increase in concentration can occur during subsequent cell development. The limiting factor appears to be total red cell riboflavin content rather than the intracellular concentration. A limited study has been undertaken to discover whether other constituents of the red cell behave in a similar manner.

*Methods.* Red cells were separated from fresh venous blood to which EDTA had been added as an anticoagulant and hemolysates were prepared in appropriate concentrations. Riboflavin was estimated by a spectrofluorometric method[4] glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase by a colourimetric method[5] and acetylcholine esterase by the method of DACIE and LEWIS[6]. Red cell folate (L. Casei factor) was measured by the method of WATERS and MOLLIN[7]. Standard hematological techniques were used[6].

The subjects studied were 54 members of the hospital staff and the local population and 27 patients with untreated iron deficiency anaemia. The range of mean corpuscular volume (MCV) was 56–107 $\mu^3$ (mean 82.3 $\mu^3$). Not all determinations were carried out in every case.

*Results and discussion.* The Table shows the relationship of red cell concentrations and red cell content to MCV for all the constituents examined. In all cases there is a significant negative correlation of cell concentration with cell volume resulting from an increased concentration in microcytic cells. The content per cell shows no significant variation with MCV for folate or the transaminases. In the case of riboflavin and acetylcholinesterase although the concentration is greater in microcytes than in cells of normal size the total cell content is less in the smaller cells.

These findings support the view that intracellular concentration is not a limiting factor for synthetic activity in red cells except in the case of hemoglobin. An increased life span of the nucleated red cell resulting in microcytosis appears to prolong synthetic activity and leads to an increased concentration of non-hemoglobin constituents. The limiting factor for all these constituents appears to be total cell content and in the case of riboflavin and acetylcholine esterase this limit is not always reached in iron deficient microcytes. When red cell factors are estimated for the assessment of nutritional status or other reasons the expression of results in terms of cell content rather than concentration will avoid differences due to the effect of cell size[8].

*Zusammenfassung.* Der Gehalt an Riboflavin, Azetylcholin-Esterase, Glutamat-Oxalacetat-Transaminase, Glutamat-Pyruvat-Transaminase und Folinsäure wurde in menschlichen Erythrozyten verschiedener Grössenordnung bestimmt. Die Ergebnisse sprechen dafür, dass ihre intrazelluläre Konzentration, im Gegensatz zu derjenigen des Hämoglobins, keinen limitierenden Einfluss auf die Fortdauer der Synthese dieser Stoffe ausübt.

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## Sarcosine Dehydrogenase Activity in Liver Mitochondria of Infant and Adult Rats

Sarcosine dehydrogenase belongs to the group of demethylating enzymes and is the first link in the sarcosine-oxidoreductase system (E.C. 1.5.3.1) which catalyses the reaction:

$$\text{sarcosin} + H_2O + O_2 = \text{glycine} + HCHO + H_2O_2$$

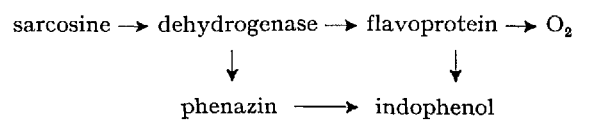
This enzyme is present only in the soluble protein fraction of liver mitochondria in adult rats[1,2]. Thus it seemed pertinent to study its activity during postnatal development since developmental changes might reflect changes in mitochondrial structure.

Sarcosine dehydrogenase activity was determined manometrically[3] and colourimetrically[4]. Liver mitochondria isolated in 0.25*M* sucrose (600–5000 *g* fraction) without the fluffy layer were washed once with 0.25*M* sucrose and then suspended in phosphate buffer 7.5m*M*, pH 7.5.

The ratio of the membrane to the soluble fraction in the mitochondria was determined by freezing and thawing them 3 times[2]. The sediment after 60 min centrifugation at 100,000 *g* (MSE Superspeed-50) was taken as the membrane fraction. Proteins were determined according to LOWRY et al.[5].

Liver mitochondria from 7-day-old rats have a sarcosine dehydrogenase activity that is 41% that of adult rats, i.e. 1.6 ± 0.68 $\mu$l $O_2$/30 min/mg mitochondrial protein against 3.9 ± 0.96 (Figure, A). Using the colourimetric method approximately the same results were obtained (Figure, B).

The electron transport flavoprotein necessary for coupling with the electron transport chain might be rate limiting for sarcosine dehydrogenase activity in 7-day-old rats. This flavoprotein can be substituted for by phenazine-methasulphate:

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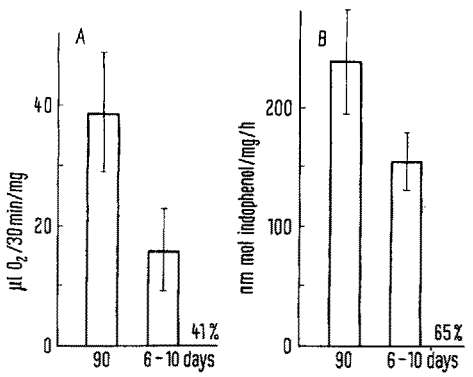
The presence of phenazine-methasulphate, however, did not decrease the difference in sarcosine dehydrogenase activity between adult and infant rats.

In 7-day-old rats mitochondrial sarcosine dehydrogenase activity represents 83 ± 17% of the total activity in liver homogenates. Hence it is unlikely that the lower activity in mitochondria from 7-day-old rats is due to the release of intramitochondrial proteins during isolation of mitochondria.




Sarcosine dehydrogenase activity of rat liver mitochondria from young and adult rats determined manometrically (A) and colourimetrically (B). Enzyme activity is expressed as $\mu$l $O_2$/30 min/mg mitochondrial protein (A) and m$\mu$moles of indophenol reduced/h/mg protein (B).

The lower activity of sarcosine dehydrogenase in liver mitochondria from infant rats might signify a change in the ratio of membrane and soluble protein fraction in them. This, however, was found not to be the case, since this ratio was found to be the same in the mitochondrial fraction (600–5000 *g*) of 7-day-old and adult rats, i.e. 64% of membrane proteins and 36% of soluble proteins.

Thus the change with age in sarcosine dehydrogenase activity described here seems to be due to a change in enzyme activity that is not related to an overall increase in soluble proteins in mitochondria.

*Zusammenfassung.* Die Aktivität der Sarcosinedehydrogenase in Lebermitochondrien 7tägiger Ratten ist um 50% kleiner als in adulten Tieren. Dieser Unterschied ist nicht durch das Elektrontransportflavoprotein limitiert und kann auch nicht durch eine Änderung in dem relativen Wert der löslichen Proteine in den Mitochondrien erklärt werden.

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## Effect of Riboflavin Deficiency on in vivo Incorporation of $C^{14}$ from Labelled Alanine into Liver Glycogen

MORGAN et al.[1,2] observed an increased gluconeogenesis in the liver at the beginning of riboflavin deficiency and a depressed gluconeogenesis in the later part of riboflavin deficiency. MOOKHERJEA et al.[3] demonstrated increased transaminase activity in the liver of riboflavin deficient rats. Recently, increase in alanine transaminase activity of the liver of riboflavin deficient rats was found to be associated with a greater deposition of glycogen in the liver[4]. NICHOL et al.[5] and EISENSTEIN[6] observed a direct correlation between alanine transaminase activity and glycogen deposition in liver through gluconeogenesis. They have studied this association in various ways, including the use of the pyridoxine deficient rats. Further studies of LONG[7] demonstrated that adrenocortical hormone administration increased body carbohydrate stores. The rise in liver glycogen and blood sugar were attributed to gluconeogenesis. HAYNES[8] and OKUNO[9] demonstrated that adrenal steroids stimulate carbohydrate synthesis from alanine when incubated in vitro with rat liver, findings which support the idea that a primary effect of these hormones is to enhance gluconeogenesis. The present experiments were therefore carried out with alanine-1-$C^{14}$ to study whether increased glycogen content in riboflavin deficient liver as reported earlier[4] resulted from increased gluconeogenesis or not.

Young male albino rats of 80–100 g were divided into 2 groups of equal average body weights. Group A consisted of control animals, and group B of riboflavin deficient rats. The animals were pair-fed on 16% protein for 45 days. Particulars regarding the diet have been reported elsewhere[10]. Water-soluble vitamins were supplied daily by s.c. injection.

After the experimental period was over, the rats were kept fasting overnight and then injected i.p. with 5 $\mu$c of alanine-1-$C^{14}$ (specific activity 1.38 mc/m*M*) and after 6h they were sacrificed. The livers were removed, cleaned of adherent blood, weighed, and chilled in ice. A weighed quantity of liver was digested in 30% KOH. The digested tissue was then subjected to processings to extract glycogen[11]. The glycogen extracted was then suspended in 2 ml of water. 1 ml of aliquot was used on planchet for radioactive counts in a windowless gas flow counter. The remaining part was used for determination of glycogen after hydrolysis[11]. Radioactive counts were subjected to

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