KK when titers in the spleen reach 10^8 per gram. Spleens of animals infected only with *P. berghei* were bacteriologically sterile.

These studies indicate that concurrent infection in mice with *P. berghei* and *S. typhimurium* is more rapidly fatal than either infection alone. The decreased survival time seems to be related to enhancement of salmonella infection and not to enhancement of malaria as the mice died with a lethal salmonella infection but with hematocrit values generally above the level at which mouse malaria was fatal.

Summary. Concurrent infection in mice with *P. berghei* and *S. typhimurium* was more rapidly fatal than either infection alone. There was no consistent difference in mean hematocrit values between mice with both malaria and salmonella infection and mice with only malaria. Spleens removed from mice dying with *P. berghei* and *S. typhimurium* infection demonstrated more than 10^8 salmonella per gram of spleen. Therefore the decreased survival time seemed to be related to enhancement of salmonella infection and not to enhancement of malaria as the mice died with a lethal salmonella infection but with hematocrit values above the level at which mouse malaria was fatal.

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Depression of Liver Affinity for Iron with Iron Overload.* (30662)

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When animals were fed a diet containing ethionine, there was an increase in the amount of iron absorbed into the body, and an increase in storage of iron in the liver(1). When *in vitro* studies were carried out in which liver slices were exposed to an iron-containing solution, it was shown that the liver from animals maintained on this ethionine-supplemented diet for 1 week had a greater affinity for the iron in the solution than did the control animals(2). It was suggested that the affinity may play a role in influencing the deposition of the iron in the liver and promoting the absorption from the gastrointestinal tract. From these experiments, it was not clear whether the change in affinity was caused by the ethionine, with secondary iron deposition, or whether the presence of iron caused the increase of the liver's affinity for the iron, leading to further iron deposition. The present experiment was designed to determine whether iron loading would, in itself, cause an alteration in liver affinity when the metabolic influence of ethionine was not introduced.

Materials and methods. Sprague-Dawley male albino rats weighing approximately 200 and 250 g were used. All animals were fed a synthetic diet of the following composition: glucose, 66.7 g; casein, 18 g; salt mixture, 4 g(3); corn oil, 11 g; choline chloride, 0.3 g. Each 100 g of diet was supplemented with approximately 60 USP units of vit. A and 1 USP unit of vit. D. Crystalline vitamins were added in the following amounts per 100 g of diet: thiamine chloride, 400 μg; pyridoxine hydrochloride, 400 μg; riboflavin, 800

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TABLE I. Iron Values, Hemoglobin, Hematocrit, and Liver Weights.

	Experiment Group	Hb (g %)	Hct (%)	Fe^{59} uptake in liver (CPM/g % standard)	Liver Fe (mg/g)	Liver wt (g)	Serum iron mcg/ 100 ml	TIBC mcg/ 100 ml	% saturation of TIBC
Part 1	I. Oral Fe	15	55	22	.22*	11.2	363*	611	60*
	II. Pair fed controls	15	54	22	.15	11.0	257	572	45
	III. Weight controls	15	53	24	.16	10.4	261	610	42
Part 2	I. Parenteral Fe	16	56	14*	1.03†	12.2	450*	640	70*
	II. Pair fed controls	16	54	21	.17	10.3	353	639	56
	III. Weight controls	16	56	26	.20	9.7	299	601	49

* $p < .05$ when compared with corresponding controls.
† $p < .01$ when compared with corresponding controls.

μg; calcium pantothenate, 1.5 mg; nicotinic acid, 2.5 mg.

In one part of the experiment the animals were divided into 3 groups of 8 each, and matched according to weight. Group I received the synthetic diet supplemented with 2% ferric citrate; Group II was pair-fed the basal diet without the iron supplement; and Group III served as the weight controls.

In the other part of the experiment, 3 groups of 8 animals each were also used. Group I received the basal diet, but in addition the iron supplement, instead of being given by mouth, was injected intramuscularly as iron dextran.† This was given in weekly doses of 10 mg iron per 100 g body weight. Group II was a pair-fed control and Group III was a weight control. The latter 2 groups did not receive iron intramuscularly.

All animals were killed after 4 weeks. Serum iron and iron binding capacity were determined by the methods of Carraway(4). Hemoglobin determinations were carried out by the cyanmethemoglobin method and hematocrits by a capillary microhematocrit method. Livers were removed, cut into slices and iron uptake studies performed by placing liver slices in 15 ml of Ringer's solution containing 10 microcuries $Fe^{59}SO_4$ and 2.8 mg of carrier iron as ferrous sulfate. After one hour of incubation at 37°C, during which period oxygen was bubbled through the incubating solution, the liver slices were removed, washed in cold Ringer's solution for ½ hour and then counted in a gamma counter for Fe^{59} activity. The results were recorded as CPM/g liver and expressed as % standard

$$\left(\frac{\text{CPM/g liver} \times 100}{\text{CPM of standard}}\right).$$

The standard used was 2 ml of fluid identical to the incubating fluid. Subsequently iron determinations were performed chemically according to the method of Kitzes, Elvehjem and Schuette(5) after digestion with nitric, sulfuric, and perchloric acids. Liver sections were fixed in acetone, stained with H&E, for iron according to Perls' method, PAS after diastase digestion (DPAS) and DPAS following treatment with 40% HCl in order to remove iron from the liver so that the DPAS positive material would not be obscured. Sections were stained for acid phosphatase according to the method of Burstone(6). To determine the relationship of iron to the DPAS positive material and the acid phosphatase, a combined technique was used whereby the same slide was stained for both DPAS positive material and iron or for acid phosphatase and iron. The data were analyzed statistically using Student's "t" test for paired comparisons. Differences were considered significant when p was less than 0.05.

Results. All animals gained weight during the experiment, and there was no significant difference in the growth pattern. There was no significant difference in the *in vitro* uptake of Fe^{59} by the liver slices of rats, in the first part of the experiment, fed the diet supplemented with 2% iron citrate when they were compared with controls (Table I). In the

† Imferon, Lakeside Laboratories, Milwaukee, Wis.

second part of the experiment, where iron dextran was injected intramuscularly, there was significantly less Fe^{59} uptake after incubation than in the control livers (Table I). On chemical analysis it was shown that the rats administered iron either orally or intramuscularly had significantly higher liver iron values than their respective controls. The liver iron values in the animals receiving the iron intramuscularly were significantly higher than those fed the iron as a dietary supplement. The hemoglobin and hematocrit levels and liver weights were not significantly different in the experimentals and controls.

The serum iron values are recorded in Table I. Serum iron levels were significantly higher in the animals receiving iron either orally or parenterally when compared with their controls. The total iron-binding capacity was not significantly altered, and as a result of this, the percent saturation of iron binding protein was significantly higher in animals receiving iron.

Histologically there was no demonstrable iron in the animals fed iron citrate. In the animals injected with iron dextran there was histochemically demonstrable iron in both the Kupffer cells and parenchymal cells. In the central areas it appeared that more Kupffer cells contained iron but the deposits within each individual Kupffer cell were less concentrated than in the Kupffer cells located elsewhere in the lobule. In addition, there was a slight increase in the parenchymal cell iron in the central areas when compared with the periphery of the lobule. In the parenchymal cells the iron was present as a blue haze and as fine granules. Occasionally the granules in the parenchymal cells showed a pericanalicular distribution. The DPAS positive material was increased in amount in the Kupffer cells and in the parenchymal cells and was intimately associated with the iron deposits. It was not possible to quantitate the overall amounts of acid phosphatase in the slide preparations, particularly in the parenchymal cells. The Kupffer cells in the central areas contained increased amounts of acid phosphatase in the animals injected with iron dextran. The increased amounts of acid phosphatase were associated with iron deposits.

Discussion. These experiments indicate that the presence of iron in the liver does not cause an increase in the affinity of the liver for more iron. As a matter of fact, when large amounts of iron were present, as in the animals injected with iron dextran, the affinity was decreased. The failure to depress the affinity in animals fed iron citrate could be related to the fact that the increase in liver iron was not sufficient to exert such effect.

It has been shown by Gedigk and Strauss (7) that following injection of colloidal iron solutions subcutaneously in mice, an organic substance developed in the local mesenchymal cells at the site of injection. This substance was similar to the ground substance of hemosiderin and consisted of protein, polysaccharide, and small amounts of lipid. The polysaccharide developed early, followed by development of the lipid component. This material had a high affinity for iron. It is possible that the DPAS positive material which is associated with iron in the present experiment is essentially similar to the substances described by Gedigk and Strauss. These authors state that this substance develops secondarily to the presence of excess iron. Our present experiments would support such a view. On the other hand, in the experiments with ethionine(8), the DPAS positive material appeared prior to iron deposition in the liver cells. The first appearance of this substance was observed at 20 hours, and not until 6 hours later could iron be visualized histologically. It is not known whether the DPAS positive substances which develop after subcutaneous injection of iron(7), ethionine feeding(8), or, in the present experiment, after iron injection, are identical.

The possibility was suggested earlier that the DPAS positive substance may be the material which is responsible for the liver affinity for iron(2). It is possible that the decreased affinity for iron in the liver from animals receiving iron parenterally is on the basis of saturation of all available DPAS positive material which could conceivably possess an affinity for iron; any new development of DPAS positive material was just

enough to hold the excess iron in the cells. The development of a DPAS positive material secondarily to the presence of iron may be a protective mechanism of the cell against excess iron. In ethionine-fed rats, on the other hand, the DPAS positive material may develop as a result of the damage caused by ethionine and only secondarily binds available iron.

In the parenchymal cells the relationship of acid phosphatase to iron and the DPAS positive substance could not be established by histologic observation. However, in the Kupffer cells, acid phosphatase in large amounts was present at the very sites where the DPAS positive material and the iron were deposited. This latter finding supports our earlier suggestion that the lysosomes may play a significant role in iron deposition(9).

Summary. When liver slices were incubated in Ringer's solution containing Fe^{59} the uptake of the radioactive iron was significantly decreased if the rats were previously overloaded with iron parenterally. Oral administration of iron did not produce the same degree of overload, and subsequently there was no significant decrease in the affinity of the liver for Fe^{59} *in vitro*. The deposition of histochemically demonstrable iron in the liver was associated with the presence of a DPAS positive material and acid phosphatase.

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Effect of Ethanol on Absorption of Iron in Rats.* (30663)

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It appears likely that alcohol may play a part in the excessive deposition of iron in the liver of humans. The large amount of iron in the beer drunk by the Bantu has been suggested as a causal factor in the production of siderosis frequently seen in that race(1). McDonald(2) feels strongly that all hemochromatosis is due to the excessive absorption of iron from alcoholic liquors with a high iron content, in the face of existing or developing portal cirrhosis. There seems to be little doubt that liver disease itself, especially fatty liver, encourages unusual deposition of iron in rats(3) and it is possible that iron overload in turn may render the liver more susceptible to injury(4) and accumulation of fat. Charlton *et al*(5) recently studied the absorption of Fe^{59} in ferrous and ferric states in humans, by collecting stools and calculating dose retention following administration of the test dose by mouth with alcohol. Their findings suggested a considerably enhanced absorption of the ferric iron when given with alcohol, but no change with the ferrous. This phenomenon appeared to be dependent on the presence of acid in the stomach. They suggested that alcohol stimulated the secretion of gastric acid which in turn facilitated the conversion of ferric to ferrous ions permitting greater uptake of the more readily absorbed ferrous ion. With these observations in mind we decided to study the effect of alcohol on the absorption of Fe^{59} as ferric and ferrous salts in rats by total body count-

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