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THE TRANSPORT OF IRON BY RAT INTESTINE

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SUMMARY

To define more clearly the mechanisms operative in the transport of iron by intestinal mucosa, the short-circuit technique has been applied to isolated sections of small intestine of rat. Parallel experiments have been carried out *in vivo* using ligated sections of the small intestine in the intact rat. Low-molecular-weight chelates are necessary to maintain iron in a soluble and permeable from. Although the rate of mucosal to serosal transport appears to be directly dependent upon the concentration of iron chelate presented to the cell, a membrane-bound facilitating carrier also participates in the process. Evidence is presented for an active serosal to mucosal component for iron transport. Metabolic inhibitors, low temperatures and anaerobiosis abolish the net serosal to mucosal flux. The chemical nature of the chelate directly affects the rate of transmembrane transport as well as the tissue distribution of the iron molecule. Iron deficiency anemia causes both a decrease in the serosal to mucosal iron flux and an increase in the mucosal to serosal component. These factors appear to be responsible for the enhanced uptake of iron observed in anemic animals.

INTRODUCTION

The regulation and control of iron transport by the intestinal mucosa has been a focal point in the study of the metabolism of this essential metal by a variety of mammalian species. Several reviews have recently been published which detail the current status of the problem[1-3]. Three rather divergent mechanisms have been proposed. The first is that of the "mucosal block" originally proposed by HAHN *et al.*[4] and extended by GRANICK[5]. This system involves the initial reduction of the iron to the ferrous form, its facilitated transport across the epithelial cells bound to ferritin, a second reduction at the plasma membrane, and, finally, re-oxidation to the ferric form when it is bound to transferrin to be transported in the plasma. A

Abbreviations: M → S, flux from mucosal to serosal surface; S → M, flux from serosal to mucosal surface.

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recent appraisal of the mucosal block hypothesis by MOORE[6] showed that it was inconsistent with the available data regarding the regulation and control of iron transport in the mucosa.

A modification of the mucosal block which has received much attention is that developed by CONRAD, WEINTRAUB AND CROSBY[7]. Some undefined fraction of the plasma iron serves as a "messenger" to regulate the uptake of the dietary iron into the mucosal cells where it is bound to protein. The rate at which this iron subsequently enters the circulation is regulated by a balance between the rate at which the epithelial cells are exfoliated and the rate at which the cellular iron enters the plasma. The actual mechanisms of uptake and movement of iron by the cells were not defined by these investigators.

A second mechanism proposed by DOWDLE, SCHACHTER AND SCHENKER[8] claimed that ferrous iron was actively transported against a concentration gradient from M → S by processes directly linked to metabolic energy. They used isolated and everted gut sacs and found profound inhibition of transport in the presence of metabolic poisons and anaerobiosis. JACOBS, BOTHWELL AND CHARLTON[9] used a loop of gut with artificial circulation and demonstrated similar inhibitions. For this reason, they supported the active transport theory of SCHACHTER. However, neither BROWN AND JUSTUS[10] nor PEARSON AND REICH[11], using everted gut loops, were able to confirm the findings of SCHACHTER. Of particular importance was the observation by PEARSON AND REICH[11] that soluble iron did not accumulate in the lumen of the everted sac, although metabolic poisons did depress the rate of uptake into the mucosal cells.

The third mechanism proposed for the regulation of iron absorption is directly related to equilibrium binding and chelation phenomena. Results from this laboratory[12], FORTH, RUMMEL AND SEIFEN[13], HOPPING AND RULIFFSON[14] and DAVIS AND DELLER[15] indicated that both ferric and ferrous iron were available for intestinal transport provided suitable chelates were present. There was no direct dependence on metabolic energy for the accumulation. The amount of iron available from the diet was related to the relative concentrations of those agents which prevent accumulation by forming insoluble or high-molecular-weight complexes, such as phytic acid and proteins, and low-molecular-weight substances able to solubilize the iron and permit its absorption.

In an attempt to clarify the mechanisms operative in the process, we have employed the short-circuit technique developed by USSING AND ZERAHN[16]. This method permits continuous monitoring of the electrochemical status of the isolated intestinal membrane, application of a short-circuit current to the system to avoid any flow of ions in response to a potential gradient, and repeated measurement of flux rates of isotopic iron both S → M and M → S. We will demonstrate that although there is an active component of iron movement S → M, regulation of iron accumulation in the circulating plasma is also a function of the chemical nature of the chelating molecule presenting iron to the system. The intestine of animals made anemic by repeated bleeding shows a significant decrease in the component of active transport, S → M, which may be important in the enhancement of iron accumulation found under such conditions.

METHODS

For all experiments *in vitro*, the buffer solution was a modified Krebs–Henseleit–Ringer solution[17]. The modifications included removal of Mg^{2+}, SO_4^{2-}, and HPO_4^{2-}; reduction of Ca^{2+} to 10 μM and K^+ to 0.1 mM; and the addition of 10 mM glucose to provide a metabolic substrate. Several other solutions were utilized for specific investigations and are described with the appropriate experiments in the text. The pH of all solutions was 7.4 and the osmolarity, 285–295 mosM.

The ^{59}Fe used as tracer was obtained as $^{59}FeCl_3$ from New England Nuclear Corp. and diluted to the desired iron concentration with ferric nitrate carrier. Approx. $12 \cdot 10^6$ counts/min · ml were initially present in the compartment to which the isotope was added. Radioactivity of the ^{59}Fe was determined on 0.1-ml aliquots using liquid-scintillation techniques. To measure *in vivo* iron uptake, radioactivity was determined using a whole animal counter equipped with two 7.5-cm diameter NaI crystals in opposition, linked to a scaler with a pulse height analyzer.

Techniques for two-way flux determination in vitro

A modification of the USSING short-circuit apparatus[18] was constructed to accomodate a section of intestine layed open as a sheet. The area across which flux was measured was 0.87 cm^2. Electrical potential across the membrane was monitored using a Keithley electrometer Model 600A *via* a pair of calomel electrodes and agar–saline bridges. Before each experiment, the resistance of the bathing solution between the bridges was measured and a correction was subsequently made to the applied short-circuit experiments. The short-circuit current was supplied from a 1.5-V dry cell using Ag–AgCl electrodes and agar–saline bridges. Circulation and aeration was achieved using 95% O_2–5% CO_2. Where anaerobiosis was desired, 95% N_2–5% CO_2 was substituted. At 30-min intervals, 0.1-ml aliquots were withdrawn from both compartments and the radioactivity determined as indicated above.

All experiments reported in this paper were carried out using male Sprague–Dawley rats, weighing approx. 400 g, which had been deprived of food and water 24 h prior to killing. Animals were killed with a blow on the head and the small intestine immediately extirpated. The first 5-cm section of small intestine distal to the pyloric musculature was washed in cold 0.9% saline, opened along the mesentery, and mounted in the apparatus which was immediately filled with Ringer solution. The iron solution containing the isotope was then added to one of the compartments and the experiments begun. Throughout the course of the experiments, short-circuit current was recorded. Periodically the current was removed in order to monitor the transmucosal potential difference. Experiments were carried out at room temperature, 25–27°.

Iron flux in vivo

The animals were anesthetized with 15 mg sodium pentabarbital, the abdomen incised and a segment of intestine, 25 cm distal to the pyloric valve, isolated by ligatures. At all times, care was taken to maintain unimpaired blood supply to the ligated segment. The experimental solution, 2 ml, containing ^{59}Fe at the desired concentration and in the desired medium, was injected with a 27-gauge needle just distal to the proximal ligature. Precautions were taken to prevent leakage from the

site of injection. The segment of intestine was returned to the abdominal cavity and the rat maintained for 2 h under a warming lamp. At the conclusion of this period, radioactivity of the whole animal was measured. The isolated intestinal segment was then excised and the animal was again counted. The amount of radioactivity within the animal with the intestinal segment removed, divided by the total radioactivity, *i.e.* the animal *plus* the intestinal segment, represented the fractional uptake of iron during the 2-h period. When transferrin was added to the system *in vivo*, 40 mg of human apotransferrin (Behringwerke, Marburg-Lahn, Germany) in 0.7 ml of 0.3 M Tris–HCl buffer (pH 7.3) was injected directly into the inferior vena cava.

The preparation of anemic animals

By cannulating the optic sinus, 5–6 ml of blood were removed from the rats every 2–3 days until a total of 25–30 ml of blood were collected from each animal. The animals were maintained in plastic cages on a low-iron diet (Nutritional Biochemicals Corp.) and distilled water. Hemoglobin levels in the anemic animals were reduced to 6.5–8.5 gram % compared to normal values of 17–18 gram %.

RESULTS

Flux of iron under short-circuit conditions

A series of iron chelate solutions were prepared containing a mole ratio of Fe: chelate of 1.05 by adding the $^{59}Fe(NO_3)_3$ to the chelate. The pH of the resulting solution was about 1.5. Solid Na_2CO_3 was then added to bring the pH to 7.3. Both chambers of the modified USSING apparatus were filled with 10 ml of bicarbonate–Ringer. The radioactive iron chelate was then added to the side of the membrane under study and the experiments begun. The membrane potential, measured in a series of over 40 intestinal segments, ranged from 3 to 5 mV (mucosa negative with respect to serosa in an external circuit). The current required to reduce the potential to 0 mV ranged from 30 to 60 μA. The average flux of iron in each direction for a series of experiments utilizing various chelates is given in Table I. Several important features should be noted. The most striking result is that the S → M transport is greater than that for M → S for all chelates studied. Further, the net S → M transport is essentially independent of the chelate. Finally, it should be noted that the addition of excess ascorbic acid as both a reducing agent and a chelator to the iron–nitrilotriacetic acid preparation while enhancing unidirectional fluxes of iron had no effect on the net transport.

TABLE I

UNIDIRECTIONAL IRON FLUX IN SHORT-CIRCUITED SYSTEM UTILIZING VARIOUS CHELATES

Iron and chelate concentrations are 1 mM in all experiments. The number of experiments is in square brackets. Variation is given as the standard error. Flux values are mμmoles/cm^2·h.

Chelate	*M → S*	*S → M*	*Net*
Nitrilotriacetic acid [16]	2.9 (± 0.3)	7.5 (± 1.0)	4.6
Nitrilotriacetic acid–ascorbate [16]	4.0 (± 0.5)	8.9 (± 1.0)	4.9
EDTA [6]	11.0 (± 0.7)	16.4 (± 1.4)	5.4
Citrate [13]	2.2 (± 0.1)	7.4 (± 0.7)	5.2

TABLE II

EFFECT OF METABOLIC INHIBITION ON FLUX OF FERRIC–NITRILOTRIACETIC ACID AND TRANSMEMBRANE POTENTIAL

Number of experimental animals is given in square brackets. Variation is given as standard error. Ferric–nitrilotriacetic acid is 1.0 mM. Flux values measured under short-circuit conditions.

Inhibition	*Iron flux (mμmoles/cm² · h)*			*Potential (mV)*
	M → S	*S → M*	*Net*	
None [16]	2.9 (± 0.3)	7.5 (± 1.0)	4.6	3.5–4.0
N_2 [13]	4.2 (± 0.8)	7.4 (± 0.7)	3.2	2.0–2.5
N_2 *plus* iodoacetic acid [7]	8.2 (± 0.6)	7.6 (± 0.5)	−0.6	0.0–0.2
4° [6]	6.2 (± 0.1)	6.2 (± 0.8)	0.0	0.0–0.2

The effect of inhibitors on net iron flux

Using the short-circuit apparatus, we measured the unidirectional transport of iron in the presence of various metabolic inhibitors. The results are presented in Table II. The substitution of N_2 for O_2 in the gas phase resulted in a moderate reduction in net iron transport. The addition of either iodoacetate or reduction of the temperature to 4°, however, resulted in complete inhibition of the net M → S flux. The observed transmucosal potential under the same conditions is also shown. The failure of N_2 to inhibit the net iron movement is not surprising in view of the fact that active sodium transport is only partially inhibited by the substitution of N_2 for O_2 in this system. The effect of iodoacetate and low temperature on net iron movement is also in agreement with the effect of these inhibitors on net sodium transport, as seen in the measured potential.

The rate of iron transport as a function of concentration in vitro

Unidirectional fluxes of iron were measured in the short-circuit apparatus over a concentration range of 10^{-4} to 10^{-2} M ferric citrate. The fraction of the presented iron transported is shown in Table III. Over the range of concentration studied, there was a slight increase in the fraction of transported iron at the highest concentration. There was a slight depression of the transmembrane potential observed at 0.01 M ferric citrate which may be an indication of loss of membrane integrity which accounts for the increase in the fraction of ferric citrate transported. These results would suggest that if a membrane-bound carrier were operative in M → S iron transport, its affinity for the complex is very low.

A more sensitive test for the operation of exchange diffusion is the stimulation of the unidirectional flux rates by the addition of unlabeled substrate to the bathing

TABLE III

FLUX M → S AS A FUNCTION OF FERRIC CITRATE CONCENTRATION

Concentration of ferric citrate (M)	*Flux (mμmoles/cm² · h)*	*% of iron transported*
10^{-4}	0.23	0.023
10^{-3}	2.3	0.023
10^{-2}	33.0	0.033

TABLE IV

TRANSMEMBRANE EFFECT OF FERRIC CITRATE ON SHORT-CIRCUIT FLUX *in vitro*

[^{59}Fe]Ferric citrate was 1 mM in all experiments. Flux values are mμmoles/cm^2·h. Number of experimental animals is given in square brackets.

Concentration of ferric citrate on "trans" side	*M → S*	*S → M*
0 [13]	2.2 (± 0.1)	7.4 (± 0.7)
1 mM [6]	4.4 (± 0.3)	9.4 (± 0.5)

solution on the opposite membrane surface to that which contains the label. Such experiments were carried out with 1 mM ferric citrate and the results are presented in Table IV. There is a marked stimulation of iron transport, both M → S and S → M, upon addition of non-radioactive ferric citrate.

The effect of chelates on the absorption of iron in vivo

Animals were prepared as described in METHODS. The results of a series of experiments with each of the chelates under investigation are shown in Table V. Unlike the systems *in vitro*, the ratio of chelate:Fe was greater than 1:1. It was

TABLE V

EFFECT OF CHELATES ON IRON UPTAKE *in vivo*

Concentration of iron was 0.1 mM in all experiments.

Chelate	*Concn. (mM)*	*Number of animals*	*Uptake (mμmole/cm^2·h)*
None	—	10	0.02
Ascorbate	30	4	0.09
Fructose	120	8	0.11
Citrate	1	8	0.18
Nitrilotriacetic acid	1	20	0.22
EDTA *plus* 3 mM Ca^{2+} *plus* 4 mM P_i	1	5	0.20

necessary to add 3 mM Ca^{2+} to the EDTA solution to prevent loss of membrane integrity*. Chelates were neutralized in the presence of ferric nitrate with NaOH, diluted to proper concentration, and injected into the washed, ligated intestinal segment. It is clear from the data that significant enhancement of iron absorption was obtained upon addition of chelating agents to the metal ion. It will also be observed that when concentration differences are taken into account, the relative and absolute rates of uptake are in good agreement with the data *in vitro*. In the absence of chelates, a colloidal suspension of $Fe(OH)_3$ was obtained upon neutralization which is not readily absorbed.

The effect of chelates on distribution of absorbed iron in vivo

A series of iron chelates were introduced into a ligated gut section *in vivo* as described. After a 2-h incubation period, the animals were sacrificed, tissues and

* Unpublished experiments from this laboratory.

TABLE VI

ORGAN DISTRIBUTION OF ^{59}Fe AS A FUNCTION OF CHELATE

2.0 ml of [^{59}Fe]ferric chelate were introduced into the lumen of the tied-off mucosal segment *in vivo*. Incubation period, 2 h. An amount of [^{59}Fe]ferric chelate equal to that taken up from the intestinal segment was infused intravenously over a 1-h period and the experiment continued for the second hour. Values are averages of three experimental animals under each condition expressed as per cent total iron in all tissues counted. Negligible amounts of activity were found in the remaining carcass.

Organ	*Intestinal*			*Intravenous*	
	Nitrilotriacetic acid	*EDTA*	*Citrate*	*Nitrilotriacetic acid*	*EDTA*
Blood	57.0	37.4	76.7	58.4	11.2
Bladder	1.6	41.3	0.4	25.0	75.6
Liver	35.9	5.1	17.7	6.3	1.1
Kidney	4.7	15.8	3.4	8.6	11.7
Spleen	0.8	0.4	1.8	0.8	0.4

organs extirpated, and the total radioactivity determined in each. The results are presented in Table VI. There are striking differences observed in the fraction of iron accumulated in a specific site as a function of various chelates. Although ferric–EDTA was rapidly absorbed, it was also rapidly excreted and not available for utilization by the animal. On the other hand, ferric–nitrilotriacetic acid was quickly taken up by the liver and spleen; little was lost *via* the kidney and bladder. It was possible that the iron initially presented to the cells as EDTA or nitrilotriacetic acid complexes did not actually enter the blood of the animal in this state. The iron may have exchanged with endogenous ligands of the mucosal cells. To test this hypothesis, parallel experiments were carried out where ferric–EDTA and ferric–nitrilotriacetic acid were introduced intravenously over a 1-h period. The amount of each iron chelate infused was the same as the quantity that was transported from the lumen *in vivo* during the same period. The distribution of intravenous iron is also presented in Table VI. The same fundamental pattern of deposition was observed as was found when the chelated iron entered the animal through the intestinal mucosa. The results suggest that these iron chelates may be transported intact across the mucosal cells without exchange.

The effect of apotransferrin on transport in vitro

In order to assess the influence of increased apotransferrin in the circulation, six rats were prepared for uptake studies *in vivo*. Three of these animals were injected intravenously with apotransferrin as described under METHODS. Three were maintained as controls. [^{59}Fe]Ferric–nitrilotriacetic acid as a solution containing 1 mM Fe^{3+} and 0.03 M nitrilotriacetic acid was introduced into the lumen. Uptake was allowed to proceed for 2 h. The animals were sacrificed and iron distribution in various organs measured. Although there was no significant difference in the total uptake with or without apotransferrin, the fraction of activity remaining in the blood was increased from 57% to 70% and the fraction in the liver reduced from 36% to 12%. Thus it is likely that the presence of increased amounts of apotransferrin in the plasma will elevate the pool of iron maintained in that compartment.

TABLE VII

FLUX OF FERRIC CITRATE BY SHORT-CIRCUITED NORMAL AND ANEMIC INTESTINE *in vitro*

Ferric citrate concentration was 1 mM. Number of experimental animals is given in square brackets.

	Flux (mμmoles/cm²·h)		
	M → S	*S → M*	*Net*
Normal [13]	2.2	7.4	5.2
Anemic [11]	3.5 (± 0.2)	5.2 (± 0.6)	1.7

The effect of anemia on iron transport

Anemic animals were prepared as described in METHODS. Intestinal segments were mounted in the modified USSING short-circuit apparatus. The unidirectional rates of iron transport were measured with [^{59}Fe]ferric citrate. The results are presented in Table VII. Two important observations should be noted. Firstly, there was a significant increase in the M → S transport rate of the ferric citrate chelate. Secondly, the net flux of iron in the anemic intestine was markedly reduced, as a result of both the increase in M → S uptake and the decrease in S → M flux.

The effect of anemia on uptake *in vivo* was also investigated. Anemic animals were prepared for uptake *in vivo* studies as described. [^{59}Fe]Ferric chelates were prepared containing 0.1 mM Fe^{3+} and either 1 mM or 0.25 M fructose and injected into the lumen of the ligated segment. The results are presented in Table VIII. In the absence of chelate, a slight increase in iron uptake was seen. However, when fructose or ferric–nitrilotriacetic acid was administered, there was a marked stimulation of uptake in the anemic animals.

TABLE VIII

EFFECT OF ANEMIA ON UPTAKE *in vivo*

Concentration of iron was 0.1 mM.

Chelate	*Uptake (mμmoles/cm²·h)*	
	Anemic	*Normal*
None	0.09	0.02
Fructose (0.12 M)	1.01	0.11
Nitrilotriacetic acid (1 mM)	0.90	0.27

DISCUSSION

The experimental results presented in this paper lead us to propose an overall scheme, Fig. 1, for the regulation and control of iron transport across the intestinal mucosa. There are two salient features in this scheme. The first is the mandatory involvement of low-molecular-weight chelating agents which are able to solubilize the iron and maintain it in a form which can pass through the membrane. The second is a membrane-bound carrier, probably at the serosal barrier, which actively transports iron chelates in an S → M direction.

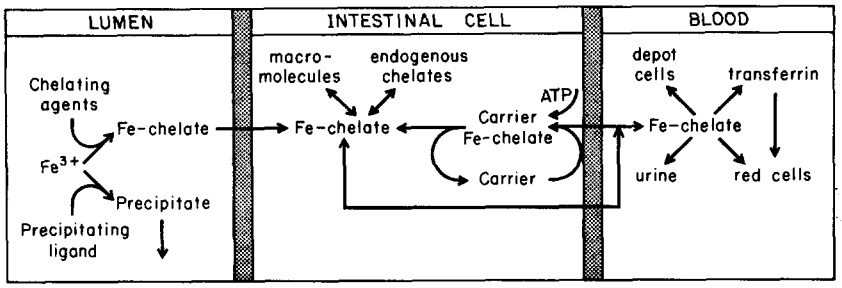

Fig. 1. Proposed mechanism for the regulation and control of iron transport by the small intestine.

There is an increasing body of knowledge concerning the fundamental importance of chelation mechanisms in trace-metal transport[18–25]. Specifically, in the case of iron, the participation of synthetic chelating agents[26] as well as endogenous ones[27] in the absorption of this metal by roots of higher plants has been demonstrated. Futher, the work of NEILANDS and his associates[28] has led to the isolation and identification of several compounds synthesized by bacteria and fungi, which solubilize iron and make it available to the cells.

It has long been recognized from nutritional studies that salts of Fe^{2+} were more effectively absorbed than of Fe^{3+}. Consideration of solubility products of these two ions shows that Fe^{2+} can exist in solution up to approximately 1 M at pH 7. On the other hand, Fe^{3+} is insoluble in concentrations greater than 10^{-17} M. The initial studies of GROEN, VAN DEN BROEK AND VELDMAN[29] using amino and organic acids to achieve iron uptake by rats were interpreted as involving the acids as substrates favoring reduction of iron. Actually, the amino acids utilized were effective chelators of iron. Recently, evidence from our laboratory[30] as well as those of HOPPING AND RULIFFSON[14] and DAVIS AND DELLER[15] have directly demonstrated the efficacy of chelating agents in the transport process. Nutritional experiments have also indicated that when ligands such as phytate, high concentrations of phosphate, or a variety of compounds which form insoluble precipitates with Fe^{3+} are present, uptake of the metal is diminished. An interesting recent discovery by DAVIS, LUKE AND DELLER[31] in Australia reveals that patients with hemochromatosis appear to lack a specific iron-binding protein which binds dietary iron and removes it from the chelatable and transportable pool.

The results of the experiments presented in this research indicate that the chemical nature of the complex affects the rate of its transmucosal transport. Of primary consideration is the stability and thus the solubility of the chelated iron complex. Many amino acids are weak chelating agents and at neutral pH's would be rather ineffective. A second consideration relates to the formation of polynuclear complexes of metal ions, particularly citrate and fructose chelates[32,33]. The rates at which these polymers dissociate may play a very fundamental role in the rate at which iron is made available to the intestinal mucosa. A third consideration is the rate at which the metal chelate interacts with the membrane or components within the cell including endogenous ligands or macromolecules such as protein and nucleic acids. The fourth consideration is the disruption of the metabolic activities of the cell or of the membrane integrity by the presence of high concentrations of either free chelating agents or its metal complexes. This is particularly noted in the case of

EDTA experiments where membrane calcium is removed by the excess chelating agents and causes an increase in the transport rate of the iron chelate, and at the same time increases the overall permeability of the membrane.

We have presented evidence for an active component of transport of iron chelate from S → M. Before discussing the nature of this active transport system, it is well to compare the advantages of the specific experimental techniques we have utilized in these experiments with the use of everted sacs, or perfusion *in vivo* by many previous investigators. The short-circuit apparatus was an adaptation of USSING's original instrument and has been described in detail elsewhere[18]. A section of the intestinal mucosa can be rapidly mounted, bathing solutions introduced on both the serosal and mucosal sides, aeration begun, and several parameters of the membrane continuously monitored, including short-circuit current and the unidirectional flux of isotopic iron in both directions. This method avoids all of the hydrostatic pressure artifacts introduced by the tied off gut loop as well as the problem of asymmetrical volumes in the compartments. Furthermore, the abolition of the transepithelial potential difference by the short-circuit current provides a more precise technique for the evaluation of transport phenomena of charged species[16]. Since the net charge on the iron chelate molecules investigated in these experiments may vary from −1 for iron–EDTA and iron citrate to 0 for iron–nitrilotriacetic acid, application of the short circuit avoids any effect introduced by an electrical gradient established by the membrane.

It is clear from the results of Table I that several chelates of iron are transported by rat small intestine with a net S → M component. This net flux was observed in the complete absence of chemical, electrical, or hydrostatic gradients. Several metabolic inhibitors including anoxia, iodoacetic acid, and low temperature either inhibit or totally abolish this net flux. In the limited series of chelates that were tested there seemed to be little specificity for the nature of the chelating agent presented to the intestine. Of particular interest is the profound inhibition of this S → M flux by animals made anemic by repeated bleedings. We believe that these experiments demonstrate the participation of a metabolically linked "pumping system" which prevents the entry of iron into the circulating blood. This system appears to be under the control of the metabolic state of the tissue as well as the iron needs of the organism.

Direct evidence for the participation of a carrier which facilitates bidirectional flux is obtained from the experiment in which the counter flow of non-radioactive chelate induced the more rapid transport of the radioactive label from the trans compartment, Table IV. All attempts to saturate this mobile membrane-bound carrier using increasing concentrations of various chelates were unsuccessful. As is seen in Table III, there is even a slight acceleration of the rate of transport at the very high concentrations of the ferric citrate.

Chelating agents are operational during transport *in vivo* as shown by similar rates observed in the system *in vitro*. Thus all the chelating agents found effective in the short-circuited system had similar activities when injected into a tied off section of the washed intestine. That the effect of repeated bleedings to produce anemia also stimulates iron uptake in the intact animals has often been observed[34]. At the present time we have no direct way of measuring the operation of the active transport component of this system *in vivo*.

The participation of the chelating agent in iron metabolism continues after it has carried the iron into the bloodstream. The rate at which the chelate transfers the metal to apotransferrin is profoundly influenced by the chemical nature of the chelate[35,36]. Thus EDTA gives up its iron very slowly, while nitrilotriacetic acid exchanges it almost instantaneously with apoprotein. The organ and tissue distribution of metal presented by various chelating agents is clearly seen in Table VI. Iron–EDTA which is slow to release its metal rapidly passes into the kidney and urine. On the other hand, iron–nitrilotriacetic acid and iron citrate readily exchange with the transferrin so that a major fraction of metal is retained in the bloodstream. If additional apotransferrin is added to the circulating plasma, the amount of iron retained by the blood is significantly increased; although the rate at which iron crosses the intestinal membrane is unchanged.

These experiments confirm and extend the participation of the chelates in the regulation and control of iron metabolism. With the discovery of a novel S → M active component of iron transport, it seems more important than ever to investigate several aspects of this system. Efforts should be devoted to the discovery of more effective chelating agents, both endogenous and synthetic, in this process. Perhaps of even greater consequence is the understanding of regulation and control within the intestinal cell and how external factors effect their operation. DAVIS, LUKE AND DELLER[31] have discovered a protein, gastroferrin, which complexes iron and makes the metal unavailable to the intestinal mucosa of normal animals. The protein appears to be absent in hemochromatosis and low in iron-deficiency anemia. The inhibition of the active S → M component by anemia is another type of regulatory mechanism. It is possible that the rate at which endogenous chelates are secreted by intestinal cells may also control the uptake process.

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