Distribution of Total Ferritin in Intestine and Mesenteric Lymph Nodes of Horses After Iron Feeding.* (18121)

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It has been postulated that a mechanism exists whereby the gastrointestinal mucosa regulates the amount of iron absorbed from the intestine. Hahn *et al.*(1) and Granick(2) suggested that the ferritin and apoferritin of the gastrointestinal mucosa are concerned with the absorption of iron. The protein apoferritin, which can combine with ferric iron to form ferritin, was thought to accumulate absorbed iron until saturated, whereupon the establishment of an equilibrium state between the ferric iron in ferritin and ferrous iron prevented further absorption of iron from the intestine. This suggestion was apparently supported by experiments conducted by Granick(3) whose semi-quantitative data indicated that only traces of ferritin are present in the duodenal mucosa of normal guinea pigs, but that after feeding iron, the ferritin content of the duodenal mucosa increases rapidly, and then falls gradually, finally reaching the level of control animals 3 to 6 days after feeding the iron. Prevention of further absorption of iron from the intestine when the apoferritin is saturated with ferric iron has been termed "mucosal block."

Gillman and Ivy(4) presented histochemical evidence indicating a progressive increase in the amount of iron in the intestinal mucosa and mesenteric lymph glands after feeding iron, but were unable to demonstrate consistently an increase in crystallizable ferritin in the intestine of the guinea pig after feeding iron, as had been reported by Granick. However, Granick's result has been reproduced in this laboratory.†

The experiments to be reported in this paper deal primarily with the determination of total ferritin‡ in the intestine and mesenteric lymph nodes of the horse by an immunochemical technic, following the ingestion of iron. We were interested in discovering whether ferritin is involved in the absorption of iron in the intestine of the horse, an animal from which ferritin can be obtained with ease and in quantity; and also in determining whether the increase in the iron content of the mesenteric lymph nodes after the ingestion of iron might be attributable to ferritin.

Experimental procedure. Three 8-year-old male horses were used in the experiments which follow. All three animals eventually were sacrificed by being shot through the head. Horse No. 1 which served as the control, was fasted for about 18 hours before being sacrificed, while horse No. 2 and horse No. 3, after an 18 hour fast, were fed gelatin-coated capsules of ferrous ammonium sulfate (containing a total of 30 g of iron) with the use of a capsule gun. Horse No. 2 was sacrificed 24 hours after the administration of iron, while Horse No. 3 was sacrificed 48 hours after the feeding of iron. The following tissue samples were taken from each horse for analysis: 3 successive portions of intestine beginning just below the pyloric sphincter,

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‡ Hereafter, unless specified otherwise, the term, ferritin as applied to these experiments, will refer to total ferritin, which includes ferritin, apoferritin, and intermediate forms.

2.5 feet, 5 feet, and 4 feet in length; the last 3 feet of intestine; and the mesenteric lymph nodes. Each sample was washed with isotonic saline.

Extracts of ferritin were prepared from the tissue to be assayed by grinding the tissue in a Waring blendor with 2 times its weight of water. The suspension was heated to 75-80°C and the coagulum was centrifuged and discarded. Sufficient acetic acid (50% by volume) was then added with stirring to adjust the pH to 4.6-4.7, and the solution was allowed to stand for 2 hours at room temperature(5). Any precipitate which formed was centrifuged and discarded. The ferritin was then precipitated by adding 35 g of $(NH_4)_2SO_4$ per 100 ml of solution, and after approximately 8 hours, the precipitate was centrifuged down and dissolved in a small amount of water. The solution was dialyzed against isotonic saline, and the clear dialyzed solution, containing ferritin, was used in the quantitative test described in the following paragraph. The procedure described in the above paragraph is based upon the work of Granick(6). The amounts of ferritin in the tissue extracts were determined quantitatively by an immunochemical technic, which was essentially the same as that described by Mazur and Shorr(5). This technic has been worked out independently in our laboratory§ but had not been published by us. In this method, the antigenic properties of ferritin are utilized in the following way. Horse spleen ferritin prepared according to the procedure of Mazur and Shorr(5) with slight modifications‖ is injected into rabbits in increasing amounts over a 4-week period. A total of 4.5 mg of antigen nitrogen is thus injected into each rabbit. The rabbits are bled 5 days after the last injection and the antisera are collected. The antigen-antibody reaction is measured by the precipitin test(8). The tissue extract is reacted with the horse spleen ferritin antiserum, and the total nitrogen content of the specific precipitate is determined by the micro-Kjeldahl method. The amount of total ferritin nitrogen present is read from a standard curve prepared with known amounts of ferritin. In our work, the colors of the specific precipitates were noted, since it has been shown(7) that the iron is precipitated quantitatively with the antigen.

Results and discussion. Since the antibody produced during the process of immunization with ferritin reacts with the protein moiety of ferritin, and since both apoferritin and ferritin react identically with the antiserum, the determinations made in this investigation measure total ferritin. The latter term includes apoferritin, iron-saturated ferritin, and any ferritin which may be only partially saturated with respect to iron. Since the colors of the specific precipitates obtained with the tissue extracts of the control horse No. 1 were paler in color than those obtained from the iron-fed horses, it is likely that in the tissues of the control horse the ratio of ferritin to apoferritin was very low, or else that a ferritin only partially saturated in iron was being precipitated.

The procedure used in this study for extracting and precipitating ferritin has not been proved quantitative over the entire range of ferritin concentrations studied, but it was the only method recorded in the literature(6) at the time this work was carried out. More recently, the procedure has been modified by Mazur and Shorr(7).

The distribution of total ferritin in the intestine and mesenteric lymph nodes of the untreated horse (No. 1) and of the horses fed iron (No. 2 and 3) is presented in Table I. (A very small amount of undissolved ferrous ammonium sulfate was found in the stomachs of the latter two animals upon sacrifice.) In the control horse (No. 1) ferritin was found to occur in the first 2.5 feet of

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‖ Mazur, A., personal communication.

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TABLE I. Distribution of Ferritin in Intestine and Mesenteric Lymph Nodes of Horses After the Ingestion of Iron.

Tissue analyzed	Total ferritin nitrogen, μg per foot of intestine, or μg per g wet mesenteric lymph node		
	Horse No. 1 (fasted control)	Horse No. 2 (fasted, then sacrificed 24 hr after feeding ferrous ammonium sulfate 30 g Fe)	Horse No. 3 (fasted, then sacrificed 48 hr after feeding ferrous ammonium sulfate 30 g Fe)
1st 2.5 ft of intestine beginning at pylorus	351	4225	2592
Next 5 ft of intestine	132	320	1546
" 4 ft " "	131	79	1717
Last 3 ft " "	205	270	783
Mesenteric lymph nodes	15	57	77

intestine beginning just below the pyloric sphincter, and to a lesser extent in the following 5 foot and 4 foot lengths of intestine, as well as in the last three feet. The presence of a small amount of ferritin was also noted in the control animal (No. 1) in the mesenteric lymph nodes.

The increase in the total ferritin content of the intestine of the horse after the feeding of iron indicates that ferritin probably is involved in the phenomenon of iron absorption in this animal. It appears that during the first 24 hours following the administration of iron, the absorption of the iron has occurred largely through the first 2.5 feet of intestine, since a profound increase in the amount of total ferritin in this portion of the intestine during the first 24 hour period has been demonstrated. However, during a 48 hour interval after feeding of iron, the iron was absorbed in the first 11.5 feet of intestine and also in the last 3 feet. Essentially then, these results are similar to those obtained by Granick(3) using the guinea pig, and that portion of the process of iron absorption which involves ferritin appears to be comparable in the two species.

Since there was a notable increase in the amount of total ferritin in the mesenteric lymph nodes of both horses No. 2 and 3 after the feeding of iron, the increase in histologically-demonstrable iron in the mesenteric lymph nodes following the ingestion of iron reported by Gillman and Ivy(4) may be attributable to an increased ferritin content of this tissue. Thus the results reported in this paper suggest that the lymphatic system is involved in iron absorption and that ferritin assumes a rôle in this case also.

Summary. 1. The distribution of total ferritin in the intestine and mesenteric lymph nodes in a control horse was compared with the corresponding distributions in 2 horses fed iron, using an immunochemical technic for determining the ferritin in extracts of the tissues in question.

2. Ferritin was found to be present in the first 11.5 feet of intestine of the control horse as well as in the last 3 feet of intestine. Small amounts of ferritin were detected in the mesenteric lymph nodes of the control animal.

3. Twenty-four hours after the oral administration of an amount of ferrous ammonium sulfate containing 30 g of iron to a second horse the total ferritin content was appreciably increased in the first 2.5 feet of intestine while 48 hours after feeding the same amount of ferrous ammonium sulfate to a third horse, there were notable increases in the amounts of total ferritin in the first 11.5 feet and in the last 3 feet of intestine.

4. Twenty-four hours after feeding iron to a second horse there was approximately a 4-fold increase in the amount of total ferritin in the mesenteric lymph nodes based on the value for the control horse, while 48 hours after feeding iron to the third horse, more than a 5-fold increase was noted, as judged by the value for the control horse.

5. The data indicate that ferritin is involved in the phenomenon of iron absorption through the intestine of the horse, and that the lymphatic system is concerned with iron absorption by some process in which ferritin also plays a rôle.

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Collagen Content of Guinea Pig Tissues. (18122)

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A chemical method has been described by which collagen, the major fibrous component of connective tissue, can be measured quantitatively in tissues(1). This technic has been used to study the effects of physiologic and pathologic processes on the formation, distribution and destruction of collagen(1-13). During the course of an investigation of the effect of ascorbic acid deficiency on the collagen content of guinea pig tissues(6), the necessity for a more detailed study of the normal pattern of connective tissue distribution in the body became more evident. Therefore, this study was undertaken.

Experimental. One hundred and eight male guinea pigs weighing between 60 and 1000 g were selected from an inbred AMC strain at this laboratory. The animals were maintained on a diet of Derwood Rabbit Chow pellets and fresh greens daily *ad libitum*. They were weaned at approximately 200 g body weight. The guinea pigs were sacrificed with a blow to the back of the skull, which occasioned a variable amount of bleeding

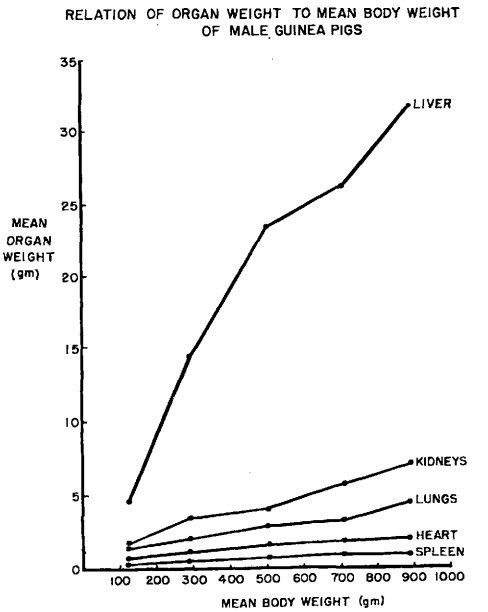

FIG. 1.

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