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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ing and on the uptake of the same salts by isolated everted pouches of rat small intestine. The plan of the experiment was as follows.

Method. Two groups of experiments were done: one was designed to determine the effect of drinking alcohol on total body absorption of ferrous and ferric iron in normal healthy rats; and the second, to compare the *in vitro* absorption rate of ferrous and ferric iron in everted gut sacs prepared from normal healthy animals with those prepared from rats drinking alcohol. The effect of both long term administration of alcohol, and of a single dose of alcohol was investigated in both groups.

Sixty female Sprague-Dawley rats weighing about 225 g and with normal hemoglobin values (Evelyn method) were randomized into 3 groups. Twenty rats were given 20% ethanol to drink as a substitute for water for 3 to 4 weeks prior to the experiment to provide animals with long alcohol exposure. Twenty rats were given alcohol at the same time the iron was given, and 20 rats were used for normal control animals. All animals were maintained on their regular diet of Purina Laboratory Chow, and fasted 18 hours before the experiments.

To compare the absorption of ferrous with ferric iron, two Fe^{59} isotopes were used. Radioactive ferrous iron was obtained from Abbott Laboratories, Oak Ridge, Tenn., as ferrous citrate. This solution contains benzyl alcohol 0.9% as preservative, ascorbic acid, 33 μg/mg and the pH is adjusted to 5-7 with sodium hydroxide. Radioactive ferric iron was obtained from Abbott Laboratories as ferric chloride. Solutions contain benzyl alcohol, 0.9% as preservative, and have a pH between 1.5 and 2.5 as adjusted with hydrochloric acid or sodium hydroxide.

To determine if oral alcohol had an effect on the total body absorption of ferrous iron in rats, 5 fasted animals which had been drinking 20% ethanol for 3 weeks or longer were each given an oral dose of 0.5 μc Fe^{59} ferrous citrate plus 0.1 mg ferrous iron carrier along with 0.5 ml of 50% ethanol; 5 normal fasted rats were given the dose with the alcohol, and 5 fasted normal control animals the dose with saline. Total body counts made immediately after the dose and again 6 days later were done in an Armac scintillation counter(6) and used to calculate the percent radioactivity remaining in each of the groups. To determine if oral alcohol had any effect on the total body absorption of ferric iron in rats, an identical experiment was done using Fe^{59} ferric chloride and ferric iron carrier.

It was of some interest to us to find if alcohol showed any *in vitro* effect on the rate of absorption of ferrous and ferric iron, since this technique has just been adapted and modified for use in our laboratory.

Our technique is a modification of the methods of Wilson and Wiseman(7) and Brown and Justus(8) and is the following: Fasted rats are killed instantly by a tap on the head and approximately 20 cm of the proximal gut tied off immediately, the first tie being placed as close to the stomach as possible. This segment of gut is removed to a bath of ice cold saline and carefully trimmed of fat, mesentery and any blood vessels. The segment is then everted over a stainless steel rod (300 × 1.5 mm) and any mucus carefully washed away. Beginning with the duodenum, double ties are placed each 4 cm down the gut and the gut divided between them. The sacs, each with one tie left long for easier handling, are placed in numbered tubes of 5 ml of thawed prepared gut wash† and placed in the 37°C shaking water bath for 30 minutes.

Seven ml of GKN‡ containing a known number of counts of Fe^{59} are pipetted into each of 4 serum tubes (rubber stoppered, 15 × 100 mm). Each tube is fitted with 2 needles; one #15 needle to which a length of polyethylene tubing was added to reach to the bottom of the tube, and one #23 needle

† It was noted during studies to be reported later, that intestinal contents of rats feeding up to the time of death regularly enhanced the uptake of Fe^{59} as ferrous iron. This is now incorporated as part of our regular procedure.

‡ Formula: 10.0 g glucose, 100 ml 0.2% phenol red, 80.0 g NaCl (Merck), 4.0 g KCl, 0.6 g KH_2PO_4, 0.6 g Na_2HPO_4 and 1000 ml deionized distilled water (dilute 1-10 with deionized distilled H_2O for use).

for use as gas exit, and through which $NaHCO_3$ can be added as necessary. The gas mixture (95% O_2-5% CO_2) is furnished through an empty baxter bottle into which gas is introduced from the cylinder to provide a very slow bubbling up through the liquid in the tubes. After the 30-minute pre-incubation in the gut wash, each numbered sac is washed in clean GKN and placed in its tube of GKN Fe^{59}, the gas flow adjusted, and incubated in the shaking water bath (37°C) for 2 hours. The minute amount of phenol red indicator in the GKN can be watched carefully for changes in pH, and as far as is possible, all tubes are kept neutral with small additions of 5% $NaHCO_3$. At the end of 2 hours, the sacs are removed to be washed for 15-20 seconds under running deionized water, placed in numbered vials, and counted. They are then carefully freed of the suture ties, and weighed on a torsion balance. Calculations are made on counts per min per g divided by counts present in the tube multiplied by 100. This is designated as the index of absorption.

The *in vitro* absorption index of ferrous iron was determined in 20 segments prepared from 5 rats which had been drinking 20% ethanol for 3 or 4 weeks, and in 20 segments from 5 rats each given one ml ethanol 20 minutes before the sacs were prepared from them. These were compared with the absorption indices of sacs prepared from 20 segments from 5 normal rats using Fe^{59} as ferrous citrate in the GKN solution. This series of experiments was repeated using Fe^{59} as ferric chloride to determine the absorption index of ferric iron.

Results. Our condensed results are recorded in Table I and II. It is evident from Table I that short or long term alcohol had no significant effect on the uptake of ferrous^{59} or ferric^{59} salts by normal healthy rats. Table II records the mean values of the uptake indices of groups of 20 rat intestinal segments. Each 20 segments were obtained from 5 separate rats each providing 4 contiguous segments of 4 cm each starting from the gastroduodenal junction. The uptake indices of segments 3 and 4 were always greater than segments 1 and 2 and often by margins of 100% providing the wide standard deviation in this Table. It can be seen in this Table that the segmental absorption of Fe^{59}-Cl_3 in rats drinking 20% alcohol for 3 weeks before the study was slightly but definitely increased over the controls and rats given a single dose of alcohol. As expected the indices for ferric absorption in general were much less than for ferrous.

TABLE I. Per Cent Total Body Radioactivity Remaining in Rats After Oral Fe^{59} (groups of 5 rats).

	Ferrous^{59} (mean ± 1 S.D.)	Ferric^{59} (mean ± 1 S.D.)
Rats on 20% ethanol for 21 days	17 ± 7.9	16 ± 10
Rats with single dose of 50% ethanol	19.3 ± 7.38	16.2 ± 9.1
Control rats	15.1 ± 14.1	14.3 ± 10.9

TABLE II. Segmental Absorption Index of Fe^{59} in Rats.*

	Ferrous^{59} (mean ± 1 S.D.)	Ferric^{59} (mean ± 1 S.D.)
Rats on 20% ethanol for 21 days	57.4 ± 19.8	35.4 ± 16.6
Rats with single dose of 50% ethanol	41.9 ± 13.9	22.8 ± 7.97
Control rats	58.3 ± 29.4	18.6 ± 15.4

* Each result = mean of 20 observations in 4 standard segments.

Conclusion. Long or short term alcohol administration appeared to have no effect in normal healthy rats on either total body uptake or the isolated intestinal segment uptake of Fe^{59} in its ferrous or ferric form with the possible exception of the effect of long term alcohol on the segmental absorption of ferric Fe^{59}.

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Streptococcal and Staphylococcal Endocarditis and Renal Lesions in Rats After Epinephrine in Oil and Dibenamine. (30664)

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Epinephrine in oil, 5 mg/kg subcutaneously daily in rats, produced a myocarditis, usually most pronounced 24 hours after the second dose(1). The myocarditis was associated at times with a severe valvulitis and with sterile vegetations involving the left atrium and occasionally the left ventricle. These changes were prevented or mitigated by prior treatment with an adrenergic blocking agent such as Dibenamine hydrochloride. The purpose of this study was to determine the susceptibility of such rats to bacterial endocarditis and the effect of pretreatment with Dibenamine.

Materials and methods. Five lots of young male Sprague-Dawley rats, 90-100 days of age and 250-350 g in weight, were housed, 4-6 per metal cage, and given a Purina Laboratory Chow diet and water *ad libitum*. Each lot included about 50 rats and was divided into 3 groups. All rats in the 3 groups were given in random order on the same day or on 2 successive days, an injection in the tail vein of 0.5 ml of a 5-hr specified bacterial broth culture(2). One control group received only bacteria. Another group received 2 subcutaneous doses of 5 mg/kg epinephrine in oil at 24-hour intervals, one dose 24 hours before and the second immediately after the first bacterial injection. The third group was treated like the second except that the rats were given in addition, 2 subcutaneous doses of 50 mg/kg of Dibenamine hydrochloride (N-(2-Chloroethyl) dibenzylamine Hydrochloride), one dose 48 and one 24 hours before the first dose of epinephrine in oil.

The bacteria selected were *Streptococcus mitis*, JH-26 and *Staphylococcus aureus*. In previous studies, it was found that intravenous injection of cultures of these bacteria produced a low incidence of endocarditis in normal animals and a high incidence in animals rendered susceptible by prior exposure to simulated high altitude(2,3,4,5) or a cold environment(6) or by prior surgical induction of aortic insufficiency(7). In the initial experiments, the animals received one injection of *S. mitis*. Since the organisms appeared less virulent after repeated subcultures, the rats in later experiments were given a broth culture of *S. mitis* or *Staph. aureus* on each of 2 successive days.

Autopsies were made on surviving rats 6 days after the first bacterial injection and on those that died after surviving at least 48 hours, a period sufficient for development of bacterial endocarditis(5). The heart and kidney were fixed in a 10% aqueous solution of buffered (pH 7.0) formalin. After fixation, the heart was bisected frontally through all 4 chambers. Paraffin sections were prepared in a routine manner. Skip serial sections from each half of the heart and sections of the kidney, made through the hilus and grossly visible lesions, were stained with azure eosin.

Results. The results are summarized in Table I. The mortality rate in rats given epinephrine in oil and *S. mitis* was significantly greater statistically, if the 2 streptococcal groups are combined, than in control rats given *S. mitis* alone; the mortality rate was significantly greater also in rats given epi-