Another series of experiments was carried out where ADH was irradiated in solutions containing sodium iodide labelled with I^{131}; the enzyme was then separated and tested for any bound radioactivity. The experimental technique can be summarized as follows: carrier free NaI^{131}, was diluted with inert NaI to achieve a molar excess of 100:1 with respect to ADH, whose concentration was adjusted to $6.5 \cdot 10^{-6} M$. The samples were then irradiated with a dose of 15.4 Krad of X-rays (residual enzymatic activity less than 1%), or left unirradiated in ice for the same time as control. Thereafter the enzyme was separated from the radioactive solution either by passage over Sephadex G-25 (Pharmacia, Sweden) or by precipitation with 60% ammonium sulphate or 10% trichloroacetic acid and washed by centrifugation until no radioactivity was detectable in washings. In no case did the separated enzyme reveal any radioactivity. This finding does not essentially contradict the hypothesis that iodine may be involved in the sensitizing effect, because the lack of iodine binding could be due to oxidation of sulphydril groups to disulphides. Experiments are at present in progress to control whether this may actually be the case[13].

Riassunto. L'alcool deidrogenasi del lievito irradiata con raggi X in soluzioni contenenti ioduro di sodio, acido 3-iodopropionico o ioduro di metile, è inattivata con un rendimento più elevato che in assenza di tali sostanze. L'effetto sensibilizzante dello ioduro di sodio e dell'acido iodopropionico è proporzionale alla loro concentrazione nell'ambito di 0–16 molecole di essi per molecola di enzima. L'alcool deidrogenasi irradiata in presenza di ioduro di sodio marcato con I^{131} non rivela la presenza di iodio legato alla sua molecola.

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The Effect of Microcytosis on Red Cell Constituents

The onset of iron deficiency anaemia in human subjects is first marked by the development of microcytic red cells with a normal hemoglobin concentration and later by the appearance of hypochromic cells with a low mean corpuscular hemoglobin concentration (MCHC)[1]. This situation is in accord with the hypothesis of Stohlman and coworkers that the total maturation time of the red cell is dependent on the rate of hemoglobin synthesis. When a critical MCHC is reached this triggers a feed back mechanism which stops further DNA synthesis and cell division[2]. If hemoglobin synthesis is impaired the time taken to achieve the critical MCHC is prolonged and an increased number of cell divisions occur, resulting in microcytosis. The critical MCHC is never exceeded whatever the duration of red cell development.

If the MCHC plays this crucial role in determining the end of nuclear activity then it must also be a limiting factor for other cell functions. Estimation of red cell riboflavin in iron deficiency anaemia shows that when microcytosis occurs the intracellular concentration is increased[3]. This suggests that if a normal concentration is attained before hemoglobinization is complete a further

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Correlation of red cell concentration (a) and total red cell content (b) with mean cell volume for 5 constituents

Constituent measured	No. of cases	Correlation coefficients r	p	Regression coefficients
Riboflavin (a) µg/100 ml	74	− 0.52	< 0.001	− 0.15
(b) µg/10^{14}RC's		+ 0.32	< 0.01	+ 0.06
Cholinesterase (a) units/100 ml	36	− 0.432	< 0.01	− 0.87
(b) units/10^{10}RC's		+ 0.407	< 0.05	+ 0.66
Glutamic-oxaloacetic transaminase (a) µmole/100 ml	81	− 0.475	< 0.01	− 2.75
(b) µmole/10^{12}RC's		+ 0.015	n.s.	+ 0.06
Glutamic-pyruvic transaminase (a) µmole/100 ml	79	− 0.369	< 0.01	− 0.94
(b) µmole/10^{12}RC's		− 0.120	n.s.	− 0.22
Folate (a) µg/ml	49	− 0.486	< 0.01	− 4.99
(b) µg/10^{14}RC's		− 0.164	n.s.	− 0.11

n.s., not significant.

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 μ^3 (mean 82.3 μ^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:

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

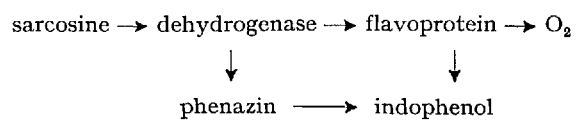
This enzyme is present only in the soluble protein fraction of liver mitochondria in adult rats[1,2]. Thus it seemed pertinent to study its activity during postnatal development since developmental changes might reflect changes in mitochondrial structure.

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 μl 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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