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# Haemoglobin Present in the Nuclear Fraction of the Liver

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The nuclear fraction of 1 g of rat and rabbit liver contains 0.34 and 0.58 mg of in aqueous medium non-extractable haemoglobin. The corresponding figure for the nuclei of the red corpuscles of 1 ml of hen's blood is 0.9 mg.

The haemoglobin content of the nuclear fraction of 1 g of foetal rabbit liver is 100 times as large as that of 1 g of maternal liver. The ratio of their $^{59}Fe$ content 11 to 17 h after labelling the maternal plasma iron, works out to be 100.

One day or more after exposure of the rat to 500 r, incorporation of $^{59}Fe$ into the non-extractable haemoglobin of the liver nucleus fraction is strongly depressed.

As mentioned in a previous note (Bonnichsen *et al.*[1]) the nuclear fraction of the liver of the guinea-pig contains small amounts of haemoglobin not extractable by saline or other aqueous solutions. This paper contains data on the incorporation of $^{59}Fe$ into this fraction isolated from the liver of adult rats and rabbits and of that of the rabbit embryo. This fraction was furthermore located in the nuclei of the erythrocytes of the hen.

That in the basophilic erythroblasts of human and rat bone marrow the site of haemoglobin synthesis is primarily the nucleus, was repeatedly suggested. The methods applied in these investigations were ultraviolet absorption microspectroscopy and cytochemical staining procedures [2–9].

## EXPERIMENTAL

In 15 experiments with male rats an aggregate number of 173 animals, weighing between 150—280 g were injected intraperitoneally with 0.25 ml of a 3.8 % ammonium-citrate solution containing 0.5—12 $\mu$g of with $^{59}Fe$ labelled iron of 0.1—12 $\mu$C activity. Half the number of rats was exposed to 150 *r* — 500 *r* of X-rays. Injection took place 15 min to 5 days after exposure.

The animals were killed from 2 to 48 h after injection. The livers were perfused first with 0.145 M NaCl and then with 0.25 M sucrose containing 0.018 m $CaCl_2$. The weighed livers were homogenized in 9 vols. of 0.25 M sucrose — 0.0018 M $CaCl_2$. The further procedure was carried out according to Hogeboom *et al.*[10]

The nuclear fraction isolated by this procedure was cytologically inhomogeneous. The fraction contained about 60—80 % of the cell nuclei, it also contained erythrocytes, connective tissue, residual intact cells and free mitochondria.

In order to haemolyze the erythrocytes, the fraction was homogenized with 10 vols. distilled water and allowed to stand several hours. This last step was repeated at least three times more.

After clearing the suspension of nuclei obtained in this way by adding 3 % of deoxycholate, the haemoglobin bands were steadily visible in the handspectroscope. The CO-band was that of haemoglobin. The pyridine-haemochromogen band was located at 557 mμ.

The haemin was extracted with a mixture of acetone and HCl (10 ml 20 % HCl in 1 l of acetone). After filtration, the acetone was evaporated *in vacuo*. The haemin was crystallized twice from conc. acetic acid, and the crystals washed with 1 N HCl. The haemin was then combusted and the solution analyzed as described by Bonnichsen *et al.*[11]

In order to know in which part of the above nuclear fraction the non-extractable haemoglobin is located, we separated the fraction, prior to haemolyzing the red cells with distilled water, in the counter-streaming centrifuge of Lindahl [12] in five fractions containing particles of different size and different specific gravity. The fractions were examined with the phase-contrast microscope and with the handspectroscope. Thereafter, the specific activity of their haemin was determined. The result of one of these experiments is seen in Fig. 1.

In five of the above experiments of the liver nuclei have been isolated both in aqueous medium and in organic solvents of low polarity according to the methods described by Dounce *et al.*[13] and Allfrey *et al.*[14] The nuclear preparations obtained in this way were treated and analysed as described above. No difference was found in the properties of the non-extractable haemoglobin of the nuclear fraction prepared either in aqueous or in organic medium.

The liver ferritin was prepared as previously described by Loftfield *et al.*[15]

In experiments with 2—3 kg rabbits 8 animals were investigated. Their plasma was labelled with $^{59}Fe$ as described by Ehrenstein *et al.*[16] and reinjected. The animals were killed 3—24 h after injection.

Simultaneously, in all our experiments haemin of the circulating haemoglobin was analysed as well.

In two experiments with hens 100 ml of hen blood were incubated *in vitro* for 3 h at 37° C with 2 ml of 3.8 % ammonium citrate solution containing 2 μg labelled iron of 25 μC activity. The plasma was centrifuged off and the red cells washed four times with 5 vols. or more of isotonic saline.

The erythrocyte nuclei were prepared according to Hogeboom *et al.*[10] However, as the red cells are not sufficiently broken down by the homogenization procedure usually employed for the disintegration of other tissues, we haemolyzed the cells by freezing at —20° C and thawing them three times or more, suspended in a mixture of 0.25 M sucrose — 0.00018 m $CaCl_2$. The nuclei were then centrifuged down (International Refrigerated centrifuge head No. 269) for 10 min at 2 000 r.p.m. Thereafter, the procedure of Hogeboom *et al.*[10] was applied. Samples were taken from the whole washed red cells, the stroma-free haemolysate and from the final nucleus preparation. The haemin was extracted as described above.

## RESULTS AND DISCUSSION

a) *Experiments with rats.*

The amount of $^{59}Fe$ present in one μg of iron of the nuclear haemoglobin fraction of the liver varied in experiments on rats between $0.6 \times 10^{-4}$ and $10 \times 10^{-4}$ % of that injected.

The liver of our rats contained 10 μg of nuclear haemin iron out of 2.5 mg of total iron present in the liver. This fraction makes out 0.4 % of its total iron content.

In the course of the purification process in aqueous medium extractable haemoglobin may have been removed. However, the non-extractable haemoglobin content of the nuclei was not found to be larger when the nuclei were

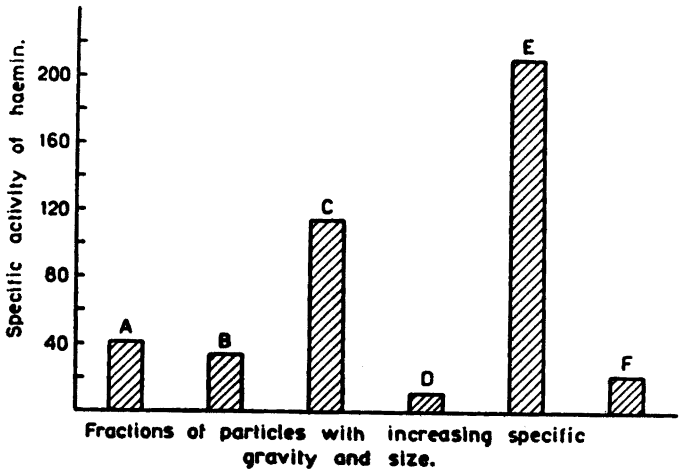


*Fig. 1.* Results of separation of nuclear components of the liver cells of the rat in Lindahls counter-streaming centrifuge.

A = Total nuclear fraction, prepared according to Hogeboom.
B = Impure mitochondria containing erythrocytes.
C = Erythrocytes and small fibers of connective tissue.
D = Cell membranes.
E = Small nuclei.
F = Large nuclei.

isolated after lyophilisation in organic medium. In the haemoglobin content of the nuclear fraction of the red cells of the hen, when these were isolated in an organic medium, Stern *et al.*[17] found a haemoglobin-iron content of 19 % of that of the total red corpuscle, while we find after isolation of the nuclei in an aqueous medium 1 % of the total haemoglobin-iron content to be non-extractable. This 1 % compares with 0.4 % found by us in the liver nuclei of rats and 2 % in those of the rabbit.

We compared also the effect of irradiation with 500 *r* of X-rays on rats on the incorporation of $^{59}Fe$ into the liver nuclear haemoglobin fraction. As seen in Table 1 the X-ray effect is shortly after exposure a very restricted one for both haemoglobin fractions. The X-ray effect as seen in Fig. 2 doesn't seem as pronounced as that on the circulating haemoglobin. If we, however, take into account the enhanced activity of the liver of the exposed animal, this difference is strongly reduced.

b) *Experiments with rabbits.*

The amount of $^{59}Fe$ present 4 h after injection in one $\mu$g iron of the non-extractable haemoglobin of the nuclear fraction of the rabbit liver varied between $2 \times 10^{-4}$ and $6 \times 10^{-4}$ % of that injected.

The iron content of the non-extractable liver nucleus fraction haemoglobin varied between 0.5 and 3.8 $\mu$g with a mean value of 1.9 $\mu$g per one g liver.

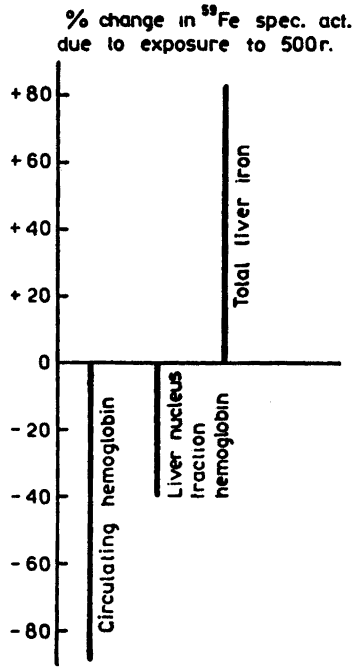


*Fig. 2.* Effect of exposure of the rat to whole body irradiation on the incorporation of intraperitoneally injected $^{59}$Fe as citrate into liver fractions and the circulating haemoglobin.

For the nuclear haemoglobin content of 1 g of rat and rabbit liver the corresponding figures are 0.38 mg and 0.56 mg.

We also carried out experiments with pregnant rabbits 21 days after mating. A plasma sample of the mother was labelled as described by Ehrenstein *et al.*[16] The livers of the mothers and foetuses were investigated 11 to 17 h after labelling the maternal plasma. The results are seen in Table 2.

*Table 1.* Ratio of specific activities of iron fractions of to 500 *r* exposed and control rats.

| Injected with $^{59}$Fe shortly after exposure | | | Injected with $^{59}$Fe 1 day or more after exposure | | |
|---|---|---|---|---|---|
| Liver nucleus fraction haemoglobin | Circulating haemoglobin | Total liver iron | Liver nucleus fraction haemoglobin | Circulating haemoglobin | Total liver iron |
| 1.16 | 0.53 | 1.60 | 0.915 | 0.161 | 1.15 |
| 0.97 | 1.04 | 1.41 | 0.503 | 0.063 | 2.48 |
| 1.15 | 0.84 | 0.93 | 0.570 | 0.238 | 1.72 |
| 0.78 | 0.46 | 1.27 | 0.727 | 0.072 | 1.92 |
| | | | 0.318 | 0.029 | 2.44 |
| Mean value 1.01 | 0.72 | 1.30 | 0.605 | 0.112 | 1.94 |

*Table 2.*

| | Mother | Foetus |
|---|---|---|
| Total liver iron | 13.9 mg | 1.5 mg |
| Total non-extractable liver nuclear haemoglobin iron | 24 μg | 41 μg |
| Total liver activity in % of that injected | 8 | 20 |
| Total non-extractable liver nuclear haemoglobin activity in % of that injected | $3.4 \times 10^{-3}$ | $3.4 \times 10^{-1}$ |

Both the iron content of the nuclear fraction and its specific activity is much higher in the foetus than in the mother.

Three of the pregnant rabbits were exposed 1 day prior to injecting them with $^{59}Fe$ to an X-ray dose of 500 *r*. The effect of the radiation on the incorporation of $^{59}Fe$ into the liver nuclear fraction is discussed in a paper by Ehrenstein *et al.*[16]

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