juvant effect in production of circulating anti-heart antibodies. This effect was greater than that of Freund's complete adjuvant and was abolished when the endotoxin was given separately, before or after the homogenate. Focal infiltrative myocarditis was seen in some of the animals but could be attributed equally to the endotoxin as to the administered antigen. It is suggested that the autoimmune process can be partially explained on the basis of modification of tissue antigen by linkage to endotoxin.

Thanks are due to Mr. Eliahu Lazarov for his continued assistance.

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Received September 3, 1963. P.S.E.B.M., 1963, v114.

## Influence of β-mercaptoethylamine, Mesenteric Vessel Clamping and pH on Intestinal Absorption of $Fe^{59}$ in Rats.* (28722)

KEDAR N. PRASAD† AND JAMES W. OSBORNE
(Introduced by T. C. Evans)
*Radiation Research Laboratory, College of Medicine, University of Iowa, Iowa City*

Iron absorption from the small intestine is oxygen dependent(1), but the mechanism is not known. Hypoxia has been reported to increase iron absorption in rats(2,3,4,5). It has been postulated that $O_2$ content of the intestinal mucosal cell may influence absorption of iron by modification of the redox potential within the cell(2,4).

β-mercaptoethylamine (MEA) has been reported to reduce tissue oxygen tension(6, 7). Clamping of the superior mesenteric vessels produces circulatory hypoxia in the gut (8). Therefore, the effects of MEA and mesenteric vessel clamping on intestinal iron absorption were studied. As a byproduct of the investigation to see whether MEA and mesenteric vessel clamping would influence iron absorption, the effect of pH on the process was studied. A perfusion technique was used to determine the effects of MEA, mesenteric vessel clamping, and pH upon iron absorption from the small bowel.

*Materials and methods.* Male Holtzman rats weighing from 200 to 250 g were used. In one group of animals, MEA (100 mg/kg) was given intraperitoneally fifteen minutes before perfusion of $Fe^{59}SO_4$. In another group, superior mesenteric vessels were

* This investigation was supported in part by research grant from Division of Radiological Health, Bureau of State Services, P.H.S.

† Present address: Medical Research Center, Brookhaven National Laboratory, Upton, N. Y. The study was conducted in partial fulfillment of the requirements for a Ph.D. degree in Radiation Biology at Univ. of Iowa.

clamped by a small serrefine during the entire perfusion period.

The perfusion solution contained 136 mM $Na^+$, 4 mM $K^+$, 28 mM $HCO_3^-$, 112 mM $Cl^-$, and 60 microcuries of ferrous sulfate ($Fe^{59}SO_4$) of specific activity 30.1 mc/mg Fe. The solution was adjusted to a desired pH by addition of 12 N HCl and 1 N NaOH so that the final electrolyte concentration in the solution was about 300 mM/liter. pH values used were 2, 4, and 6. All containers and rubber tubing utilized were soaked overnight in 10% stable ferrous sulfate, then rinsed with distilled water before use. At the end of each perfusion experiment, the tubing was rinsed with distilled water and dried. Before each subsequent experiment, the tubing was rinsed with stable ferrous sulfate and stock $Fe^{59}SO_4$ solutions before it was introduced into the gut. The procedures described minimized the loss of $Fe^{59}$ onto the tube or container walls.

All animals were fasted for 24 hours before perfusion. A metal screen was inserted into the bottom of each cage to prevent ingestion of fecal material by the animals. Animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg) which was supplemented by ether as needed.

Small slits were made lengthwise in the small bowel 3 cm below the duodeno-jejunal flexure and 3 cm above the caecum. Inlet and outlet tubes were introduced into the slits and held in place with silk sutures. A 3-way stopcock was inserted into the inlet tube near the point where the tube entered the gut. This made it possible to obtain a 2 cc reference standard just before the perfusion solution entered the bowel. The abdomen was covered during perfusion with gauze sponges soaked in warm saline. Occasionally during perfusion, gentle intestinal massage was necessary to free the gut of fecal material. The stock solution was maintained at 36° ± 1°C. It was pumped through the entire ileum and jejunum for one hour at a rate of 0.84 ± .06 ml/min by a Bowman infusion pump. The time of appearance of the first drop in the collection flask was considered as zero time. The pH at the point of entry of the solution into the gut was 6 except in those experiments designed to show differences due to pH.

Immediately after one hour of perfusion, 2 cc of blood were drawn by cardiac puncture and assayed for radioactivity. The animals were then killed with ether. The perfused segment was freed of mesentery, opened lengthwise, and the residual perfusate recovered. The segment was then cut into 3 or 4 pieces; each piece was held at one end by a forceps and subjected to 6 consecutive saline rinses of 30 seconds each. Upon removal from the last solution, each segment of the gut was transferred without blotting to a plastic test tube. In each case, the volume of the intestinal strip was about 2 cc and constant counting geometry was maintained throughout.

Assay of $Fe^{59}$ activity was done by integral counting using a scintillation well counter, a single channel gamma spectrometer, and a scaler. The per cent of the total amount of $Fe^{59}$ perfused which was found in the entire ileum and jejunum after washing was considered to represent mucosal uptake and was calculated as follows:

$$\frac{\text{Counts/min in the entire jejunum and ileum}}{\text{Total counts/min perfused}} \times 100.$$

For histological studies, intestines from animals were treated as previously described (8). The Student t test was employed to determine the level of significant difference between two means.

*Results.* It was found that a given volume of a mucinous substance from the gut lumen had "trapped" about 8 times as much $Fe^{59}$ as existed in the same volume of the reference standard. No epithelial cells were found in the mucinous substance.

Fig. 1 shows that the mucosal uptake of $Fe^{59}$ was significantly below control values ($P \leq .05$) when MEA (100 mg/kg) was given 15 minutes before perfusion. Consistent values for blood activity could not be obtained. No histological changes in the intestines were seen after one hour of perfusion in MEA-treated animals. The mucosal uptake of $Fe^{59}$ was reduced to 2.1% when the

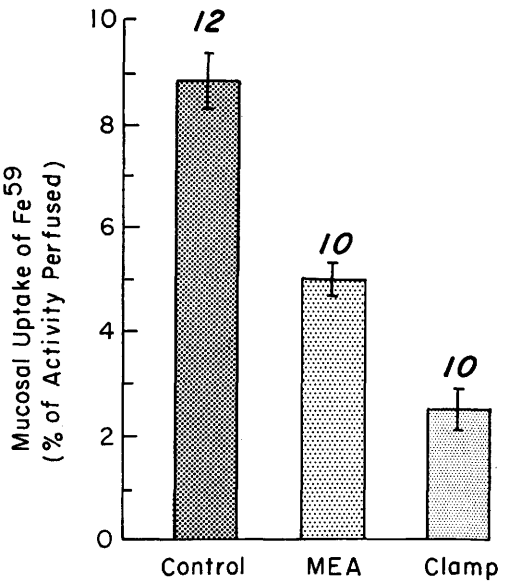


FIG. 1. Effect of MEA and mesenteric vessel clamping on mucosal uptake of $Fe^{59}$ in rats. The number at top of each bar indicates number of animals. Vertical line at top of bar indicates standard error of mean.

mesenteric vessels were clamped during the entire period of perfusion. This was significantly different from the control ($P \leqq .05$). The blood activity was significantly higher ($P \leqq .05$) than in non-clamped animals. Histologically, the gut (Fig. 2) showed acute congestion in the lamina propria. Villi were devoid of epithelial cells, but the crypts appeared normal.

Table I shows that the mucosal retention was higher at pH 4 than at pH 2 and pH 6. The blood activity was 20 times higher at pH 2 and 7 times higher at pH 4 than at pH 6. Histologically, the gut perfused at pH 2 showed no apparent degenerative changes in the mucosa. When the pH of the solution at point of entry into the gut was 2, 4, and 6, the pH at the point of exit was found to be 6.4, 7.2, and 7.2 respectively.

*Discussion.* Some investigators(10,11) who used the perfusion technique to study absorption *in vivo* considered the amount which disappeared from the lumen as the amount absorbed. Had this criterion been used in this study, it would have given a false impression of iron absorption mainly because of the $Fe^{59}$ which was "trapped" by the mucinous substance found in the gut lumen. Disappearance of a compound from a perfusion solution without absorption by the gut has been noted before. It was reported(12) that small losses of polyvinylpyrrolidone-I-131 in normal subjects and large losses in patients with sprue were due to binding of the polymer by the intestinal mucous.

In defining mucosal uptake as the per cent of the total amount of $Fe^{59}$ perfused which was present in the entire ileum and jejunum after perfusion, the small amounts transferred to the blood were so minute that they have been ignored. Thus for the purpose of determining the capacity of the mucosa for mucosal uptake, the method seems satisfactory.

TABLE I. Influence of pH on $Fe^{59}$ Absorption in Rats.

| No. of animals | pH | % of activity perfused in: Mucosa | Blood* ($\times 10^{-3}$) |
|---|---|---|---|
| 10 | 2 | 12.0 ± .9† | 1.4 ± .2 |
| 10 | 4 | 20.8 ± 1.3 | .5 ± .04 |
| 12 | 6 | 8.9 ± .5 | .07 ± .01 |

* Blood volume was estimated to be 5.1% of body wt(9).
† Standard error of mean.

The reduced mucosal uptake of $Fe^{59}$ as a result of MEA administration is in agreement with the hypotheses that MEA reduces tissue oxygen tension(6,7) and reduced oxy-

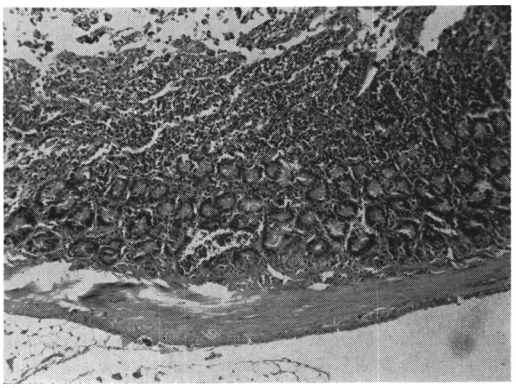

FIG. 2. Histological appearance of ileum immediately after perfusion. Mesenteric vessels were clamped during the one hour period of perfusion. Villi are devoid of epithelial cells, but crypts appear normal. There are congested areas in the lamina propria.

gen tension decreases mucosal uptake of iron (1). The mucosal uptake of $Fe^{59}$ could be used as a procedure for determining whether chemical compounds suspected of having a role as an oxygen competitor really have that property.

The extent of reduction of mucosal uptake of $Fe^{59}$ during mesenteric vessel clamping was much greater than in animals given MEA 15 minutes before perfusion. The marked reduction in mucosal uptake of $Fe^{59}$ could have been due to reduced oxygen tension *per se* plus the clamping-induced sloughing of epithelial cells from the villi. Blood activity in the clamped animals was twice that in the sham-clamped group. Thus, under reduced oxygen tension, $Fe^{59}$ presumably was transferred to the blood at a higher rate than in the sham-clamped animals, and likely occurred during the initial clamping period when the epithelial cells lining the villi were still viable. A change in redox potential which favors conversion of $Fe^{+++}$ to $Fe^{++}$ (2,4) during the clamping period could account for an increased iron transport from mucosa to the blood. Since only divalent $Fe^{59}$ is transported to the blood(13), the hypothesis of increased rate of conversion from the ferric to ferrous form under reduced $O_2$ tension seems to be a reasonable one to account for the augmented transfer of $Fe^{59}$ to the blood.

In the experiment reported here, the reference standard at the point of entry into the gut gave a higher count rate at pH 2 than at pH 4 or pH 6. An increase in $H^+$ concentration in an aqueous medium decreases the conversion of $Fe^{++}$ to $Fe^{+++}$. This may be the reason why at pH 2 there was reduced adsorption of iron by the walls of the rubber tubing and containers.

As shown in the diagrammatic representation of pathways for iron absorption (Fig. 3), the influence of low pH on processes A, B, and C, and on the cell membrane (M) might result in a "mucosal error" and thus a large build-up of $Fe^{++}$ within epithelial cells lining the villi. The scheme proposed thus could account for the augmented transfer of $Fe^{59}$ from mucosa to the blood at low pH.

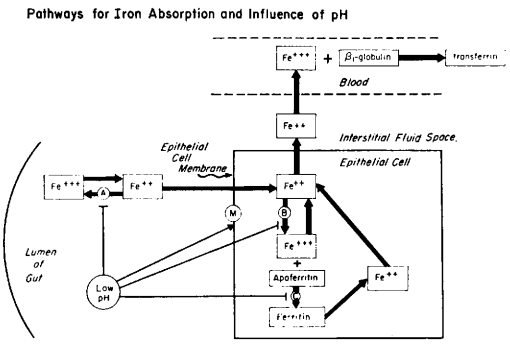


FIG. 3. Pathways for iron absorption based on "mucosal block" hypothesis and influence of pH. ⟶ stimulatory effect ⟊ inhibitory effect

Mucosal retention of $Fe^{59}$ was higher at pH 4 than at pH 2, presumably because less $Fe^{59}$ was transferred to the blood at pH 4 than at pH 2. The high rate of $Fe^{59}$ transfer from mucosa to blood at pH 2 was likely due to availability of more intracellular $Fe^{++}$.

The fact that low pH increases iron absorption may help in understanding the etiology of hemochromatosis. Whether the "mucosal error" which leads to increased iron absorption is a hereditary defect as suggested by some authors(14,15) and/or an acquired one is unknown. We have shown that a perfusion solution delivered to the small bowel at a low pH does induce a mucosal error which increases iron absorption in the rat. The mechanism of pH-induced "mucosal error" and its persistence deserve further investigation.

*Summary.* Mucosal uptake of $Fe^{59}$ by the small intestine of rats was below normal when MEA (100 mg/kg) was given intraperitoneally 15 minutes before perfusion. The clamping of the mesenteric vessels during the entire period of perfusion reduced mucosal uptake even more than MEA. Activity of $Fe^{59}$ in the blood of the clamped group was twice the normal value. Consistent blood values were not obtained in the MEA group. Mucosal retention of $Fe^{59}$ was much higher at pH 4 than at pH 2 and pH 6, but blood activity was considerably higher at pH 2 than at pH 4 and pH 6.

The authors are grateful to Dr. Titus C. Evans, Radiation Research Laboratory for reading the

manuscript and to Drs. H. P. Schedl and H. Hamilton, Dept. of Internal Medicine, for several stimulating discussions and use of some equipment. The authors are thankful to Mr. George Nokes for advice on some aspects of the perfusion technique. The technical help of Miss Kathleen Masterpole is also much appreciated.

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Received September 3, 1963. P.S.E.B.M., 1963, v114.

## Distribution of $Se^{75}$ in Serum Proteins of Chicks and Influence of Selenium on Albumin Recovery.* (28723)

ELMER D. WALTER, LEO S. JENSEN AND JACK S. DUNLAP
*Nutrition Laboratory, Department of Animal Sciences and Department of Veterinary Pathology, Washington State University, Pullman*

Time-distribution studies on dog serum proteins(1) have demonstrated that albumin is the immediate acceptor of injected $Se^{75}$. After some time, however, the selenium is released to or bound by other serum proteins, chiefly alpha-2 and beta-1 globulins, indicating that albumin may act in the transport of selenium. Similar studies have not been conducted with chicks, although it has been shown(2) that $Se^{75}$ retention in various tissues, including blood, is markedly influenced by the level of selenium in the diet. A high incidence of exudative diathesis(3), which is accompanied by serum protein changes, characterized by lowered A/G ratios(4) is produced by feeding chicks a torula yeast-glucose type diet. Vitamin E or selenium will prevent this condition(5). In studying serum protein changes in chicks recovering spontaneously from exudative diathesis, Bieri and Pollard(6) indicated that total protein concentrations were usually elevated, while the A/G ratios varied from normal to very low. Thus, it appeared that during recovery, globulin synthesis proceeded faster than albumin synthesis.

It is shown here that serum albumin is not the immediate acceptor of $Se^{75}$ in chicks, and that oral administration of selenium appears markedly to influence albumin synthesis (or turnover) in Vit. E and selenium depleted chicks.

*Methods.* Two groups of New Hampshire $\times$ Delaware cross-bred male chicks were reared in battery brooder compartments for 3 weeks. One group was fed a basal diet composed principally of torula yeast and glucose monohydrate, similar to that used by Scott *et al*(3). The second group was fed the same diet, supplemented with selenium (1 mg/kg) as selenious acid. At 3 weeks, 2 chicks were randomly selected from each group and moved to small wire-floored cages

* Scientific Paper No. 2375, Washington Agri. Exp. Sta., Pullman. Project 1706.