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Effect of Intestinal Contents on Uptake of Radioiron by Everted Rat Gut Sacs.* (32106)

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Although much attention has recently been directed to the effect of gastric(3) and pancreatic(5) secretions on the absorption of iron, the role of intestinal contents has been neglected. Iron absorption can take place along the whole gastrointestinal tract (8) and even the colon can absorb ferrous iron(7). It seems likely that when there is an increased demand for iron these potential sites of absorption may play an important part. If this is so, then intestinal intraluminal factors may also be important in this process. During the investigation of the uptake of Fe^{59} by everted intestinal sacs obtained from rats, we noticed that sacs prepared from feeding rats ("unfasted") took up more isotope than sacs from fasted rats. We decided to investigate further the effect of intestinal contents and other materials on the *in vitro* uptake of Fe^{59} by everted gut sacs.

Method. The gut sacs were prepared and used according to the method we have already described(6), using the first 20 cm of gut beyond the pylorus to provide 4 segments of 4 cm from each rat. At least 4 such sequential segments from one or more rats were used to study a single test material. The segments were first exposed to the prepared secretion or test material at 37°C for 30 minutes, then washed in ice cold phosphate buffer at pH 7.2. The uptake of Fe^{59} by the segments as ferrous citrate was then determined and recorded as an index of uptake(6). The secretions tested and their methods of preparation were as follows:

(1) Unfasted gut wash consisted of the contents of the first 60 cm of gut beyond the stomach washed into 5 ml of buffer, homogenized in a Waring blendor and neutralized with 5% sodium bicarbonate.

(2) Fasted gut wash was prepared as in (1) from rats fasted 18 hours.

(3) Fasted stomach contents were obtained by emptying the stomach into 5 ml of buffer and treating as in (2).

(4) Unfasted stomach contents were obtained and prepared as in (1).

(5) Histamine stimulated stomach contents were obtained by injecting 1 mg of histamine intravenously into fasting rats and collecting the secretions as in (1).

(6) Fasted rats were given 0.245 units of pancreozymin, 0.24 units of secretin and 1 mg of histamine intravenously and the gut contents collected as in (5).

(7) Five grams of Laboratory Chow were homogenized with buffer, filtered through gauze and neutralized with sodium bicarbonate.

(8) Ascorbic acid 1 mg was added to 7 ml of buffer and neutralized with sodium bicarbonate.

(9) Unfasted gut washed was prepared as in (1), and then boiled for 30 minutes.

All intestinal and gastric contents were adjusted to pH 7.2 to avoid the effect of varying pH. Sixty fasted Sprague-Dawley rats weighing approximately 225 g and with normal hemoglobins were used to provide 240 gut sacs. Segments from "unfasted" rats were also used as further controls.

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TABLE I. (Arranged in order with Figure 1.)

	No. samples	Iron Fe^{59} Index	S. E. M.	P. value
Sacs from unfasted rats	16	58.1	3.0	<.001
Sacs from fasted rats—untreated	16	21.6	2.8	control
Sacs from fasted rats pretreated with Laboratory Chow	4	20.8	3.5	.4
Sacs from fasted rats pretreated with gut wash from fasted rats given pancreozymin, secretin and histamine	4	24.1	3.7	.3
Sacs from fasted rats pretreated with gut wash from fasting rats	4	25.4	5.7	.35
Sacs from fasted rats pretreated with boiled gut wash	4	28.3	8.7	.25
Sacs from fasted rats pretreated with Ascorbic acid	4	28.4	6.0	.2
Sacs from fasted rats pretreated with stomach contents from unfasted rats	12	34.5	3.7	.01
Sacs from fasted rats pretreated with stomach contents from fasted rats	16	47.8	4.3	<.001
Sacs from fasted rats pretreated with stomach contents from fasted rats given histamine	16	47.6	4.7	<.001
Sacs from fasted rats pretreated with gut wash from unfasted rats	16	70.8	2.6	<.001

Results. The columns in the histogram (Fig. 1) record the mean uptake indices of the everted gut sacs for Fe^{59} (ferrous citrate). Table I records the mean data, standard errors of the mean and the significance of the variations in uptake of the treated sacs over the controls. "Treated sacs" refer to sacs pre-exposed to the variety of materials listed while "control sacs" refer to sacs obtained from fasted rats without pre-exposure to any of the listed materials. The following observations were made. Intestinal contents from fasted animals had no significant effect on uptake of Fe^{59} by gut sacs from fasted rats, but intestinal contents from unfasted animals increased the index considerably $p = <0.001$. Stomach contents from fasted animals or from animals stimulated with histamine also increased the indices, but not as greatly as unfasted intestinal contents. No other test solution had significant effect on the segments and it is noteworthy that boiling destroyed the ability of unfasted intestinal contents to potentiate iron absorption. Sacs from unfasted rats without pretreatment with test solution also showed considerable increase in uptake over segments from fasted rats $p = <0.001$.

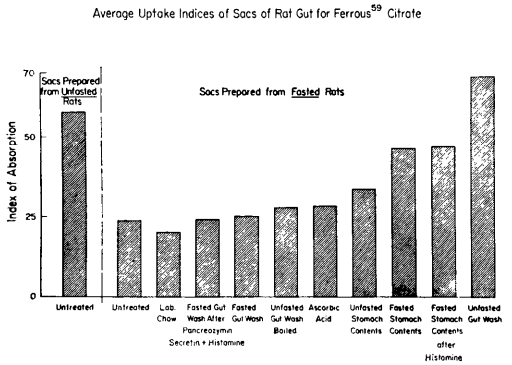

FIG. 1.

Discussion. Our observations suggest: (1) That there is a factor in intestinal contents of feeding animals which facilitates the uptake of ferrous iron by everted gut sacs. (2) That there is a factor in neutralized gastric juice from fasted animals or animals stimulated with histamine which has a similar but less powerful effect. (3) The gastric factor cannot be the same as the intestinal factor as the latter appears only in the feeding animals and the former in fasting or stimulated animals. (4) That pancreozymin, plus secretin, plus histamine stimulation of fasting animals does not potentiate iron absorption, suggesting that pancreatic secretions *per se* do not influence iron absorption in sacs. (5) The intestinal factor appears to be the result of the action of intestinal secretions on food and can be destroyed by heat.

Phosphate cannot play an important role in these studies, because (1) as a buffer medium for these studies it has been shown no different in its effect than bicarbonate(1),

(2) the same buffer was used throughout all our studies, (3) the diet of the feeding animals (Laboratory Chow), did not have an unusually high or low phosphate content (.96%), (4) phosphate could not be responsible for the heat lability of the intestinal factor.

It is clear that iron absorption can take place without either gastric(2) or intestinal contents(4), but it may be difficult for the animal in the face of greater increased demand for iron to increase absorption without these factors. Recently Koepke and Stewart(3) have suggested that there is a factor in the gastric juice of anemic dogs which potentiates the absorption of iron, and this is supported by the findings of Whitehead and Bannerman(9) that the rats following total gastrectomy cannot increase their uptake of Fe^{59} in the face of anemia. It is possible that the intestinal absorption, as distinct from duodenal absorption, is a mechanism for increasing iron absorption when the demands are high. Ordinarily, sufficient iron for daily metabolic needs is absorbed in the duodenum, but under demanding circumstances iron may be absorbed along the whole length of the gut providing that the necessary factors for promoting absorption are present. Our findings are strictly *in vitro* observations, and as such they cannot be interpreted as what happens in the living animal. It is interesting to speculate, however, that a similar mechanism may operate *in vivo*.

Summary. Our observations suggest that there is a factor in the intestinal contents of feeding rats not present in fasting rats, which potentiates the uptake of $Ferrous^{59}$ by everted gut sacs. This factor can be destroyed by heat. There is also a factor in fasting gastric juice other than acid which has a similar effect.

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Experimental Eosinophilia XI. Cell Responses to Particles of Delineated Size. (32107)

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Eosinophilia associated with immune reactions and hypersensitivity states may be explained in some species by the nature of cell responses to antigen-antibody complexes(1-4) and to some antigens(5-7). In specific instances our earlier findings(3,8) suggested relationships between eosinophil appearance and the molecular aggregated state of the protein antigen. Reported phagocytic functions(9,10) of eosinophils for immune reactants and products of antigen-antibody union may be germane to a consideration of a possible unifying concept of responsible eosinotactic mechanisms. We have pursued this question further through the study of eosinophil leukocyte responses to inflammatory challenge with particles of delineated size and character in the guinea pig.

Materials and methods. Experimental procedures were based upon the development