Transferrin and the Absorption of Iron

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MOST DIETARY IRON is not absorbed even though it is in a form which makes it available for absorption.\(^1\) The reasons for this have intrigued investigators for many years. The relative saturation of iron-binding protein, transferrin, has been proposed as one controlling mechanism.\(^2,3\) To evaluate the influence of saturating transferrin on the absorption of iron, the following experiment was devised. The procedure is similar to one used for the study of amino acid absorption.\(^9\)

METHOD

Mongrel dogs, of 9 to 20 Kg. with hematocrits of 42 or more, were selected for the study. After an overnight fast they were anesthetized with pentobarbital. The duodenum was brought out through an abdominal incision and a segment varying from 10 to 20 Cm. was isolated from the proximal and distal bowel with clamps. The dog was heparinized with 50 mg. of sodium heparin. The mesenteric vein draining the isolated segment of bowel was cannulated with a polyethylene catheter. The arterial supply of the loop was preserved. Small venous tributaries from the isolated loop were closed with ligature or cautery to prevent absorbed iron from leaking back to the animal. An infusion of normal saline was started into the loop, entering through a 16 or 20 gauge needle and exiting through a soft rubber catheter; both needle and catheter were secured in parallel in the antimesenteric border of the gut; the catheter was held by a purse string suture (fig. 1). The efferent catheter was elevated 1–3 cm. to induce continuous, slight distention of the loop. The loop was covered with gauze, moistened at approximately 5-minute intervals with normal saline at 37 C. and warmed continuously with a 100 watt bulb kept about 12 cm. from the surface of the gauze.

Just prior to the start of the absorption study, an exchange transfusion was performed between the experimental dog and a reservoir of heparinized blood collected from two or three fasting, anesthetized, heparinized donor dogs. The exchange, 750–1000 cc. in volume, was performed to avoid the possibility of factors in the donor blood influencing the iron absorption study after its start. During the experiment, measured and estimated blood loss was replaced from the blood reservoir.

A solution consisting of ferrous sulfate 7.5 mg., ascorbic acid 2.4 mg., iron\(^{59}\) citrate 150–1200 \(\mu\)g., and normal saline 750 cc. was prepared. The iron concentration of this solution was 2 \(\mu\)g. per cc. The pH was 5. The solution was warmed before entering the gut lumen by passing through plastic tubing coiled in a 37 C. water bath. The absorption study was begun with the rapid infusion of 200 cc. of this iron-containing solution through the gut lumen, followed by a continuous drip of approximately 1 cc. per minute for the remainder of the 4- to 5-hour experiment.

The effluent from the mesenteric vein was collected in timed fractions and its volume measured in heparinized graduates. Duplicate 1 cc. aliquots were pipetted into counting tubes. All tubes were counted in a Packard Auto Gamma crystal scintillation counter. The counting error was \(\pm 2\) per cent or less for most samples. Samples with lowest activity had a maximum counting error of \(\pm 12\) per cent.

At the end of each experiment the duodenal loop was excised, freed of its mesentery,
Fig. 1.—Illustrates arrangement of gut loop for study of iron absorption. The duodenum has been brought out through an abdominal incision and isolated from the proximal and distal bowel with clamps. The mesenteric vein draining the isolated segment has been cannulated with a polyethylene catheter.

opened, blotted and weighed. Absorption was calculated as µg. of iron absorbed per Gm. of gut.

When an arterial infusion was to be given, the major artery supplying the isolated loop was cannulated in retrograde fashion through a small, distal branch. The lesser arterial supply of the isolated loop was closed by ligature. The arterial infusate consisted of either ferrous ammonium sulfate or ferrous sulfate in normal saline, given by constant infusion pump or gravity drip. The iron content of the infusate ranged from 125 to 287 µg. per cc.; the speed of infusion ranged from 0.05 to 0.2 cc. per minute.

In each experiment the total iron given intra-arterially was well in excess of the total iron binding capacity of the mesenteric venous effluent plasma.

Iron and total iron-binding capacity of the serum were determined by the method of Ramsey.3,4 Starch gel electrophoresis followed the method of Smithies.5 Iron59 in the gel was located by cutting serial strips perpendicular to the path of protein migration and counting these in the Auto Gamma crystal scintillation counter.

RESULTS

The method used in these experiments maintained an approximately constant concentration of iron in the intestinal lumen; a maximum of 8 per cent of available iron was absorbed in any timed interval. Absorption curves in control experiments are shown in figure 2. In each control experiment there was a maximum rate of absorption in the first half of the study, and a decline to approximately half the maximum rate at the end of the study. Spontaneous fluctuations in the rate of absorption were sometimes as great as 20 per cent in adjacent timed periods. The reasons for these fluctuations in the rate of absorption are not clear. Peristalsis, which was active but variable from time to time, could have changed available surface area. Blood flow did not seem to
Fig. 2.—Absorption of iron from isolated segments of dog duodenum. Mesenteric venous outflow from isolated segments of dog duodenum has been collected at timed intervals. The radioactivity of the sample collected in the interval during which the rate of iron absorption is maximal has been given a value of 100. The rate of each other interval is plotted relative to the maximum. Despite the relatively constant concentration of intraluminal iron, the rate of iron absorption falls from an early maximum. A similar decline in rate of iron absorption is seen in humans subjected to constant intragastric infusions of iron. This fall may reflect a process which also takes place under more physiologic circumstances and which serves to diminish the amount of dietary iron ultimately absorbed.

Dog 24M, 13 Kg. female. Gut segment 8.6 Cm. Iron absorbed (mean rate), 0.13 μg. per Cm. of gut per hour. Dog 1P7, 9 Kg. male. Gut segment 21.7 Cm. Iron absorbed (mean rate), 0.34 μg. per Cm. of gut per hour. Dog 80P, 17 Kg. male. Gut segment 18.1 Cm. Iron absorbed (mean rate), 0.29 μg. per Cm. of gut per hour.

Fig. 3.—Absorption of iron from isolated segment of dog duodenum. Infusion of normal saline by gravity drip into artery supplying isolated loop from 90–140 minutes. Infusion of ferrous sulfate in normal saline by gravity drip into artery from 140–180 minutes. Dog 97P, 11 Kg. male. Gut segment 13.3 Cm. Iron absorbed (mean rate), 0.12 μg. per Cm. of gut per hour.
Fig. 4.—Absorption of iron from isolated segment of dog duodenum. Intra-arterial infusion of ferrous ammonium sulfate by constant infusion syringe from 90–135 minutes. Dog R05, 20.5 Kg. male. Gut segment 12.5 Gm. Iron absorbed (mean rate), 0.04 μg. per Gm. of gut per hour.

Fig. 5.—Absorption of iron from isolated segment of dog duodenum. Intra-arterial infusion of ferrous sulfate by constant infusion syringe from 75–150 minutes. Dog R92, 18 Kg. male. Gut segment 19.4 Gm. Iron absorbed (mean rate), 0.05 μg. per Gm. of gut per hour.

influence absorption except to reduce it when changes were drastic because of inadvertent hemorrhage or mesenteric venous obstruction; the experiments in which these occurred were discarded.

Three experiments in which an intra-arterial iron infusion was given are shown in figures 3, 4 and 5. Iron absorption continued during these intra-
artrial infusions despite the large excess of ionic iron in the plasma perfusing the intestinal loop. Electrophoresis of plasma from the mesenteric venous effluent obtained during the latter part of the intra-arterial infusion in dog RO5 showed a diffuse distribution of iron\textsuperscript{59}. Electrophoresis of mesenteric venous effluent plasma obtained prior to the start of the intra-arterial infusion in dog RO5 showed iron\textsuperscript{59} discretely localized.

**DISCUSSION**

Absorption of iron from the isolated intestinal loop continues despite saturation or near-saturation of transferrin's iron-binding capacity. Viewed against the spontaneous fluctuations of the control curves, which are as great as 20 per cent, there is probably no significant change in iron absorption during the intra-arterial iron infusions. This finding seems incompatible with the concept that the degree of saturation of transferrin plays a direct or immediate role in regulating iron absorption.

Laurell first proposed that iron absorption might be diminished by saturation of transferrin’s iron-binding capacity.\textsuperscript{10} Hallberg and Sövell in an elegant series of human studies found that saturating transferrin with ionic iron suppressed iron absorption. They suggested that previous studies which had failed to demonstrate this effect were invalid because colloidal iron, rather than ionic iron, had been used to saturate the iron-binding protein.\textsuperscript{2} Their method is indirect, however, and assumes that little or none of the absorbed iron is removed by the liver before entering the general circulation. Recently reported experiments\textsuperscript{6} have shown that rats with saturated transferrin continue to absorb iron from the small intestine but under these conditions most of the absorbed radioactive iron is immediately deposited in the liver. It seems likely that Hallberg’s subjects did the same and that the apparent decrease in iron absorption associated with saturation of transferrin was an artifact produced by prompt removal of absorbed radioactive iron from the portal blood by the liver.

The negligible influence of saturating transferrin on iron absorption is, perhaps, not unexpected. Hemochromatotics continue to absorb iron despite near-saturation of their total iron binding capacity.\textsuperscript{7} Moreover, Heilmeyer’s case of atransferrinemia absorbed excessive iron.\textsuperscript{8}

**Summary**

A loop isolated in situ has been used to study iron absorption in the dog. An infusion of iron salt into the artery supplying the isolated loop fails to stop the absorption of iron from the lumen of the gut. Iron absorption appears to be independent of the relative saturation of iron-binding protein.

**Summario in Interlingua**

Un ansa intestinal, isolate in sito, esseva usate pro studiar le absorption de ferro in le can. Un infusion de sal de ferro ad in le arteria alimentante le isolate ansa non arresta le absorption de ferro ab le lumine del intestino. Le absorption de ferro pare esser independente del saturation relative de proteina ferro-ligatori.
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