Influence of Transferrin Saturation on the Effect of Intraluminal Fructose or Histidine on Iron Absorption.* (32243)

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The present experiments were designed to delineate the influence of transferrin on iron absorption in the presence and absence of the intraluminal chelating agents fructose and histidine. Further, the difference in action between these two agents was studied. There is general agreement that chelation of iron enhances its absorption by the intestinal mucosa(1-8). On the other hand, the role of transferrin in regulation of the absorption of iron is still unresolved. There are two divergent opinions, *i.e.*, either there is a positive correlation between iron absorption and the availability of unbound transferrin(9,10,11) or iron absorption is unrelated to the serum iron binding capacity(12,13,14,15,16). The present studies indicate that high saturation of transferrin influences the iron absorption in the presence of intraluminal fructose but has no effect on iron absorption in the presence of intraluminal histidine or a simple salt solution.

Methods. Male albino rats of the Sprague-Dawley strain,¶ weighing approximately 200 g and with hemoglobin values of 15.5 ± 0.5 g%, were used as experimental animals. They were maintained on a standard laboratory diet (Purina Laboratory Chow‖). All animals were fasted overnight for 18-20 hours prior to use in order to eliminate the possible influence of recent exposure of the duodenum to food and dietary iron. As described previously(3), the lumen of the first 5 cm of small intestine immediately distal to the pylorus was perfused while the animal was under nembutal anesthesia. A finger-type proportioning pump continuously recycled perfusate from the reservoir through the cannulated intestinal segment, at a rate of 5.0 ml/min. The reservoir was immersed in a water bath such that the temperature of the perfusate entering the intestinal segment was maintained at 37.0 ± 1°C.

Immediately after installation of the cannulae, the intestinal segment was rinsed with 5.0 ml of isotonic saline for approximately 3 min. The rinse solution was then aspirated and the 5.0 ml of the test solution containing 25 microcuries of Fe^{59} was recycled through the intestinal segment for one hour. The specific activity of the Fe^{59} was approximately 10 millicuries per milligram of iron. Test solutions were prepared by combining 1.5 ml of 1.54 M NaCl, 0.5 ml of 1.5 M KCl, 180 microcuries of $Fe^{59}SO_4$ and distilled water to 36 ml. An aliquot of this test solution was used as standard for each set of experiments. When included in the perfusate, fructose or histidine was added to make a final concentration of 0.1 M. Throughout the perfusion time the pH was maintained between 5.0 and 6.0 by the addition of 0.05 to 0.1 ml of 0.1 or 0.5 N HCl or NaOH as indicated.

The rats were divided into 2 groups (I and II), each of which was subdivided into subgroups A, B, C, consisting of 7 animals each (Table I). The perfusates of subgroups B and C were supplemented with fructose and histidine respectively. In Group II, 55 μg of elemental iron in the form of ferrous ammonium sulfate was injected intracardially 15 minutes prior to perfusion with Fe^{59}. Two groups of non-operated rats (III and IV) were included in order to determine whether the operative technique employed influenced the

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TABLE I. Influence of Parenteral Iron on the Serum Iron, Serum Iron Binding Capacity and Saturation of the Serum Iron Binding Capacity in Rats with *in vivo* Intestinal Perfusion.

Experimental groups	Supplement to perfusate	Parenteral iron	Mean serum iron, μg %	Mean SIBC, μg %	% Saturation ± S.D.
Group I					
Subgroups					
A	None	None	138	405	34.1 ± 2.9
B	Fructose	"	153	417	36.7 ± 5.2
C	Histidine	"	149	404	36.9 ± 0.7
Group II					
Subgroups					
A	None	55 μg	297	338	87.9 ± 6.0
B	Fructose	55 "	379	491	77.2 ± 7.4
C	Histidine	55 "	365	549	67.0 ± 6.7
Group III*	Not perfused	None	195	489	39.9 ± 5.2
Group IV†	Not perfused	55 μg	372	478	77.8 ±10.0

* Normal non-perfused rats.

† Normal non-perfused rats receiving 55 μg of elemental iron parenterally. Determinations were made 60 min after injection.

serum iron (S.I.) or serum iron binding capacity (S.I.B.C.). Groups III and IV were composed of 6 animals each. The rats in the latter group received 55 μg of elemental iron parenterally 60 minutes before the S.I. and S.I.B.C. were determined.

Blood samples were taken from the tail with a hemoglobin pipette at time periods 5, 10, 15, 30, 45, and 60 minutes. At the end of one hour, blood was withdrawn from the heart for serum iron and iron binding capacity determinations(17) and the animals were sacrificed. Livers and perfused intestinal segments were removed. The carcass was considered to be the body of the animal minus the liver and perfused intestinal segment. The perfused intestinal segments were opened longitudinally, rinsed in tap water, EDTA solution, and again in tap water, and then blotted gently to remove adherent water. These segments, livers and carcasses were then weighed and counted. Livers, intestinal segments and blood samples were counted for Fe^{59} gamma activity in an Auto-Gamma** well-type scintillation counter and carcasses in an Armac scintillation counter.

The counts which were obtained in the Armac scintillation counter were converted to the number of counts which would be obtained from measuring similar activity in the Auto-Gamma counter. Total body Fe^{59} activity was derived by summing the counts per minute of the livers, carcasses and washed intestinal segments.

Three counts of one minute each were done on all samples. Fe^{59} activity of the carcasses, livers, and perfused intestinal segments were expressed as cpm/g, whereas that of the blood was expressed as cpm/ml. For statistical evaluation, all counts were converted to percent of standard by dividing the cpm/g or cpm/ml by the counts per minute of the standard (equivalent to 25 μc Fe^{59}) and then multiplying by 100. The logarithms of these values were then analyzed by the analysis of variance, and comparisons were made among the logarithms of the geometric means to ascertain where the differences had occurred when significant differences were found. A least-significant difference based on the "Studentized" range was used to make the comparison. This provides a conservative test which controls the level of significance for the whole experiment to be less than 0.5 or 0.01 for a specific variable.†† The statistical analysis for the values of the serum iron, serum iron binding capacity and iron saturation were performed using Student's "t" test for paired comparisons. In all the tech-

** Packard Instrument Co., Downer's Grove, Ill.

†† Statistical analyses performed by Dr. James Grizzle, Dept. of Biostatistics, School of Public Health, Univ. of North Carolina, Chapel Hill, under partial support of USPHS Grant GM1286-8-01 from Nat. Inst. of General Med. Sciences, Nat Inst. Health.

niques, results were considered statistically significant when the "p" was less than 0.05. However, most of the statistically significant material had a "p" of less than 0.01.

Results. Serum iron and serum iron binding capacity. The serum iron, serum iron binding capacity and saturation of the serum iron binding capacity are shown in Table I. Following the parenteral injection of elemental iron, the serum irons were significantly greater in the injected than in the non-injected animals. This difference, in all cases, was at least a 2-fold increase. Furthermore, there were no significant differences in the serum iron or serum iron binding capacities among the subgroups within Group I or within Group II. In the animals receiving parenteral iron there was a much greater saturation of the S.I.B.C. than in the animals not so treated. Comparison of the values obtained from the non-operated animals (Groups III and IV) with those of the operated rats (Groups I and II, respectively) showed that the operative technique did not significantly alter the S.I. or S.I.B.C. values.

Total body Fe^{59} activity. Table II shows total body Fe^{59} activity in various subgroups. When either fructose or histidine was present in the perfusate the total Fe^{59} activity was significantly greater than in the non-supplemented subgroups, regardless of the saturation of the serum iron binding capacity. There was no significant difference in total Fe^{59} activity between the fructose and histidine subgroups within Groups I and II. Increasing the saturation of the S.I.B.C. did not influence the total body Fe^{59} activity when the perfusate contained histidine, or when the perfusate was not supplemented. However, an increase in saturation of S.I.B.C. resulted in a statistically significant lowering of total body Fe^{59} activity in the fructose supplemented subgroup.

Fe^{59} activity in blood, liver, carcass and intestinal segment. The mean Fe^{59} activity in the blood, liver and carcass is shown in Fig. 1, 2a, and 2b, respectively. The histidine subgroups had the highest Fe^{59} activity and the non-supplemented subgroups the least. When the animals receiving parenteral iron were compared with corresponding animals not receiving parenteral iron, no statistically significant differences were found. This trend was not maintained for the perfused and EDTA rinsed intestinal segments (Fig. 2c). Here, it was noted that the fructose subgroups showed the greatest Fe^{59} activity and the histidine subgroups the least. Furthermore, the perfused intestinal segment of the fructose subgroup receiving parenteral iron contained significantly less Fe^{59} activity than the intestinal segment of the analogous subgroup not receiving parenteral iron.

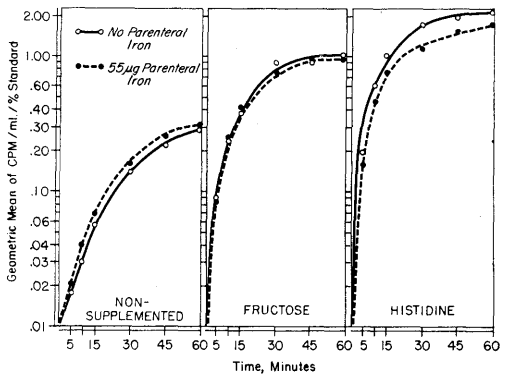

FIG. 1. Blood Fe^{59} activity at different time intervals during perfusion of an intestinal segment with non-supplemented, and with histidine and fructose-supplemented perfusates. Values are shown from animals not receiving parenteral iron and animals receiving 55 µg of parenteral iron in order to increase the transferrin saturation.

Distribution of Fe^{59}. In an attempt to determine whether the different treatments influenced the distribution of the absorbed iron, the Fe^{59} activity of the liver, perfused intestinal segment and carcass was expressed as a percentage of the total body iron Fe^{59} activity (Table II). When each subgroup of Group I was compared with its analogous subgroup in Group II, it was found that increasing the saturation of the S.I.B.C. did not influence the distribution of the Fe^{59}. The one exception was that the liver of the histidine supplemented subgroup receiving parenteral iron showed a greater percentage of Fe^{59} than the liver of the histidine subgroup not so treated.

When the fructose and histidine supplemented subgroups were compared with the non-supplemented subgroups, it was found that the former groups showed a significantly

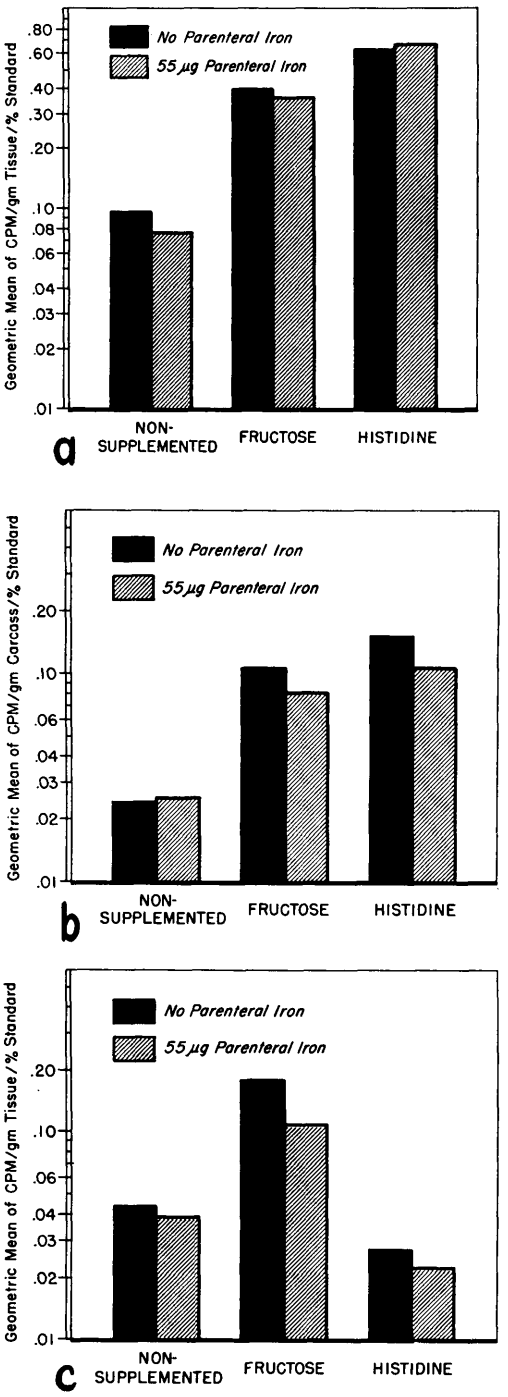

FIG. 2. Fe^{59} activity after one hour of intestinal perfusion with non-supplemented, fructose-supplemented, and histidine-supplemented perfusate. Values are shown from animals not receiving parenteral iron and animals receiving 55 µg of parenteral iron, in order to increase the transferrin saturation. a. Fe^{59} activity in liver; b. Fe^{59} activity in carcass; c. Fe^{59} activity in perfused intestinal segment.

greater proportion of Fe^{59} in the liver. This was present whether or not the animals received parenteral iron. In contrasting the fructose and histidine subgroups it was seen that the histidine subgroups showed a greater proportion of Fe^{59} in the liver, whereas the fructose supplemented animals retained a much greater proportion of the Fe^{59} in the intestinal mucosa. The proportion of Fe^{59} activity in the intestinal segments of the fructose-supplemented animals did not differ significantly from that seen in the non-supplemented animals. With regard to the carcass, the histidine supplemented animals showed the greatest proportion of Fe^{59} activity.

Discussion. It has been shown that absorption of iron from the isolated intestinal loop of a dog continues when the serum iron binding capacity is saturated or nearly saturated(12,16).

On the other hand, Charley and co-workers (1) found that in an animal whose uptake of ferrous fructose had reached a plateau, there was an immediate resumption of iron absorption following intravenous administration of unsaturated transferrin. Sölvell(11), working with human subjects, reported that only insignificant amounts of iron were absorbed into the plasma after saturating the serum iron binding capacity with an intravenous infusion of ferrous ammonium sulfate. Furthermore, administration of transferrin resulted in a marked increase in the absorption of iron into the blood.

In the present experiments a high level of transferrin saturation was associated with a decrease in iron absorption when the perfusate was supplemented with fructose. However, the level of transferrin saturation did not influence iron absorption when the perfusate was supplemented with histidine or when it contained no supplement. Therefore, under certain circumstances the level of transferrin influences transfer of iron from the mucosa to the body stores.

Both fructose and histidine enhance iron absorption and in our experiments the total absorption was the same in both instances.

TABLE II. Total Body Fe^{59} Activity and Distribution of Fe^{59} in Liver, Intestinal Segment and Carcass Following One Hour of Intraluminal Perfusion.

Exp group	Supplement to perfusate	Parenteral iron	Total body Fe^{59} activity (% std. ± S.D.)	Body Fe^{59} activity ± S.D. % in liver	% in small intestine	% in carcass
Group I						
Subgroups						
A	None	None	6.26 ± 1.38	8.1 ± 1.5	34.6 ± 8.7	57.3 ± 7.8
B	Fructose	"	28.48 ± 4.90	11.1 ± 2.4	26.7 ± 9.5	62.2 ± 7.7
C	Histidine	"	26.80 ± 4.34	14.8 ± 1.8	3.6 ± .5	81.6 ± 3.5
Group II						
Subgroups						
A	None	55 μg	7.23 ± 1.80	8.2 ± .9	26.3 ± 8.8	65.5 ± 7.8
B	Fructose	"	19.07 ± 4.68	14.2 ± 6.6	26.4 ± 9.5	59.4 ± 8.5
C	Histidine	"	22.78 ± 6.95	22.4 ± 7.5	3.6 ± 1.0	74.0 ± 7.9

Stitt and co-workers(18) have clearly shown that an iron-fructose complex is associated with increased iron absorption when compared to ferrous sulfate. A similar role in enhancement of iron absorption has been shown for histidine(3,4,5,6). There are, however, obvious differences in the distribution of the absorbed iron when the histidine and fructose-supplemented animals are compared, in that a much greater proportion of iron was retained in the perfused intestinal segment of the fructose-supplemented animals (Table II). Nevertheless, when the fructose and histidine-supplemented subgroups were compared, no significant difference was found for the total body Fe^{59}. This indicates a differential role of the two complexing agents in affecting distribution of iron. It follows, therefore, that in both instances an equal amount of radioiron is absorbed by the mucosal cells of the perfused intestine, but in the case of histidine, there is a more rapid transit of iron through the cells of the intestinal mucosa.

Summary. The influence of transferrin saturation on the enhancement of iron absorption by intraluminal histidine or intraluminal fructose was studied in rats. A loop of lumen immediately distal to the pylorus was perfused *in vivo* with a solution containing Fe^{59}. The greatest amount of Fe^{59} absorption was noted when the perfusate was supplemented with histidine or with fructose. When the perfusate in the intestinal lumen was not supplemented, or when it was supplemented with histidine, increase in transferrin saturation did not influence the iron absorption in each instance. However, when the perfusate contained fructose, the transferrin saturation had a lessening effect on the amount of iron absorbed. Histidine supplement to the perfusate caused a rapid transit of iron across the intestinal mucosa, since there was significantly less iron retained in the mucosa in the face of a high level of iron absorption.

1. Charley, P. J., Stitt, C., Shore, E., Saltman, P., J. Lab. & Clin. Med., 1963, v61, 397.

2. Brown, E. B., Rother, M. L., ibid., 1963, v62, 357.

3. Kroe, D. J., Kaufman, N., Klavins, J. V., Kinney, T. D., Am. J. Physiol., 1966, 211, 414.

4. Kroe, D. J., Kinney, T. D., Kaufman, N., Klavins, J. V., Lab. Invest., 1965, v14, 581.

5. Kroe, D. J., Kaufman, N., Kinney, T. D., Klavins, J. V., Fed. Proc., 1963, v22, 512.

6. Kroe, D. J., Kinney, T. D., Kaufman, N., Klavins, J. V., Blood, 1963, v21, 546.

7. Groen, J., van den Broek, W. A., Veldman, H., Biochim. et Biophys. Acta, 1947, v1, 315.

8. Jacobi, H., Pfleger, K., Rummell, W., Arch. exp. Path. u. Pharmakol., 1956, v229, 198.

9. Hallberg, L., Sölvell, L., Acta Med. Scand., 1960, v168, (Suppl. 358), 19.

10. Hyde, A. S., Am. J. Physiol., 1957, v191, 265.

11. Sölvell, L., Acta Med. Scand., 1960, v168 (Suppl. 358), 71.

12. Pollack, S., Balcerzak, S. P., Crosby, W. H., Blood, 1963, v21, 33.

13. Yuile, C. L., Hayden, J. W., Bush, J. A., Tesluk, H., Stewart, W. G., J. Exp. Med., 1950, v92, 367.

14. Brown, E. B., Dubach, R., Moore, C. V., J. Lab. & Clin. Med., 1958, v52, 335.

15. Bothwell, T. H., Pirzio-Biroli, G., Finch, C. A., ibid., 1958, v51, 24.

16. Wheby, M. S., Jones, L. G., J. Clin. Invest., 1963, v42, 1007.

17. Caraway, W. T., Clin. Chem., 1963, v9, 188.

18. Stitt, C., Charley, P. J., Butt, E. M., Saltman, P., Proc. Soc. Exp. Biol. & Med., 1962, v110, 70.

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Osmotic Pressure and Electrical Conductivity of Urine Under Drug-Induced Diuresis.* (32244)

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Investigation of the electrical conductivity and relative salinity of normal human urine by Hull(1), prompted further study of these during the diureses of saluretic agents. Mercaptomerin (THIOMERIN) and ethacrynic acid† were selected to show how measurements of urinary concentrative properties (2), specifically osmotic pressure and electrical conductivity, may be used to analyze pharmacologic actions beyond the possibilities afforded by measurement of individual electrolytes alone.

Methods. Standard analytic technics were used: sodium and potassium by flame photometer; chloride by Cotlove titrimeter; osmotic pressure by Fiske osmometer; electrical conductivity by RC 16B2 bridge.‡

Six unanesthetized female dogs (10-20 kg) with implanted cystostomy tubes(3) were maintained on standard laboratory rations for at least seven days before experimentation. In addition, 6 other female dogs of similar weight were anesthetized with sodium pentobarbital, urine collections being made by catheter. A control urine sample was collected, followed by administration of either intravenous mercaptomerin (4 mg Hg per kg) or ethacrynic acid (1 mg per kg). Successive, timed collections were made and the urine analyzed.

Conductivity and osmotic pressure of urine were expressed, respectively, as condosity (T = millimolarity or millinormality of NaCl solution having the same specific conductance as the urine) and osmosity (S = millimolarity or millinormality of NaCl solution having the same freezing point), the conversions being obtained by standard tables(1,4,5). The ratio of these, the relative salinity ($\Sigma = T/S$), measures the fraction of the total number of dissolved particles in the urine which behave as if they were NaCl. The difference, S — T, is a defined measure of the total nonelectrolyte of the urine (noncondosity); and by subtracting actual urinary sodium concentration (in equipollent units of mEq/l) from S or T, we obtain defined measures of the total "nonsodium" solute concentration of the urine and the total "nonsodium electrolyte" concentration, respectively.

Results. With no significant differences in pharmacologic action manifest between anesthetized and unanesthetized dogs, the results in these series were combined as averages. Fig. 1 and 2 illustrate the flow and concentrative changes in the urine. Tables IA and IIA provide average excretion rates; Tables IB and IIB, ratios of electrolytes and concentrative properties. Statistically significant changes between control and experimental values with both drugs were determined by t-test ($P < .05$) for urine flow and all excretion rates except for total nonelectrolytes; among ratios, for relative salinity (T/S) and relative nonsalinity, (S — T)/S. Significant changes with mercaptomerin were Na/S and (Na + K)/S; and K/T with ethacrynic acid. Other ratios changed as shown in the Tables but too few measurements were available to decide statistical significance. In the case of

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