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Changes in the Blood and Iron Metabolism in Rats with Alcoholic Fatty Livers (39073)

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Chronic ingestion of alcohol in excessive quantity frequently brings about fatty liver which is the hallmark of liver disease (1). The degree of fat deposition in the hepatocyte depends upon the quantity of alcohol ingested and whether or not it is complicated by malnutrition and avitaminoses (2). Very often, the effects of alcohol and nutritional defects are concurrent in action and are difficult to distinguish as individual causes of the disease. In addition to causing fatty liver, alcohol has a multiplicity of effects on haematopoietic tissue (3). Also iron metabolism and cirrhosis of the liver have been closely linked by investigators since the recognition of haemochromatosis as a clinical entity. Nevertheless, reports on the changes in blood picture together with iron metabolism remain sketchy and incomplete. The present study was designed to investigate the changes in haematological parameters in correlation with the compartmental shift of iron in rats with fatty livers induced by chronic consumption of alcohol.

Materials and methods. Animals used in the following experiments were adult albino rats of either sex from the same colony. Their weights ranged from 180 to 200 g before the experiment. A total of 40 rats were fed with 20% alcohol and commercially prepared rat pellets *ad libitum*. They were paired with equal number of control animals. The control rats were allowed drinking water instead of alcohol and were kept at the same conditions. After a period of 12 months, the alcoholic rats, in batches of 10 and paired with equal number of controls, were subjected to the following investigations. Group I animals were sacrificed for the study of the haematologic parameters which included haematocrit, haemoglobin concentration, reticulocyte and red cell count (4) and the storage iron. Spleen, small intestine and liver were assayed for non-haem iron using a method described by Kaldor (5). To assess the histopathological condition of the liver from these alcoholic rats, a fresh specimen of liver tissue were divided into two, one part was fixed in formalin, sectioned and stained with haematoxylin and eosin. The other section was processed by freeze-sectioning technique and stained with oil red O. Total liver lipids were extracted and measured chemically from another small specimen of liver tissue by the method of Folch *et al.* (6).

Group II animals were subjects of the plasma iron turnover study. Plasma iron disappearance rate and plasma volume were obtained following an intravenous injection of 2–4 μCi of tracer ^{59}Fe in the form of ferric chloride (Radiochem, Amersham) through an exposed femoral vein (7). Plasma iron and total plasma iron-binding capacity (TIBC) were determined spectrophotomatically (8). Measurement of red cell life span was performed in Group III using chromium ^{51}Cr labelling method (7). The alcoholic and control animals in Group IV were used for the intestinal iron absorption study. The percentage of radio-iron ^{59}Fe absorbed was determined through whole body counting the percentage of the radioactivity of an intragastric test doses retained in the body a week after the administration (9). Test doses in volume of 1 ml containing 50 μg of ferric chloride and 0.5 μCi of radio-iron $^{59}FeCl_3$ (Radiochem, Amersham) was given to each rat. Statistical analysis of the differences between sample means were compared by a small sample method of Student's t test (10).

Results and discussion. Gross and microscopic examination of the livers from alco-

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TABLE I. CHANGES IN THE IRON STORES IN RATS WITH ALCOHOLIC FATTY LIVERS.

	Control (n = 10)[a]	Alcoholic (n = 10)	Student's t test
Body weight (g)	230[b] (±17)	204 (±6)	$P < 0.01$
Liver			
Weight (g)	5.2 (±0.6)	6.4 (±0.8)	$P < 0.01$
Total lipid (μg/g dry)	27 (±6)	67 (±16)	$P < 0.01$
Nonhaem iron (μg/g)	393 (±39)	411 (±42)	NS[c]
Spleen			
Weight (g)	0.4 (±0.1)	0.4 (±0.1)	NS
Nonhaem iron (μg/g)	645 (±82)	1374 (±302)	$P < 0.01$
Small intestine			
Weight (g)	4.1 (±0.4)	3.9 (±0.3)	NS
Nonhaem iron (μg/g)	120 (±24)	346 (±47)	$P < 0.01$

[a] n denotes the number of animals.
[b] Mean ± standard deviation.
[c] Not significant ($P > 0.05$).

holic rats revealed a slight accumulation of fat but no sign of fibrosis. The quantitative measurement of the total liver lipids (Table I) shows a more than twofold increase from a normal value of 27 to 67 μg/g dry weight ($P < 0.01$). Excepting for the liver, increased nonhaemoglobin iron stores were found in the spleen and small intestine in the alcoholic rat. These differences were statistically significant ($P < 0.01$).

There were no marked changes in haemoglobin concentration, haematocrit and erythrocyte count between the alcoholic and control animals (Table II). Similarly, the red cell volume and cellular haemoglobin so calculated in the alcoholic rats did not differ significantly from those in the control group. There was a 20% reduction in the erythrocyte survival time but the rate of erythropoiesis, as indicated by the reticulocyte count, was also accelerated ($P < 0.01$).

Table III shows the ferrokinetic and iron absorption studies in rats with alcoholic fatty livers. The plasma volume was slightly expanded in the alcoholic rats, a finding not uncommon in the alcoholic human patients (11). The plasma iron concentration did not vary, however, the plasma iron-binding capacity (TIBC) fell, probably as a result of decreased production of β_2-globulin or transferrin by the liver (12). The rate of plasma ^{59}Fe clearance as well as the total plasma iron turnover rate were found to be significantly lower in the treated animals ($P < 0.01$).

Although an increased rate of iron absorption is frequently reported in patients with liver cirrhosis (13, 14), it is now clear that the rate depends on whether the cirrhosis is complicated by the occurrence of anaemia or not and on the status of the body iron stores (15). If the body iron stores are depleted and anaemia arises secondary to the cirrhosis of the liver, an increase in intestinal iron absorption is not an uncommon feature. On the other hand, patients or experimental animals with liver cirrhosis together with siderosis may frequently have a low absorption rate (15, 16). In the present chronic alcoholism study, the rats with slight siderosis, especially in the spleen, absorbed less iron. However, this difference was not significant.

Normally, about 70% of the total plasma

TABLE II. THE HAEMATOLOGICAL PARAMETERS IN RATS WITH ALCOHOLIC FATTY LIVERS.

	Control (n = 10)[a]	Alcoholic (n = 10)	Student's t test
Haematocrit (%)	46.4[b] (±3.7)	46.6 (±4.5)	NS[d]
Haemoglobin (%)	12.2 (±2.2)	12.9 (±1.5)	NS
RBC count ($\times 10^6$)	7.3 (±0.9)	7.5 (±0.9)	NS
Reticulocyte count (%)	1.0 (±0.5)	3.1 (±1.2)	$P < 0.01$
RBC life-span[c] (T/2, days)	13.7 (±2.1)	10.5 (±0.5)	$P < 0.01$

[a] n denotes the number of animals.
[b] Mean ± standard deviation.
[c] Animals studied were from Group III.
[d] Not significant ($P > 0.05$).

TABLE III. FERROKINETIC STUDIES AND IRON ABSORPTION IN RATS WITH ALCOHOLIC FATTY LIVERS.

	Control (n = 10)[a]	Alcoholic (n = 10)	Student's t test
Plasma Fe (μg/100 ml)	113[b] (±27)	118 (±19)	NS
Plasma total iron binding capacity (μg/100 ml)	260 (±50)	218 (±29)	$P < 0.05$
Plasma volume (ml/100 g b wt.)	2.0 (±.17)	2.2 (±.14)	$P < 0.01$
Plasma ^{59}Fe clearance ($T/2$, min)	44 (± 5)	70 (± 7)	$P < 0.01$
Plasma iron turnover per day (μg)	240 (±19)	151 (±14)	$P < 0.01$
^{59}Fe absorbed (%)[c]	8.7 (±2.8)	7.1 (±2.5)	NS

[a] n denotes the number of animals.
[b] Mean ± standard deviation.
[c] Animals studied were from Group IV.
[d] Not significant ($P > 0.05$).

iron leaves for the bone marrow for erythropoiesis (17), 10% to the liver (18) and the rest, to the unidentified soft tissues. The reticulo-endothelial system is the major supplier of iron to the plasma pool (19). The body handles and conserves the iron extremely well and there is little excretion of body iron by any pathway. Any loss of iron is restored by dietary iron supply through the process of intestinal absorption of iron. It seems that in the rats with alcoholic fatty livers, a reduction in the red cell life span is well compensated by an elevation in the erythrocyte production so that the number of the circulating erythrocytes remains within the normal range. To keep up with this increased erythropoiesis, more iron is required by the bone marrow. One would, therefore, expect a higher plasma iron turnover in this case. We reported here a lower turnover rate and the implication of this finding is dubious. Perhaps, the clearance of plasma iron through other channels may be drastically curtailed. Some of the body iron stores we had examined here (Table I) showed no sign of depletion, instead, an increased pool size was generally observed. Therefore, it is rather unlikely that there would be a marked reduction in the rate of iron transport from the plasma pool to these iron depots. The uncertainty arises in the present study is the quantity of plasma iron moving into the unknown soft tissues.

Summary. The changes in the haematological parameters and iron metabolism in rats with fatty livers after they had been fed with 20% alcohol and normal standard diet for a period of 12 months were studied. The haematocrit, haemoglobulin concentration and erythrocyte count were within the normal range. The erythropoietic rate was elevated, probably as the result of the shortened life-span of the erythrocytes. The rate of iron absorption in the alcoholic rat did not vary. The iron stores in the spleen and small intestine were, however, markedly increased. The ferrokinetic study shows that the alcoholic rat had a lower plasma iron turnover rate. In view of the elevated erythropoiesis the implication of this finding is difficult to interpret when the efflux of plasma iron to the unspecific soft tissues is not available.

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