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Research Article

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ROLE OF TRANSFERRIN IN IRON ABSORPTION *

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Regulation of intestinal absorption of iron remains an unsolved and interesting problem. Conflicting evidence has been presented regarding the role of transferrin in this regulation. Earlier findings (1–3) indicated that iron absorption was not influenced when saturation of transferrin was produced. In more recent reports (4, 5) it was concluded that the iron-transferrin system probably formed the basis for regulation of iron absorption. The studies in the present report were conducted in an attempt to clarify further this problem.

METHODS

A. *Rat absorption studies*

Albino rats of the WRCF strain [1] weighing 350 to 400 g were used. All but the iron-deficient rats were fed ad libitum a balanced diet (Walter Reed rat and mouse diet). Iron deficiency was produced in some by repeated bleeding and feeding a milk-powder iron-poor diet. Groups I and II were untreated controls. Group III was given 10 mg of phenylhydrazine hydrochloride intraperitoneally 4 days before study to produce hemolytic anemia. Group IV was bled a total of 12 ml in 4 bleedings; the first was 20 weeks, and the last was 8 weeks before the study. Since the hematocrits had returned to practically normal levels, the animals were considered to have deficient iron stores but little or no anemia. Group V was bled the same amount over a 3-week period ending 5 days before study. They were considered to have acute blood-loss anemia and iron deficiency.

For 16 hours preceding the experiment only distilled water was allowed. Rats from the same cage were used for each study, and by drawing numbers they were separated at random into 2 groups, control and intravenous iron-treated. Anesthesia was induced with intraperitoneal sodium pentobarbital, 6 mg per 100 g of body weight. As soon as they were unconscious, blood for a microhematocrit was taken from the orbital plexus with a capillary tube. The left jugular vein was cannulated with no. 50 polyethylene tubing and through this an amount of ionic iron estimated to saturate the iron-binding capacity was given with a constant infusion pump,[2] over a 5-minute period. Throughout the remainder of the study a maintenance infusion of iron was given. The iron solution consisted of ferrous ammonium sulfate, 3 moles of ascorbic acid for each mole of iron in 0.9% saline. Control rats were done alternately and received in an identical manner the ascorbic acid-saline solution without iron. Fifteen minutes after the infusion was started, the absorption dose containing Fe^{59} was injected into closed duodenal loops made as described below. Fe^{59} as ferrous citrate and carrier iron as ferrous sulfate were mixed in the desired ratio in 0.005 N HCl in 0.9% saline (pH 2.5). In this solution iron remained in the ferrous state up to 24 hours. The absorption dose administered was calculated in one of two ways. *1)* By counting in a small-animal counter (6) the syringe containing the test dose before and after injection into the loop. The validity of this method was established by counting syringes before and after injection of the contents into a 250-ml water-filled polyethylene bottle that was also counted. *2)* By weighing the syringes before and after injection to determine by weight the volume injected. The volume injected multiplied by the radioactivity of a standard solution made from the test solution gave the total dose administered.

The duodenal loop. After an abdominal incision was made along the linea alba, the duodenum was located, and with an atraumatic curved needle a suture was placed approximately 6 cm distal to the pylorus and tied. A proximal suture was placed around the pylorus and left loose until a blunted 22-gauge needle could be inserted through a previously prepared gastrotomy; then the suture was drawn tight. The test solution containing Fe^{59} was then injected through the needle with a 1-ml plastic tuberculin syringe; the needle was removed and the suture tied. Great care was taken not to occlude observable vessels. The color of the loops remained normal throughout the study. Checks for leakage were made by blotting the injection site with gauze and screening this for radioactivity. Time was measured from completion of the injection to a preset end-point. At that

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[1] The Walter Reed Carworth Farm strain is derived from the Wistar strain and, as supplied, is pathogen free.

[2] Harvard Apparatus Co., Inc., Dover, Mass.

time the chest was opened, and blood for plasma iron (7), total iron-binding capacity (8), and radioactivity determination was immediately drawn from the heart into plastic syringes lightly wetted with heparin. Simultaneously, the duodenal loop was amputated and entirely removed as a closed sac. It was trimmed of mesentery, cut across the sutures, opened lengthwise, washed 4 times in 5-ml volumes of a buffered, isotonic, pyrophosphate solution (pH 7.4), gently blotted, and weighed in a preweighed counting tube. After weighing, 0.5 ml of saline was pipetted into the tube in preparation for counting. The liver of the dead animal was dissected free, blotted of excess blood, trimmed of nonhepatic tissue, wrapped in a gauze sponge, and replaced in the upper abdomen in approximately normal position. The animal was placed in a 1-quart ice-cream carton for counting in a 4-Pi, small-animal, whole-body, liquid-scintillation counter, the characteristics of which have been described previously (6). After counting the carcass with the liver in place, the liver was removed and the carcass recounted. The liver radioactivity determined in this way did not differ significantly from that obtained when the liver was digested and put into solution in a 250-ml polyethylene bottle in order to obviate differences in the geometry of counting. Plasma samples and gut segments were counted in a well counter along with a standard solution made from the test dose solution. In all instances, including the dog studies below, sufficient counts were obtained to give a counting error of not over 1%. The radioactivity of the carcass, liver, and gut were each related to the total radioactivity administered in order to calculate absorption. By using the specific activity of the test dose, results were converted to micrograms of iron and expressed as micrograms of iron absorbed per 500 mg of gut. Absorption into carcass was corrected for the amount of radioactivity removed with the blood.

B. *Dog studies*

Healthy appearing dogs weighing approximately 15 kg were given no food after the morning preceding the study. Anesthesia was induced with intravenous sodium pentobarbital, 25 mg per kg body weight. A no. 50 polyethylene catheter was inserted intravenously through a needle into the right foreleg. Blood for base-line studies was drawn, and a saturating dose ranging from 800 to 3,500 μg of ionic iron was given through the catheter over a 5-minute period, followed by a maintenance dose ranging from 6.5 to 35 μg per minute throughout the experiment. In one absorption study the saturating dose was given into the portal venous system. Control dogs received the same amount of saline and ascorbic acid.

1. *Absorption studies.* After the abdomen was opened, a firm tie was placed around the pylorus and at the ileocecal valve. Thirty minutes after intravenous iron had been started, and while the maintenance dose of iron was being infused, the test solution prepared as described above but containing 200 μg of iron labeled with 2 μc of Fe^{59} was injected into the duodenum with a no. 27 gauge needle. Careful observation of the puncture site showed no leakage, and gauze used to wipe the injection site revealed no radioactivity. One hour later the animal was killed rapidly by injecting intravenously 5 ml of a saturated solution of potassium chloride. This produced cessation of heart beat within 10 seconds. The entire small intestine was immediately removed, opened, washed 4 times in normal saline, and placed, for counting, into a 2.5-L polyethylene bottle which was then filled with formalin. The liver was removed, dissected free of nonhepatic tissue, squeezed, and washed to remove as much blood as possible. It was then wrapped in a towel and replaced in the abdominal cavity in approximately normal position. The carcass was placed into a plastic bag in a fiber carton for counting in a human whole-body, liquid-scintillation counter (9, 10). Counts were made on the carcass before and after removal of the liver. A standard solution was made by placing an accurately measured sample of the test solution into a water-filled 2.5-L plastic bottle. The dose administered was calculated from this count, and the volume of the test dose administered. Absorption was determined as in the rat studies. Plasma was obtained from blood removed from a jugular vein at intervals during the experiment; 1-ml samples were counted in a well-counter, and the remainder used for duplicate serum iron and total iron-binding capacity determinations. Duplicate microhematocrits were measured at the beginning and end of each experiment.

2. *Portal infusion studies.* After the abdominal incision was made, a loop of small intestine was exposed and covered with moist towels. A mesenteric vein was striped of its sheath and cannulated with no. 50 polyethylene tubing through which saline was infused while a careful check was made for leakage. Saline was continued until the saturating and maintenance dose of ionic iron had been infused into the leg vein for 30 minutes. At this time the portal infusion of ferrous sulfate labeled with Fe^{59} as ferrous citrate was begun and continued for 30 minutes at a constant rate by using a constant infusion pump which was calibrated before each experiment. The labeled iron was in a solution of acidified saline, or previously incubated with and thus bound to the transferrin of dog plasma. Concentrations and volumes of the portal infusion were adjusted to deliver approximately 10 μg of elemental ferrous iron and 2 μc of Fe^{59} in 2 ml of acidified saline. The 4.5-ml infusion of labeled plasma contained 8 μg of iron, 0.6 μc of Fe^{59}, and had a transferrin saturation of 50%. Ten minutes after the portal infusion was stopped, the dog was killed and handled as in the absorption studies. For the last 12 experiments, only the liver was counted in a small-animal, whole-body counter (6) along with a suitable standard solution. During the experiments, blood samples for radioactivity and iron studies were taken usually before the saturating dose of iron, immediately before starting the Fe^{59} infusion, and then 5, 15, 30, and 40 minutes after beginning Fe^{59}. In three experiments, blood was taken simultaneously from the jugular and portal

TABLE I

*Iron absorption and distribution studies in control and iv iron-treated normal rats**

Hematocrit	Absorption: Total	Absorption: Gut	Absorption: Carcass and liver	Absorption: Liver	Liver, absorbed activity excluding gut	Plasma: Radio-activity	Plasma: Iron	Plasma: TIBC	Plasma: Satu-ration
%		*μg Fe/500 mg gut*			%	*cpm/ml*	*μg/100 ml*	*μg/100 ml*	%
Control									
	8.7	5.9	2.7	0.1	3.7				
	7.6	3.0	4.6	1.0	21.7				
	6.4	5.2	1.2	0.1	8.3	1,410			
			2.7	0.3	11.1				
Mean	7.6	4.7	2.8 ± 0.7†						
Iron, iv									
51	6.2	4.7	1.5	1.4	93.3				
	4.7	2.7	2.0	1.9	95.0	26	327	440	74
55	8.6	5.5	3.1	3.0	96.8	42	382	429	89
49.5	11.6	4.6	7.0‡	6.7	95.7	98	437	650‡	67
	10.1	5.5	4.6	4.4	95.7	6	470	396	100
Mean	8.2	4.6	3.64 ± 1.0† (2.8 ± 0.5†)						

* Group I. Absorption dose and time were 50 μg of iron for 35 minutes. Saturating dose of iv iron was 50 μg, and maintenance, 7 μg per minute. Two rats not shown here were bled 5 minutes and 15 minutes after starting the saturating dose of iv iron. Saturation levels were 87 and 79%, respectively. TIBC = total iron-binding capacity.

† SE of mean.

‡ The TIBC indicates that this rat was iron deficient. If it is eliminated, the mean is as shown in parentheses.

veins at the end of the study. Slight modifications of this basic technique will be noted under results.

RESULTS

Tables I through V contain the results of absorption studies done in rats. In some of the early experiments, difficulty was encountered in obtaining adequate amounts of blood for complete iron studies. The results indicated that increasing the saturation level of transferrin to between 60 and 100% (Tables I and III) and even exceeding the saturation limit (Tables IV and V) did

TABLE II

*Iron absorption and distribution studies in control and iv iron-treated normal rats**

Hematocrit	Absorption: Carcass and liver	Absorption: Liver	Liver, absorbed activity	Plasma: Radio-activity	Plasma: Iron	Plasma: TIBC	Plasma: Satu-ration
%	*μg Fe*		%	*cpm/ml*	*μg/100 ml*	*μg/100 ml*	%
Control							
47	1.68	0.26	15.5	2,730	142	438	32
44	0.69	0.09	13.0	1,710		451	
47	0.45	0.10	22.2	1,190	119	345	34
48	0.83	0.34	40.9	1,940			
Mean	0.91 ± 0.27†						
Iron, iv							
43	1.59	1.48	93.1	160	363		
49	1.03	0.95	92.2	100	381		
49	2.17	2.06	94.9	170	338		
48	0.46	0.44	95.6	40	289		
Mean	1.31 ± 0.37†						

* Group II. Absorption dose and time were 5 μg for 1 hour. Saturating dose of iv iron was 62.5 μg, and maintenance was 1.9 μg per minute. In this experiment, a closed loop was not made. The distal suture was applied at the ileocecal junction, and at the end of the hour the entire small intestine was removed and discarded. TIBC = total iron-binding capacity.

† SE of mean.

TABLE III

*Iron absorption and distribution studies in control and iv iron-treated, phenylhydrazine-treated rats**

Hematocrit	Absorption: Total	Absorption: Gut	Absorption: Carcass and liver	Absorption: Liver	Liver, absorbed activity excluding gut	Plasma: Radioactivity	Plasma: Iron	Plasma: TIBC	Plasma: Saturation
%		*μg Fe/500 mg gut*			%	*cpm/ml*	*μg/100 ml*	*μg/100 ml*	%
Control									
41	8.80	3.83	4.97	0.89	17.9				
39	5.52	3.23	2.29	0.61	26.6	314	227	667	34
43	7.78	4.57	3.21	0.66	20.6	658			
44	10.49	6.50	3.99	0.77	19.3	228	243	756	32
42	4.41	2.04	2.37	0.62	26.2	472	236	616	38
Mean	7.4	4.0	3.37 ± 0.51†						
Iron, iv									
41	9.98	5.95	4.03	3.69	91.5	46	494	822	60
42	6.87	3.47	3.40	3.14	92.3	61	379	581	65
43.5	4.90	2.52	2.38	2.26	94.9	18	400	546	73
44.5	7.64	2.20	5.44	5.14	94.4	53	393	554	71
42.5	7.27	2.65	4.62	4.52	97.8	18	436	713	61
Mean	7.3	3.4	3.97 ± 0.52†						

* Group III. Absorption dose and time were 50 μg for 35 minutes. Saturating dose of iv iron was 50 μg, and maintenance was 10 μg per minute. The high total iron-binding capacities in these rats are probably a result of phenylhydrazine treatment as reported previously (11).

† SE of mean.

not significantly decrease iron absorption. The mean and 1 SE of the mean are shown for absorption into carcass and liver, which represents iron absorbed across the intestine. No significant differences were seen between the control and intravenous iron-treated rats in any of the five groups. In Tables IV and V, the slight differences between the means of the control and intravenous iron-treated rats were not significant at the 10% level when compared by the *t* test (12). This was true even when the animal with only 44% saturation was excluded from the iron-treated group

TABLE IV

*Iron absorption and distribution studies in control and iv iron-treated rats with chronic iron deficeincy**

Hematocrit	Absorption: Total	Absorption: Gut	Absorption: Carcass and liver	Absorption: Liver	Liver, absorbed activity excluding gut	Plasma: Radioactivity	Plasma: Iron	Plasma: TIBC	Plasma: Saturation
%		*μg Fe/500 mg gut*			%	*cpm/ml*	*μg/100 ml*	*μg/100 ml*	%
Control									
47.5	6.48	0.57	5.91	0.56	9.5	38,861	165	565	29
45	4.77	0.48	4.29	0.35	8.2	35,087	238	640	37
43	5.33	0.45	4.88	0.25	5.1	32,738	131	577	23
44	8.61	1.13	7.48	0.87	11.6	43,191	200	681	29
Mean	6.3	0.66	5.64 ± 0.7†						
Iron, iv									
43.5	5.15	0.64	4.51	3.58	79.4	2,788	635	606	>100
44	3.41	0.44	2.97	2.43	81.8	421	808	436	>100
40	5.38	0.77	5.24	4.81	91.8	964	785	721	>100
44	7.82	1.00	6.82	5.94	87.1	2,084	731	698	>100
Mean	5.4	0.71	4.89 ± 0.8†						

* Group IV. Absorption dose and time were 13 μg for 15 minutes. Saturating dose of iv iron was 125 μg, and maintenance was 12.5 μg per minute. TIBC = total iron-binding capacity.

† SE of mean.

TABLE V

*Iron absorption and distribution studies in control and iv iron-treated rats with acute iron deficiency**

	Absorption				Liver, absorbed activity excluding gut	Plasma			
	Total	Gut	Carcass and liver	Liver		Radio-activity	Iron	TIBC	Satu-ration
	μg Fe/500 mg gut				%	*cpm/ml*	*μg/ 100 ml*	*μg/ 100 ml*	%
Control									
	9.30	1.88	7.42	0.53	7.1	9,095	130	563	23
	8.99	1.85	7.14	0.53	7.4	6,202	86	486	18
	6.52	1.23	5.29	0.46	8.7	7,002	81	486	17
	10.82	2.56	8.26	0.65	7.9	10,099	92	580	16
	7.93	1.45	6.48	0.58	9.0	7,504	111	566	20
Mean	8.7	1.8	6.92 ± 0.5†						
Iron, iv									
	8.72	2.58	6.14	0.66	10.7‡	7,091	248	563	44‡
	9.40	2.39	7.01	5.84	83.3	241	548	566	97
	6.31	1.21	5.10	3.96	77.6	306			
	7.50	1.55	5.95	4.81	80.8	207	581	566	>100
	8.01	2.21	5.80	4.76	82.0	326	551	547	>100
Mean	8.0	2.0	6.0 ± 0.6†						

* Group V. Absorption dose and time were 19 μg for 15 minutes. Saturating dose of iv iron was 125 μg, and maintenance was 12.5 μg per minute. TIBC = total iron-binding capacity.

† SE of mean.

‡ Owing to a technical problem, this rat received only a small amount of iv iron.

(Table V). In addition, no significant differences were found in the amount of iron absorbed by but not transported across the intestinal segment (listed in the columns under "gut," Tables I to V). That the method employed is sensitive enough to differentiate normal absorption from the increased absorption found in the iron-deficient and the decreased absorption found in the iron-loaded animal was shown in previous work (13). Although the experiments in the present report were not designed to make comparisons between groups, it appeared that the phenylhydrazine-treated rats, group III, did not absorb more iron than the normal rats, group I. This may have resulted from performing the absorption studies too soon after administration of phenylhydrazine.

As the saturation of the plasma transferrin increased (last column, Tables I to V), the portion of absorbed radioiron found in liver also increased from 4 to 22% to 78 to 98%. The distribution of absorbed iron found in group III is shown in Figure 1. Noted also was the marked difference in plasma radioactivity between the intravenous iron-treated group and the control rats (Tables I to V).

Table VI shows the results of the absorption studies in dogs. Sufficient studies were not done to allow conclusions to be drawn regarding the effect of transferrin saturation on the amount of iron absorbed; rather, the purpose of these studies was to see if the phenomenon observed in rats

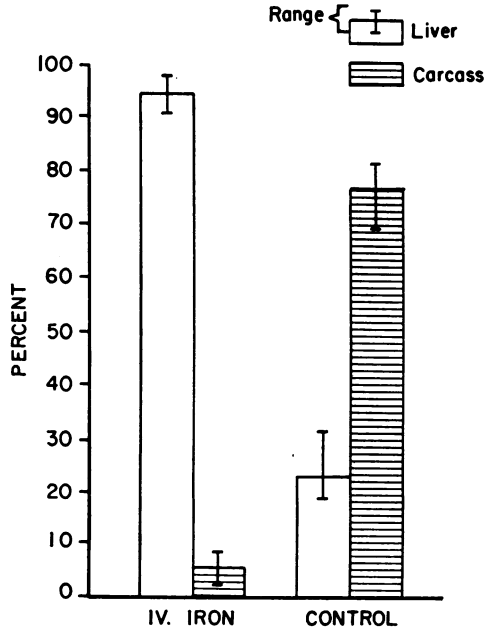

FIG. 1. DISTRIBUTION OF ABSORBED IRON, EXCLUDING GUT, FOUND IN RAT GROUP III. The clear bar indicates liver content; the hatched bar, carcass content.

TABLE VI

*Iron absorption and distribution studies in control and iv iron-treated dogs**

Hematocrit	Plasma Iron C†	Plasma Iron E‡	Plasma TIBC C†	Plasma TIBC E‡	Plasma Saturation C†	Plasma Saturation E‡	Absorption Gut	Absorption Carcass and liver	Liver, absorbed iron excluding gut
%	μg/100 ml		μg/100 ml		%		μg Fe/200 μg dose		%
Control									
44	154	156	316	341	46	50	24	9	14.5
Iron 1, iv									
36.5	107	312	310	332	32	96	26	72	83.7
Iron 2, iv									
35	69	271	314	390	22	71	22	13	89.0
Iron 3, iv§									
39	84	326	406	471	21	76		75	64.7

* Absorption dose and time were 200 μg of iron for 1 hour. Saturating dose of iv iron was 3,000 μg, and maintenance was 35 μg per minute. TIBC = total iron-binding capacity.
† Control.
‡ During absorption period.
§ Saturating dose given into portal venous system.

would occur. It was found that despite almost complete saturation of transferrin (96%), iron absorption was not blocked. As seen in the rat studies, most of the iron absorbed under these conditions was found in liver.

Tables VII contains the results of the infusion studies, examples of which are shown in Figures 2 and 3. In the control dogs (dogs 1, 2, and 3), the results were the same whether the portal infusion of Fe^{59} was in saline or bound to plasma. Liver content of infused radioactivity was approximately 10%. In the intravenous iron-treated dog infused with Fe^{59} bound to dog plasma (dog 4), a result identical to that in dogs 1, 2, and 3

TABLE VII

*Plasma and liver content of radioiron infused into portal vein of control and iv iron-treated dogs**

Dog	Hematocrit	Portal infusion	Plasma Iron C†	Plasma Iron E‡	Plasma TIBC C†	Plasma TIBC E‡	Plasma Saturation C†	Plasma Saturation E‡	Peak radioactivity§	Liver, infused radioactivity
	%		μg/100 ml		μg/100 ml		%			%
Control										
1	35.5	Fe-saline	87	140	308	349	28	42	42‖	9.8
2	49.5	Fe-saline	105	115	319	353	32	35	17	10.9¶
3	42.0	Fe-plasma	98	128	296	304	33	42	20	8.2
Iron, iv										
4	46.0	Fe-plasma	86	368**	364	390	22	93	20	11.1
5	44.0	Fe-saline	117	341	359	386	33	88		80.5
6	41.5	Fe-saline	133	313**	316	353	38	95		78.5
7	42.0	Fe-saline	123	182	166	181	74	100	1.3	82.6¶
8	37.0	Fe-saline	69	374	355	394	20	98	1.0	92.4
9		Fe-saline	105	340	327	347	32	98	2.0	91.7
10	36.5	Fe-saline	69	274	269	286	26	97	8.1	56.8
11	40.0	Fe-saline	88	227	226	242	36	99	2.4	78.0
12	43.0	Fe-saline	83	232	286	313	30	75	14	8.4
13	34.5	††	97	211	286	324	34	70	28	45.6

* The experiments on dogs 2 and 9 and dogs 3 and 4 are also shown in Figures 2 and 3, respectively. TIBC = total iron-binding capacity.
† Control.
‡ During infusion period.
§ Expressed as percentage of infused radioactivity × 100.
‖ Portal blood taken at same time had same radioactivity and iron content.
¶ Spleen had less than 1% of infused radioactivity.
** Portal blood taken at same time had same iron and TIBC content.
†† Fe-saline infused into leg vein.

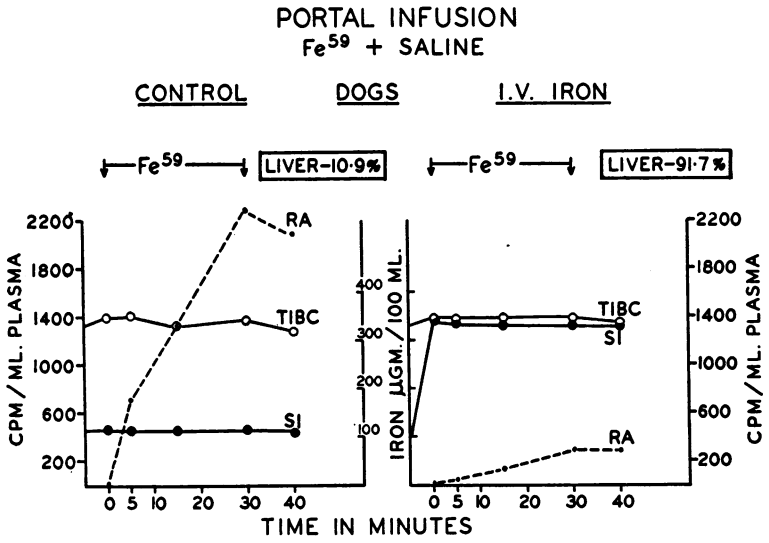

FIG. 2. TWO PORTAL-INFUSION EXPERIMENTS IN WHICH THE INFUSED MATERIAL WAS IRON IN SALINE. On the left is shown the result in the control dog (dog 2, Table VII). Plasma radioactivity is indicated by RA, iron by SI, and total iron-binding capacity by TIBC. The arrows indicate the duration of portal infusion. On the right is shown the result when an identical portal infusion was given to a dog with almost complete transferrin saturation (dog 9, Table VII). Plasma radioactivity in the control animal reached approximately 10 times that of the iron-treated dog. Liver content of infused iron is shown for each dog.

was found (Table VII, Figure 3). In contrast, when transferrin saturation was over 75% and the portal infusion was Fe^{59} in saline (dogs 5 to 11), 56.8 to 92.4% of the radioactivity was in the liver. In addition, very little of the portally infused radioactivity reached peripheral blood (Table VII; Figure 2). This indicates that the Fe^{59} was lost into liver on its first passage through. When

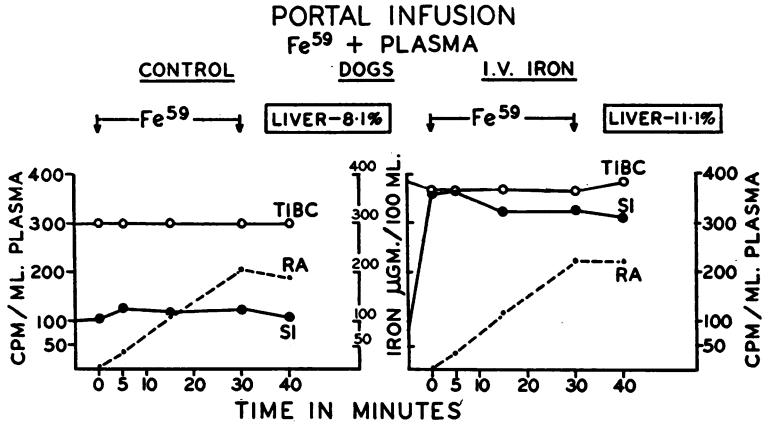

FIG. 3. TWO PORTAL-INFUSION EXPERIMENTS IN WHICH THE INFUSED MATERIAL WAS IRON BOUND TO DOG PLASMA. On the left is shown the result in the control dog (dog 3, Table VII). Plasma radioactivity is indicated by RA, iron by SI, and total iron-binding capacity by TIBC. The arrows indicate the duration of portal infusion. On the right is shown the result when an identical portal infusion was given to a dog with almost complete transferrin saturation (dog 4, Table VII). Plasma radioactivity and liver content were almost identical in the two dogs.

transferrin saturation was raised, in this case to 70%, and the Fe^{59} in saline was infused into a peripheral vein, 45.6% of the radioactivity was found in the liver (dog 13, Table VII). Although this result is similar to that seen in the portal infusion studies with high transferrin saturation, the peak plasma radioactivity achieved was quite different, reaching 10 to 20 times that found during portal infusion.

When, in addition to the usual saturating dose of ionic iron, an animal was given 3,000 μg into the portal system 20 minutes before the Fe^{59} infusion, there was no effect on the subsequent hepatic uptake of radioactivity (dog 5, Table VII). It would appear that most of this additional iron was also lost extravascularly into the liver, since transferrin saturation never reached 100%.

In one experiment, the transferrin saturation was raised to 75%, but most of the infused iron was not deposited in the liver (dog 12, Table VII). The liver content of 8.4% did not differ from control animals, and it is possible that the critical saturation level for this phenomenon to occur in dogs is around 75%.

DISCUSSION

In 1947, Laurell (14) postulated that the saturation level of transferrin might regulate iron absorption from the intestinal tract. He accepted the theory that iron leaves and enters the blood stream in ionized form and explained the possible influence of transferrin on iron metabolism by the following reversible reaction: Fe^{+++} + Fe-free transferrin $\rightleftharpoons$ Fe-transferrin. The equilibrium of this reaction is expressed by the equation: (Fe-transferrin) / (Fe-free transferrin) = $K \cdot (Fe^{+++})$. He believed that this hypothesis explained why during periods of increased iron demand, reflected by a decreased serum iron, or increased transferrin level, or both, iron is mobilized from stores and intestine. Subsequent studies did not confirm this hypothesis. Yuile and associates (1) concluded that the relative saturation of transferrin played little role in the control of iron absorption in dogs. Brown, Dubach, and Moore (2), from their study on a normal human subject, reached a similar conclusion. These experiments were later criticized (5) because the iron used in attempting to saturate transferrin was in a complexed, nonionized form (iron-gelatin, saccharated iron oxide). Since plasma iron determinations would have included iron in the injected complex (15), it is possible that in spite of a high plasma iron concentration, transferrin might not have been saturated. Bothwell, Pirzio-Biroli, and Finch (3), however, produced transferrin saturation in rabbits and humans by injecting nonviable erythrocytes and were also unable to show that this influenced iron absorption. Recently, Hallberg and Sölvell (4) presented evidence which suggested that Laurell's hypothesis regarding iron absorption was correct. These investigators utilized a double-isotope technique for determining the rate of iron absorption in humans. One iron isotope was given orally and the other intravenously, the latter to determine continuously plasma iron turnover. Since in their studies on the effect of transferrin saturation, the intravenous isotope used to determine plasma iron disappearance was bound to transferrin prior to injection, and since an antecubital vein was the site for blood sampling, two assumptions had to be made: *1*) that all absorbed iron was bound to transferrin and *2*) that blood removed from the antecubital site was representative of portal blood and thus truly reflected total outflow of iron from plasma, including the first passage of absorbed iron through the liver. Sölvell (5) found that when transferrin was completely saturated by intravenously administered elemental iron before oral iron was given, no absorption of iron from intestine to plasma was evident during the study. If absorption actually had taken place, however, and if the absorbed iron remained in the liver on its initial flow through, then this iron would not have been detected at a peripheral site. The experiments in the present report indicated that this is what occurred in both rats and dogs. Infusion of intravenous elemental iron in quantities sufficient to raise transferrin saturation in rats to above 60% in some experiments, and in excess of the saturation limit in others, did not decrease iron absorption significantly, but did result in deposition in the liver of approximately 90% of the iron absorbed from intestine (Tables I to V). The same phenomenon of iron deposition in liver occurred in dogs when transferrin saturation was raised above 70%. A comparison was not made between control dogs and dogs with transferrin saturation regarding amount of iron absorbed, but in one experiment, absorption took place despite 96% saturation (Table VI). Furthermore, when to simulate absorption, small

amounts of ionic iron (6 to 12 μg) not bound to transferrin were infused over a ½-hour period into the portal venous system of dogs with transferrin saturation raised above 75%, most of the infused iron was deposited in liver and very little remained in the blood. This was in contrast to control dogs, where only 10% of similarly infused unbound iron was found in liver. Along this line, it is of interest that in Hallberg's and Sölvell's studies with constant intragastric infusion of iron (4), the iron absorption rate, as determined from peripheral venous blood, decreased when transferrin saturation reached approximately 70%. It is possible that the same phenomenon we observed in animals occurred in their human studies. If so, they would have obtained a falsely low estimate of absorption rate.

When, in one experiment, transferrin was saturated and the portal infusion of iron was bound to plasma prior to infusion, the amount of iron passing through the liver and reaching peripheral blood did not differ from that found in the control dog with normal transferrin saturation. This suggested that under the conditions of our studies, when iron was absorbed from the intestine, it did not enter plasma already bound to transferrin, for had it been so, loss from plasma on its initial passage through the liver would not have occurred. Iron was absorbed whether or not transferrin was available for binding it. These findings were compatible with recent evidence that intestinal absorption of iron involves an energy-dependent transport process that is able to move iron against a concentration gradient (13, 16). On the basis of our studies, it would appear that the function of transferrin may not be that of an essential acceptor of iron at the epithelial mucosal cell level. Rather, it seems to be necessary for rapidly and firmly binding iron entering plasma from epithelial mucosal cells, thereby preventing its loss extravascularly, and for transporting it to cells that synthesize iron-containing substances (17). Jandl, Inman, Simmons, and Allen have shown the specificity of the transport function of transferrin (18). In their experiments, "nonspecific" unloading of transferrin-bound iron took place onto mature erythrocytes, reticulocytes, or rat liver slices when transferrin saturation was raised above 60%. This does not appear to explain our results since the infusion of iron bound to plasma into the portal system of a dog with almost fully saturated transferrin did not result in unusual loss of the iron to liver (Figure 3). It should be noted that the infused plasma had a transferrin saturation of 50%. It seems unlikely that rapid deposition in liver would occur with even higher levels of saturation if the iron was bound to transferrin. If it did occur, then one would expect to see a great increase in total outflow of iron from plasma as plasma iron concentration was raised. This was not found in human subjects (5). As seen in these experiments and as reported previously (5, 9, 20), however, ionic iron not bound to transferrin was quickly lost from plasma, and it is likely that a rapid distribution of the ionized iron between plasma and extracellular fluid occurred in addition to adsorption onto cell membranes (18). When this takes place in the portal venous system, unbound iron will be deposited in the liver. In our studies, this was apparently the fate of iron absorbed from intestine while large amounts of ionic iron were being infused intravenously. Infused iron attached to many of the available binding sites on transferrin, and as a result, newly absorbed iron was presumably not bound before it reached liver, where it was lost from blood. The ultimate distribution and utilization of iron handled in this manner are not known.

The findings in the patient with congenital atransferrinemia reported by Heilmeyer and associates (21) are what one would expect if the main function of transferrin is iron transport and if it is not essential for iron absorption. The patient, a 7-year-old girl, had an iron-deficiency anemia in the presence of excessive iron deposits in liver, heart, and spleen. An iron absorption study showed greater absorption than normal. This finding supports the results in the present report and indicates that transferrin need not be present for iron absorption to occur. Our results also suggest that in these short-term experiments in rats, the level of transferrin saturation did not have an important role in the regulation of iron absorption. A recent report of studies in dogs was in agreement with this conclusion (22).

SUMMARY

1. Iron absorption studies were done in rats by utilizing Fe^{59} and whole-body counting. These studies indicated that the level of transferrin saturation had no important role in the regulation of

iron absorption during these short-term experiments.

2. Raising transferrin saturation to above 60% in some experiments and in excess of the saturation limit in others with intravenous infusion of ionic iron did not significantly influence the amount of iron absorbed by rats.

3. Most of the iron absorbed when transferrin saturation was raised to over 60% in rats and 70% in dogs was deposited in liver apparently on the first circulation through the portal system.

4. When Fe^{59} in saline was infused into the portal venous system of dogs with saturated transferrin, most of the Fe^{59} was lost from blood during the first circulation through liver. This did not occur in an experiment in which the isotope was bound to dog plasma before it was infused into portal circulation. These studies strongly suggested that when iron entered plasma from sites of intestinal absorption, it was not already bound to transferrin.

5. These results confirmed the importance of transferrin in iron transport.

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ADDENDUM

Preliminary studies in human subjects showed results similar to these reported in animals, i.e., accumulation in the liver of iron absorbed while transferrin was saturated (23).

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