen über Kinetik und Konstanten. Es wurden verhältnismässig niedrige Transportgeschwindigkeiten gemessen für die N-azetylierten und Hydroxyanalogen von Methionin.

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## Intracellular Distribution of Folic Acid in Mouse Liver

WRIGHT and ANDERSON[1,2] found a pterin reductase in *Clostridium stricklandii* which can be coupled to the oxidation of pyruvate. They also noted that under anaerobic conditions the conversion of serine to glycine in the same organism required NAD $Mn^{+2}$, inorganic phosphate, pyridoxal phosphate and any of a number of polyglutamyl pteridines. On oxidation of pyruvate, a compound identified as dihydroteropterin was formed. These experiments suggested to us the possible importance of folate in mitochondrial oxidation of pyruvate. Further, SCRIMGEOUR et al.[3] have investigated the reduction of folate by ferredoxin. MACLEAN et al.[4] showed the importance of folate in photophosphorylation by sonicated chloroplasts. The initiation of protein synthesis through the use of N-formylmethionine and the role of folic acid in the metabolism of $C_1$ units, may suggest a role for folate in protein synthesis as well. With this in mind we studied the relative distribution of folate in the mitochondrial and microsomal fractions of mouse liver. We also investigated the uptake and distribution of radioactive folate in the subcellular fractions of mouse liver homogenates. These results show higher concentration of folate in mitochondria than may be expected from the known role of folate in the metabolism of $C_1$ units, and in increasing the levels of RNA in kidneys[5].

*Materials and methods.* In studies on the subcellular distribution of folate, the different cell fractions were obtained by differential centrifugation of mouse liver homogenates according to standard methods, all work having been done at 2–4 °C. The sucrose homogenate was centrifuged at 600 *g* to obtain nuclei and cellular debri. The supernatant was centrifuged at 10,000 *g* for 30 min to obtain mitochondria. The mitochondrial fraction was resuspended in 0.25 *M* sucrose and recentrifuged to obtain purer mitochondria. The first mitochondrial supernatant was centrifuged at 100,000 *g* for 60 min to obtain the microsomes.

Folic acid was assayed using *Lactobacillus casei* ATCC 7469. The cell fractions were frozen and thawed and then subjected to ultrasonication followed by dilution before they were assayed for folic acid.

In the studies on uptake from blood and distribution of radioactive folic acid-2-$C^{14}$, 1 μC of the folate was administered per 30 g of body weight to each of 6–9 mice i.v. The distribution over different periods of time was studied. At the end of the stated length of time after i.v. administration, the organs were isolated, homogenized and were fractioned at 2 °C as per the method described above. The different fractions were counted for radioactivity in a planchet type counter, making the necessary radiochemical corrections. The radioactive folic acid-2-$C^{14}$ was obtained as potassium salt from Amersham-Searle and has a specific activity of 31.4 μc/μ*M*.

*Results and discussion.* The levels of folic acid in the different subcellular fractions of mouse liver are shown in Table I. Out of a total of 14.23 μg of folic acid per gram of mouse liver, 2.73 μg or approximately 19% is found

Table I. Folic acid content of subcellular fractions of mouse liver (assayed with *Lactobacillus casei*)

| Cell fraction | Folic acid (μg/g fresh total tissue) |
|---|---|
| Mitochondria | 2.73 |
| Microsomes | 0.75 |
| Cell sap (100,000 *g* supernatant) | 10.75 |

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in the mitochondria. The microsomal content is much lower and is only about 5% of the total cellular folic acid. In view of the known functions of folic acid, the higher content of folic acid in mitochondria is striking. This is further corroborated by the higher uptake of radioactive folate by mitochondria (Table II). The ratio of mitochondrial to microsomal folic-2-$C^{14}$ acid content in these uptake experiments varies from about 5–8. The same ratio in the normal mouse is approximately 3.5. SELIG and SANKAR[6] found that addition of folate enhanced the mouse liver mitochondrial oxidation of pyruvate in the presence of NAD. This leads us to wonder whether mitochondrial folate may not be involved in electron transport or in its regulation. In view of the fact that dihydrofolic reductase may use NADP and/or NAD depending on the pH of the reaction medium[7], it is conceivable that folate may be involved in the reactions that govern the NADPH/NAD ratios in mouse liver. Many folate-linked enzymes have higher activity in cytoplasm[8] than in the mitochondria. This adds support to the concept of a role for folate in mitochondrial electron transport rather than in mitochondrial folate-linked enzymes. Further studies to answer this question are in progress.

Table II. Percentage distribution of radioactive folate in mouse liver subcellular fractions

| Cell fraction | % Distribution at time elapsed after administration of folate-2-$^{14}$C | |
|---|---|---|
| | 30 min | 120 min |
| Nuclei | 19.5 | 14.9 |
| Mitochondria | 14.3 | 26.0 |
| Microsomes | 1.6 | 4.6 |
| Cell sap | 64.6 | 54.5 |

*Zusammenfassung.* Eine bedeutende Menge der gesamten Folsäure der Mäuseleberzellen ist in den Mitochondrien vorhanden. Auch nach Inkubation des Leberhomogenats mit radioaktiver Folsäure wird der Grossteil der Radioaktivität im mitochondrialen Anteil gefunden.

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## Changes in Free Sugar During the Germination of Pea Seeds

Among the free oligosaccharides, sucrose, raffinose, and stachyose have been found in seeds of Leguminosae[1], Cruciferae[2], and Liliaceae[3]. A large amount of stachyose was found in peas[4], and the distribution of these oligosaccharides of the raffinose family in peas and lima beans during the course of ripening periods has been studied[5,6]. In experiments with pea extracts, it has been suggested that the sucrose is synthetized from UDPG and D-fructose[7,8]. In analogy with the formation of sucrose in plants, D-galactose-1-phosphate or D-galactose nucleotides have been suggested as intermediates in the formation of raffinose and stachyose from sucrose by enzymatic systems carrying on transgalactosylation reactions[9]. In the course of a study on germination of soybeans, α-galactosidase which is responsible for the splitting of D-galactose from the reserve oligosaccharides of the raffinose family, and enzymes which participate in the metabolsim of D-galactose were found[10].

In the study described here, individual free sugars were analyzed during the course of the germination of pea seeds and we observed the reverse of the process by which the reserve oligosaccharides of the raffinose family are formed during the ripening process.

Dry pea seeds (*Pisum sativum*, variety Early Perfection) produced at this Station were selected and soaked in water containing 10% (v/v) of Clorox for 18 h at room temperature. The rehydrated seeds were placed in petri dishes to germinate at room temperature (23 °C). Germinated peas were taken at intervals from 0 to 100 h and extracted with 80% alcohol for the analysis of individual free sugars by a quantitative paper chromatographic method[5,11].

It was observed (Figure) that the decrease in the absolute amount of stachyose and raffinose is accompanied by a corresponding increase in sucrose during germination of pea seeds. The amount of the only reducing hexose (glucose) monitored held constant at 0.5–1.0 mg/grain. Fructose and galactose were observed in trace amounts only.

Apparently α-galactosidase and galactokinase systems were very active at the early stages of germination of pea seeds and reversed the transgalactosylation reaction of the ripening process of peas. Therefore, stachyose and raffinose were hydrolyzed to galactose and sucrose, and the liberated galactose was converted rapidly into the

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