

# Competitive Nature of the Intestinal Transport Mechanism for Cobalt and Iron in the Rat

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**ABSTRACT** Dose- and time-response studies were performed in iron-loaded and iron-deficient rats in order to define, (a) the kinetics of absorption of cobalt and iron, (b) the nature of the inhibitory effect of one metal on the absorption of the other, and (c) the effect of variations in body iron stores on these processes. The duodenum was perfused for 5–90 min with labeled solutions containing 5.0 mM iron or 5.0 mM cobalt. In iron-loaded rats, the rate of cobalt absorption was constant for 90 min whereas the rate of iron absorption fell after 30 min. In comparison to these results, the rate of absorption of both metals was increased in iron deficiency, and was more rapid in the first 30 min than in the 30–90 min period.

To determine the response to varying doses of metal, we perfused duodenal loops for 30 min with 0.1–10.0 mM solutions of either iron or cobalt. In both iron-loaded and iron-deficient groups, a greater proportion of the metals was absorbed from smaller than from larger doses. When iron and cobalt were perfused together in iron-deficient animals, cobalt competitively inhibited iron absorption, and conversely, iron reduced cobalt absorption. The apparent maximum transport velocity was similar for both metals, but the affinity for cobalt was greater than iron.

The results suggest that the absorption of cobalt and iron is mediated by a transport system in which two processes operate simultaneously; the first is limited largely by the concentration of available metal in the lumen of the intestine, whereas the second process depends upon the activity of a mechanism which displays saturation kinetics and competitive inhibition. The former process prevails when iron stores are replete, whereas the latter predominates when there is a need for iron, such as in iron deficiency.

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## INTRODUCTION

The absorption of both iron and cobalt is increased in man and in the rat with iron deficiency (1–3). The predominant site of absorption of both elements in the rat is the proximal intestine, and the absorption of iron is proportional to the absorption of cobalt (3). The addition of cobalt to increasing concentrations of iron inhibits the transfer of iron from the lumen to the carcass (4, 5). Furthermore, the administration of iron with an oral dose of cobalt reduces cobalt transfer (6). These features suggest that iron and cobalt share at least part of a common absorptive pathway.

The aim of this investigation was (a) to define the absorption kinetics for iron and cobalt; (b) to investigate the nature of the inhibitory effect of one metal upon the absorption of the other; and (c) to study the effect of varying body iron stores on these processes.

The term *transfer*, when used in the text, is synonymous with *absorption*.

## METHODS

**Animals and diets.** Iron deficiency was induced in one group of pathogen-free albino rats by raising them from weaning on a semisynthetic diet containing 10 mg/kg of iron (3, 7, 8). Iron overload was produced in another group of animals by supplementing the semisynthetic diet with 150 mg/kg of iron, and by the injection of 15 mg of intramuscular iron in the form of Imferon (Benger Laboratories, Toronto, Ontario) when the animals were 28 days old. The hematological picture and iron status of animals raised on this regime have been reported previously (3). When the animals were between 60 and 90 days of age, studies of intestinal absorption were carried out. The principles of laboratory animal care, as promulgated by the Canadian Federation of Biological Societies, were observed.

**Test solutions of iron and cobalt.** As described previously (5), test solutions of iron and cobalt, labeled with  $^{59}\text{Fe}$  or  $^{60}\text{Co}$  respectively, were prepared so as to contain 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0  $\mu\text{moles}$  of metal per ml of 154 mM NaCl in iron-free water (9). For each

micromole of iron or cobalt, 2  $\mu$ moles of sodium ascorbate were added to maintain the metals in a soluble form (3).

**Intestinal perfusion technique.** 12 hr before each experiment, food was removed and replaced with 5% glucose in iron-free water. The animals were anesthetized with sodium pentobarbital, and test solutions were pumped through open-ended loops of duodenum (5); the outflow tubing was set 15 cm above the loop to maintain uniform pressure. Following the perfusion period, the duodenal loop was excised and the lumen was flushed with three washings of 10 ml of normal saline, each followed by 10 cc of air. To determine the completeness of the washing technique in removing unabsorbed radioactivity from the lumen, the mucosa of two groups of animals was flushed with an additional three washings of either 10 ml of normal saline, or 10 ml of 0.01 M EDTA, an iron-chelating agent. The radioactivity removed by additional washings with either technique represented less than 0.3% of the intestinal activity remaining in the wall of the intestine.

The methods employed for the measurement of radioactivity have been reported earlier (3, 5). The radioactivity was measured in the carcass and remaining intestinal tract after excision of the perfused duodenal loop; absorption was defined as that amount of metal (millimicromoles per duodenal segment) which was transferred to the carcass. The radioactivity in the washed duodenal segment was also measured; uptake was defined as metal absorbed plus that amount of metal remaining in the wall of the perfused duodenal loop. In addition, absorption was expressed as a percentage of uptake according to the following:

$$\frac{\text{Absorption (carcass, } \mu\text{moles)}}{\text{Uptake (carcass, } \mu\text{moles} + \text{duodenal wall, } \mu\text{moles)}} \times 100$$

The validity of the technique for the measurement of intestinal uptake and transfer depends upon the passage of minimal radioactivity from the carcass back into the intestinal lumen. It was established previously that in a 30 min period, only small amounts of iron and cobalt passed from the carcass into the wall and lumen of the duodenum (5). Additional studies, in which the portal vein of 15 animals was injected with 0.5 ml of 1.6 mM iron or cobalt test solution, revealed that an average of 2.9% (0.4-4.1%) of the  $^{59}\text{Fe}$  and 3.0% (1.4-5.3%) of the  $^{57}\text{Co}$  passed from the carcass into the duodenal perfusate during a 90 min perfusion period; less than 0.4% of the test dose of either iron or cobalt was detected in the wall of the duodenal

segment. Even with this long perfusion period, secretion of injected material into the duodenum was small, and therefore reentry of labeled metal was considered to be negligible.

To ascertain the viability of the duodenum throughout the rate study, one group of seven iron-deficient animals was perfused for 30 min with 5.0 mM iron; an average of  $2078 \pm 226$  (SE)  $\mu$ moles were taken up, and  $643 \pm 67$   $\mu$ moles were transferred to the carcass. In a second group of seven iron-deficient animals, duodenal loops were perfused with saline for 60 min, and then with the 5.0 mM iron test solution for 30 min; an average of  $2169 \pm 126$   $\mu$ moles were taken up and  $932 \pm 55$   $\mu$ moles were transferred. Since no significant decline was found in either uptake or transfer between the first 30 min and the last 30 min of the perfusion period, it was assumed that the absorptive capacity of the intestine did not diminish throughout the rate study.

To investigate the initial uptake and absorption of iron and cobalt, duodenal loops of three groups of eight rats were perfused for 2 min with 0.85% NaCl, unlabeled 5.0 mM iron, or unlabeled 5.0 mM cobalt test solution respectively. The loops were flushed with 10 ml of normal saline, followed by 10 cc of air, and then were quickly filled with labeled 5.0 mM iron or cobalt test solution. One minute later, the test dose was flushed from the lumen and the mucosa was washed as described previously. The radio-cobalt uptake in 1 min represented 20% of the cobalt uptake in 30 min, whereas over 40% of the 30 min iron uptake occurred within 1 min (Table I). Rapid uptake of a 0.05 mM dose of iron has been described previously by Wheby and co-workers (10).

The initial rapid uptake was unlikely to be due to absorption, since further washing of the mucosa failed to remove greater than 0.3% of the intestinal activity in the wall of the intestine, and since prior perfusion of the duodenum with unlabeled iron or cobalt solution failed to diminish significantly the subsequent uptake or transfer of a labeled test dose.

## Experimental design

A sequential experimental design was employed in an attempt to reduce any bias introduced into the results by the possible effects of changes in body weight, the time of performing the experiments, and day-to-day variations in iron or cobalt absorption. In the time, dose, and inhibition

TABLE I  
*Intestinal Uptake and Transfer in 1 min of 5.0 mM Solutions of Iron and Cobalt*

Test solution	No. of rats		Intestinal uptake		Transferred to the carcass		Amount of uptake transferred		Retained in the mucosa	
	S	M	S	M	S	M	S	M	S	M
			$\mu$ moles		$\mu$ moles		%		$\mu$ moles	
Cobalt	8	9	346 $\pm$ 33	379 $\pm$ 40	167 $\pm$ 42	187 $\pm$ 38	45 $\pm$ 7	46 $\pm$ 6	179 $\pm$ 15	194 $\pm$ 21
Iron	7	7	791 $\pm$ 112*	668 $\pm$ 58*	174 $\pm$ 55	137 $\pm$ 40	20 $\pm$ 5*	21 $\pm$ 6*	617 $\pm$ 73	531 $\pm$ 63

Mean values  $\pm$  SE. The values in the animals perfused with saline are represented as S, and the values in the animals preperfused with unlabeled iron or cobalt test solution are represented as M. The differences between the mean values of the S groups, and the mean values of the corresponding M groups were not statistically significant,  $P < 0.05$ .

\* Indicates that the difference between the mean value for cobalt and iron in either the S or M group is statistically significant,  $P < 0.05$ .

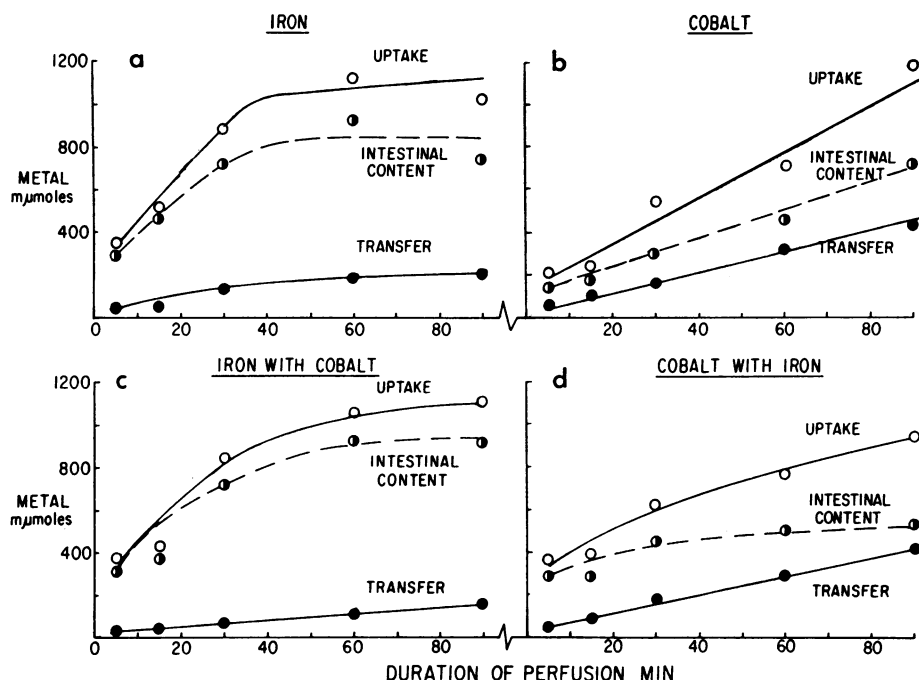


FIGURE 1 Time-response study of the intestinal uptake and transfer of 5.0 mM iron and 5.0 mM cobalt from the duodenum in iron overload. Uptake and transfer refer respectively to the amount of metal from the perfusate that was taken up by the duodenal mucosa, and the amount that passed from the mucosa to the carcass. Intestinal content refers to the amount of labeled metal from the perfusate that was present in the duodenal wall at the termination of the experiment.

studies, each variable was tested once; then another rotation was begun, starting with the second variable and finishing with the first. This pattern was continued until approximately eight rotations were completed and eight animals were studied in each group.

To determine the effect of duration of perfusion on the absorption of iron and cobalt, duodenal loops of groups of animals with iron overload or with iron deficiency were perfused with a test solution containing 5.0 mM iron, 5.0 mM cobalt, or 5.0 mM iron together with 5.0 mM cobalt, for periods of 5, 15, 30, 60, or 90 min. To establish the effect of dose on the absorption of these metals, animals with loaded or with deficient iron stores were perfused for 30 min with a test solution containing either iron or cobalt in concentrations of 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, or 10.0 mM.

To investigate the inhibitory effect of cobalt on iron absorption, groups of animals were perfused for 30 min with solutions containing either 2.0 mM cobalt together with iron in concentrations varying from 0.1 to 10.0 mM, or with equimolar amounts of iron and cobalt in concentrations varying from 0.1 to 5.0 mM.

The retention of iron and cobalt in the intestinal mucosa was studied in animals raised on a commercial diet.<sup>1</sup> They were given an oral dose of 1 ml of 0.2 mM iron plus 0.2 mM cobalt with 0.4 mM sodium ascorbate. Groups of animals were killed 30 min, 6 hr, and 24 hr later. The small intestine was removed, washed with saline, and divided into three segments each of which were assayed for radioactivity as described previously (3, 5).

To detect whether any test solution leaked from the in-

testinal loop into the carcass, the peritoneal cavity was washed at the end of each experiment, and the radioactivity was measured (3). Out of a total of 542 experiments, contamination of the carcass was found in only 16 animals, and they were excluded on the basis that greater than 5% of the carcass radioactivity was present in the peritoneal wash. Although the remaining experiments appeared to be acceptable from the technical point of view, the carcass counts in 10 animals were greater than twice the mean value of their respective groups, even though their peritoneal wash was not contaminated. The statistical criteria of Grubbs (11) for testing outlying observations were applied to these extreme values, and they were excluded on the basis of  $T_1 = P < 0.01$ .

**Statistical methods.** The statistical significance of the difference between any two mean values in an experiment was evaluated by Student's *t* test (12). The equality of the variances was determined by the *F* test (12), and whenever a significant difference was found, the Cox-Cochrane correction (13) was applied. The kinetic constants,  $V_{max}$  and  $K_m$ , of the iron and cobalt dose-response studies were determined by the least squares method for nonlinear regression (14, 15). The equations describing the relationship between uptake, transfer, or intestinal activity and metal concentration in the perfusate were determined by the method of linear regression or curvilinear regression for a second degree polynomial (15). Analyses of variance were used to test for the significance of curvilinearity. The square of the multiple correlation coefficient ( $R^2$ ) was calculated to express the proportion of the total variability which was accounted for by curvilinear regression.

<sup>1</sup> Rockland, Teklad Inc., Monmouth, Ill.

## RESULTS

### Effect of time

*Iron and cobalt administered separately.* Duodenal loops of animals with iron overload or with iron deficiency were perfused with a 5.0 mM test solution containing either iron or cobalt for periods of 5–90 min. In iron-loaded rats, the absorption of cobalt occurred at a constant rate for 90 min, whereas iron absorption was more rapid in the first 30 min than from 30 to 90 min (Fig. 1 *a* and 1 *b*).<sup>2</sup> The uptake of both metals followed a similar pattern to transfer, but the uptake of iron in the first 30 min and of cobalt throughout was more rapid than transfer, and as a consequence both metals accumulated in the mucosa. Iron uptake exceeded cobalt uptake,

<sup>2</sup> Tables showing the complete data for the Time-Response Studies (absorption of 5.0 mM iron and cobalt) and the Dose-Response Studies (absorption of iron and cobalt) will be made available by the authors on request from interested persons.

whereas the transfer of cobalt was slightly greater than iron; thus, the quantity of the iron label in the mucosa at any one time was greater than cobalt.

In iron deficiency, the intestinal uptake and transfer of iron and cobalt were increased well above the levels in iron overload. In the first 30 min, the rate of uptake of both metals exceeded the rate of transfer; from 30 to 90 min the rate of both uptake and transfer declined, and little further <sup>56</sup>Fe or <sup>57</sup>Co accumulated in the intestinal mucosa (Fig. 2 *a* and 2 *b*). The rate of iron transfer was similar to the rate of cobalt transfer, but the uptake and thus the intestinal content of <sup>56</sup>Fe was greater than <sup>57</sup>Co.

*Iron and cobalt administered together.* A test solution containing both 5.0 mM iron and 5.0 mM cobalt was perfused through duodenal loops for periods varying from 5 to 90 min. In the iron-deficient animals, and to a lesser extent in the iron-loaded ones, the administration of cobalt with labeled iron was associated with a diminution in iron transfer (Figs. 1 *c* and 2 *c*); iron uptake was unaffected in the former group (Fig. 1 *c*),

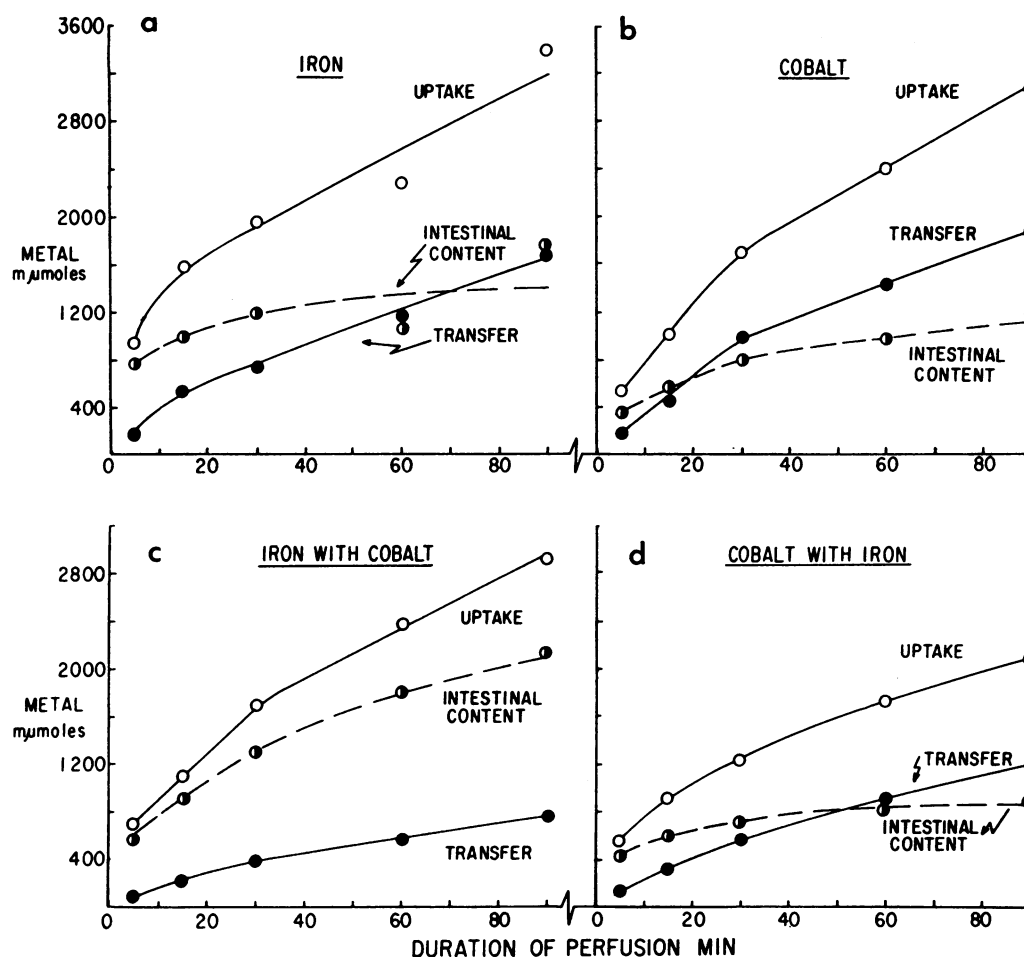


FIGURE 2 Time-response study of the intestinal uptake and transfer of 5.0 mM iron and 5.0 mM cobalt from the duodenum in iron deficiency.

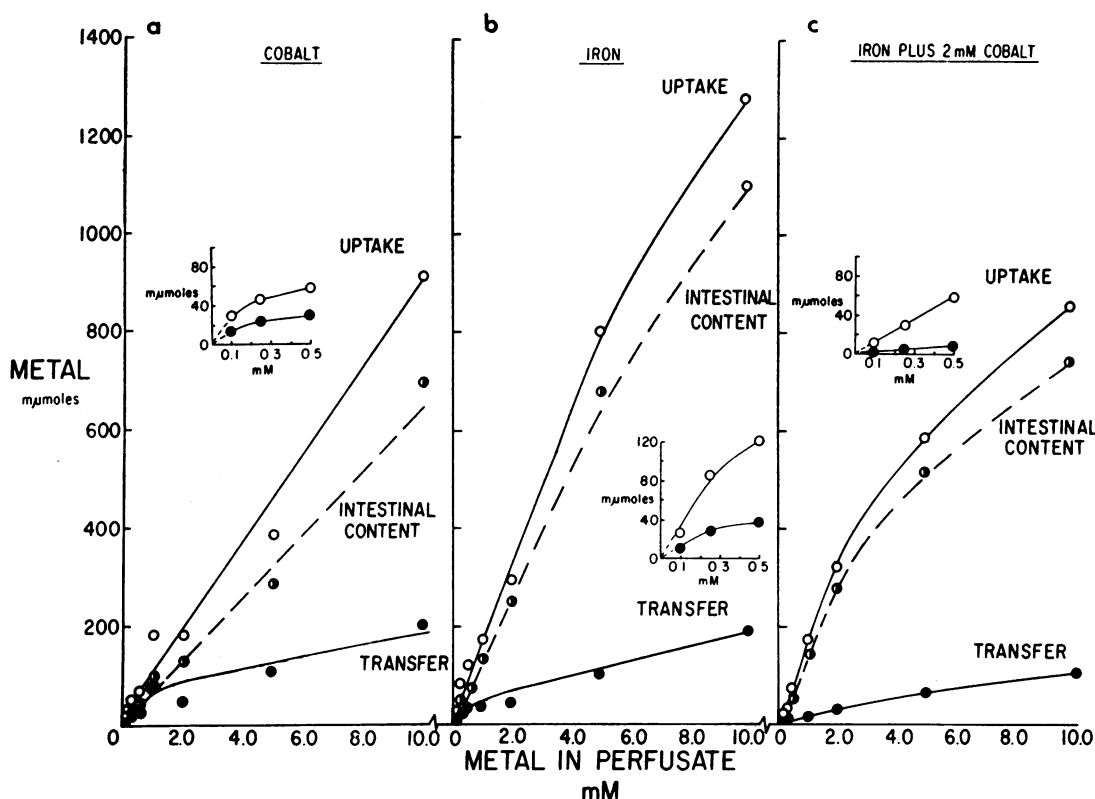


FIGURE 3 Dose-response study of the intestinal uptake and transfer of iron and cobalt with a 30 min perfusion of the duodenum in iron overload. The insert in each figure shows the uptake and transfer at the three smallest doses on an expanded scale. The equations for the regression of uptake, transfer, or intestinal content against the concentration of metal in mM ( $x$ ) of cobalt (Co), iron (Fe) or iron plus 2.0 mM cobalt (FeCo) are given. The variance ratio ( $F$ ) describes the significance of the curvilinearity of the regression; the  $R^2$  value represents the proportion of the sum of the squared deviations accounted for by the curvilinear regression. Uptake Co =  $46.0 + 55.6x + 3.2x^2$ ,  $F = 17.1$  ( $P < 0.01$ ),  $R^2 = 0.912$ ; uptake Fe =  $4.3 + 177.4x - 4.4x^2$ ,  $F = 10.9$  ( $P < 0.01$ ),  $R^2 = 0.876$ ; uptake FeCo =  $158.4x - 7.2x^2 - 2.9$ ,  $F = 400.0$  ( $P < 0.01$ ),  $R^2 = 0.914$ . Transfer Co =  $28.5 + 13.4x + 0.5x^2$ ,  $F = 10.7$  ( $P < 0.01$ ),  $R^2 = 0.822$ ; transfer Fe =  $12.1 + 21.6x - 0.23x^2$ ,  $F = 10.7$  ( $P < 0.01$ ),  $R^2 = 0.650$ ; transfer FeCo =  $11.8 + 0.11x + 1.1x^2$ ,  $F = 35.6$  ( $P < 0.01$ ),  $R^2 = 0.879$ . Intestinal content Co =  $17.3 + 42.4x + 2.7x^2$ ,  $F = 15.0$  ( $P < 0.01$ ),  $R^2 = 0.907$ ; intestinal content Fe =  $155.9x + 4.2x^2 - 7.5$ ,  $F = 11.0$  ( $P < 0.01$ ),  $R^2 = 0.881$ ; intestinal content FeCo =  $142.4x - 6.6x^2 - 2.9$ ,  $F = 36.2$  ( $P < 0.01$ ),  $R^2 = 0.908$ .

but it was reduced in the latter (Fig. 2c). The addition of iron to labeled cobalt was associated with a reduction in cobalt uptake and transfer in the iron-deficient but not in the iron-loaded animals (Figs. 1d and 2d). Neither metal had any consistent effect upon the proportion of the other that accumulated in the intestinal mucosa during the 90 min perfusion.

#### Effect of dose

*Iron and cobalt administered separately.* Solutions containing either iron or cobalt in concentrations varying from 0.1 to 10.0 mM were perfused for 30 min through the duodenal loops of animals with increased or with decreased iron stores. In the iron-deficient group, and

to a lesser extent in the iron-loaded group, a greater proportion of the iron and of the cobalt was absorbed from smaller than from larger doses (Figs. 3a, 3b, 4a and 4b). The uptake of each metal was greater than transfer, and thus both metals accumulated in the mucosa. In iron-loaded animals, the uptake of iron exceeded the uptake of cobalt at all doses except the smallest, 0.1 mM, and more iron than cobalt activity was present in the mucosa. In iron-deficient rats, the rate of uptake of iron and cobalt increased in a similar manner with doses of 0.1 to 2.0 mM. At higher doses, more iron was taken up than cobalt, and iron activity progressively increased in the mucosa whereas cobalt activity remained approximately constant.

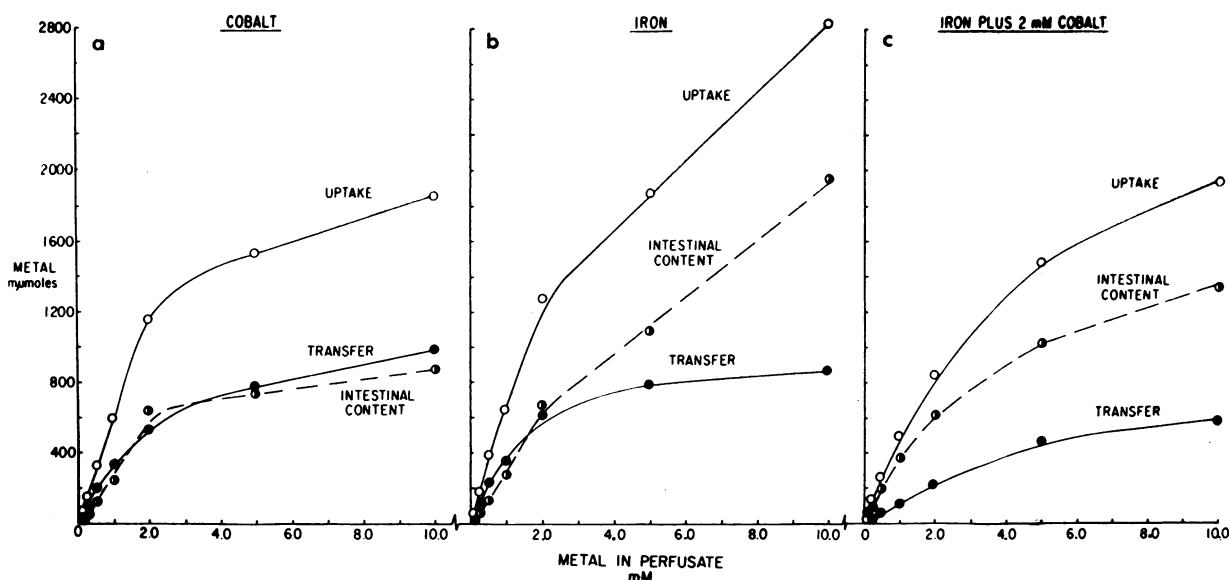


FIGURE 4 Dose-response study of the intestinal uptake and transfer of iron and cobalt with a 30 min perfusion of the duodenum in iron deficiency. The equations for the regression of uptake, transfer, or intestinal content against the concentration of metal in mM ( $x$ ) of cobalt (Co), iron (Fe), or iron plus 2.0 mM cobalt (FeCo) are given. The variance ratio ( $F$ ) describes the significance of the curvilinearity of the regression; the  $R^2$  value represents the proportion of the sum of the squared deviations accounted for by the curvilinear regression. Uptake Co =  $105.0 + 466.1x - 29.4x^2$ ,  $F = 33.7$  ( $P < 0.01$ ),  $R^2 = 0.834$ ; uptake Fe =  $119.6 + 501.8x - 22.8x^2$ ,  $F = 10.2$  ( $P < 0.01$ ),  $R^2 = 0.848$ ; uptake FeCo =  $21.5 + 456.5x - 26.9x^2$ ,  $F = 72.7$  ( $P < 0.01$ ),  $R^2 = 0.941$ . Transfer Co =  $80.1 + 215.3x - 12.5x^2$ ,  $F = 52.5$  ( $P < 0.01$ ),  $R^2 = 0.878$ ; transfer Fe =  $103.2 + 234.4x - 15.7x^2$ ,  $F = 73.5$  ( $P < 0.01$ ),  $R^2 = 0.853$ ; transfer FeCo =  $148.8x - 8.7x^2 - 22.2$ ,  $F = 236.2$  ( $P < 0.01$ ),  $R^2 = 0.978$ . Intestinal content Co =  $11.4 + 250.5x - 16.3x^2$ ,  $F = 78.3$  ( $P < 0.01$ ),  $R^2 = 0.850$ ; intestinal content Fe =  $26.2 + 253.1x - 5.6x^2$ ,  $F = 8.02$  ( $P < 0.01$ ),  $R^2 = 0.841$ ; intestinal content FeCo =  $31.5 + 322.3x - 19.0x^2$ ,  $F = 49.3$  ( $P < 0.01$ ),  $R^2 = 0.894$ .

The data for iron and cobalt absorption in iron-deficient animals were analyzed by the method of least squares for nonlinear regression (14, 15), to determine the approximate maximum transport velocity ( $V_{\max}$ ) of these metals (Table II). With concentrations of 5.0 mM and 10.0 mM the rate of absorption approached but did not achieve a plateau (Fig. 4). Therefore, the calculated values given for  $V_{\max}$  and  $K_m$  in Table II for cobalt and iron under different experimental conditions are only approximate. The absence of a distinct plateau is probably due to the concomitant passive diffusion of small amounts of metal (17). This would have its greatest effect on the estimation of  $V_{\max}$  when absorption was low, such as in iron overload, and therefore these values were not calculated.

#### Effect of administering iron and cobalt together

To investigate the inhibitory effect of cobalt on iron absorption, test solutions containing 2.0 mM cobalt with

iron in concentrations ranging from 0.1 to 10.0 mM were perfused in groups of iron-loaded and iron-deficient animals. This dose of cobalt was chosen as it represented a concentration above the  $K_m$  calculated for cobalt when given with iron. The addition of cobalt was associated with a decrease in iron uptake and transfer in both the iron-loaded and iron-deficient animals (Figs. 3c and 4c). The  $V_{\max}$  for iron alone,  $1008 \pm 53$  (sd)  $\mu\text{moles}$ , was similar to the  $V_{\max}$  for iron with 2.0 mM cobalt,  $986 \pm 99$   $\mu\text{moles}$ ; in contrast, the  $K_m$  for iron with cobalt,  $6.3 \pm 1.3$  mM, was significantly greater than the  $K_m$  for iron alone,  $1.6 \pm 0.4$  mM (Table II). This indicates that cobalt competitively inhibited iron absorption.

Varying concentrations of iron from 0.1 to 10.0 mM had no effect on the intestinal uptake and absorption of 2.0 mM cobalt in iron-loaded animals. In iron deficiency, however, the addition of iron in doses greater than 0.5 mM was associated with a progressive decline in cobalt uptake and transfer (Fig. 5).

TABLE II  
Kinetic Constants for Iron and Cobalt Absorption in Iron Deficiency

Experiment	$V_{\max}$		$K_m$	
	Iron	Cobalt	Iron	Cobalt
	$\mu\text{moles}/30 \text{ min per duodenal segment } (\pm\text{SD})$		$\text{mM metal in perfusate } (\pm\text{SD})$	
Perfusion of either iron or cobalt alone, in concentrations varying from 0.1 to 10.0 mM (Fig. 4 a, 4 b)	$1008 \pm 94$	$1221 \pm 77$	$1.6 \pm 0.4$	$2.6 \pm 0.4$
Effect of 2.0 mM cobalt on the absorption of concentrations of iron varying from 0.1 to 10.0 mM (Fig. 4 c)	$986 \pm 99$	—	$6.3 \pm 1.3$	—
Perfusion of iron and cobalt together in equimolar concentrations varying from 0.1 to 5.0 mM (Fig. 6 b, 6 c)	$426 \pm 82$	$505 \pm 39$	$1.7 \pm 0.7$	$0.4 \pm 0.1$

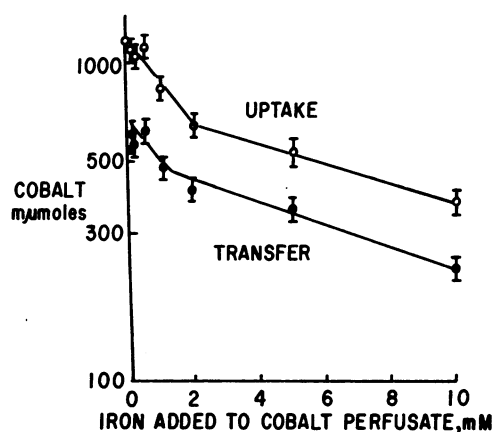


FIGURE 5 Effect of varying concentrations of iron on the uptake and transfer of 2.0 mM cobalt in the duodenum of iron-deficient rats.

To determine the relative affinity of the transport mechanism for iron and cobalt, test solutions containing both metals in equimolar concentrations ranging from

0.1 to 5.0 mM were perfused through duodenal loops of iron-deficient animals. A similar increase in the uptake of both metals was observed (Table III, Fig. 6 a); at a concentration of 5.0 mM, cobalt uptake was less than iron, but the difference was not statistically significant ( $0.10 < P < 0.05$ ). In contrast to uptake, the transfer of cobalt greatly exceeded iron (Fig. 6 b); a Lineweaver-Burk plot revealed a common intercept on the metal-transfer axis, but different intercepts on the metal-concentration axis (Fig. 6 c). The  $V_{\max}$  for iron,  $426 \pm 82$  (SD)  $\mu\text{moles}$ , and the  $V_{\max}$  for cobalt,  $505 \pm 39$   $\mu\text{moles}$ , were similar, but the  $K_m$  values for iron and cobalt were different,  $1.7 \pm 0.7$  and  $0.4 \pm 0.1$  mM respectively (Table II). The difference in the  $K_m$  suggests that the affinity of the transport mechanism for cobalt was much greater than for iron. That there was no statistically significant difference between the  $K_m$ 's for iron and cobalt in the study where the metals were perfused separately is probably due to the wide range of the data. This was reduced in the above experiment in which the results obtained in the same animals were compared.

TABLE III  
Absorption of Equimolar Amounts of Iron and Cobalt when Perfused Together in Iron Deficiency

Concentration of metal in perfusate	No. of rats	Intestinal uptake		Transferred to the carcass		Amount of uptake transferred		Retained in the mucosa	
		FeCo	CoFe	FeCo	CoFe	FeCo	CoFe	FeCo	CoFe
mM		$\mu\text{moles}$		$\mu\text{moles}$		%		$\mu\text{moles}$	
0.1	7	$78 \pm 8$	$104 \pm 12$	$43 \pm 4$	$85 \pm 10^*$	$56 \pm 3$	$80 \pm 2^*$	$35 \pm 5$	$20 \pm 2^*$
0.25	7	$162 \pm 7$	$222 \pm 11^*$	$64 \pm 5$	$168 \pm 12^*$	$39 \pm 3$	$75 \pm 2^*$	$98 \pm 6$	$54 \pm 4^*$
0.5	7	$307 \pm 20$	$342 \pm 29$	$96 \pm 6$	$236 \pm 20^*$	$32 \pm 3$	$69 \pm 3^*$	$210 \pm 19$	$106 \pm 12^*$
1.0	8	$582 \pm 47$	$650 \pm 51$	$165 \pm 17$	$458 \pm 45^*$	$28 \pm 2$	$70 \pm 3^*$	$417 \pm 34$	$192 \pm 19^*$
2.0	10	$760 \pm 43$	$717 \pm 30$	$208 \pm 20$	$410 \pm 25^*$	$27 \pm 2$	$57 \pm 2^*$	$552 \pm 31$	$307 \pm 16^*$
5.0	6	$1575 \pm 197$	$1229 \pm 97$	$330 \pm 76$	$426 \pm 33$	$20 \pm 3$	$35 \pm 2^*$	$1245 \pm 106$	$803 \pm 79^*$

Mean values  $\pm$ SE. The values for iron in the presence of an equimolar amount of cobalt are represented as FeCo; the values for cobalt in the presence of an equimolar amount of iron are represented as CoFe.

\* Indicates that the difference between the mean of FeCo and CoFe is statistically significant,  $P < 0.05$ .

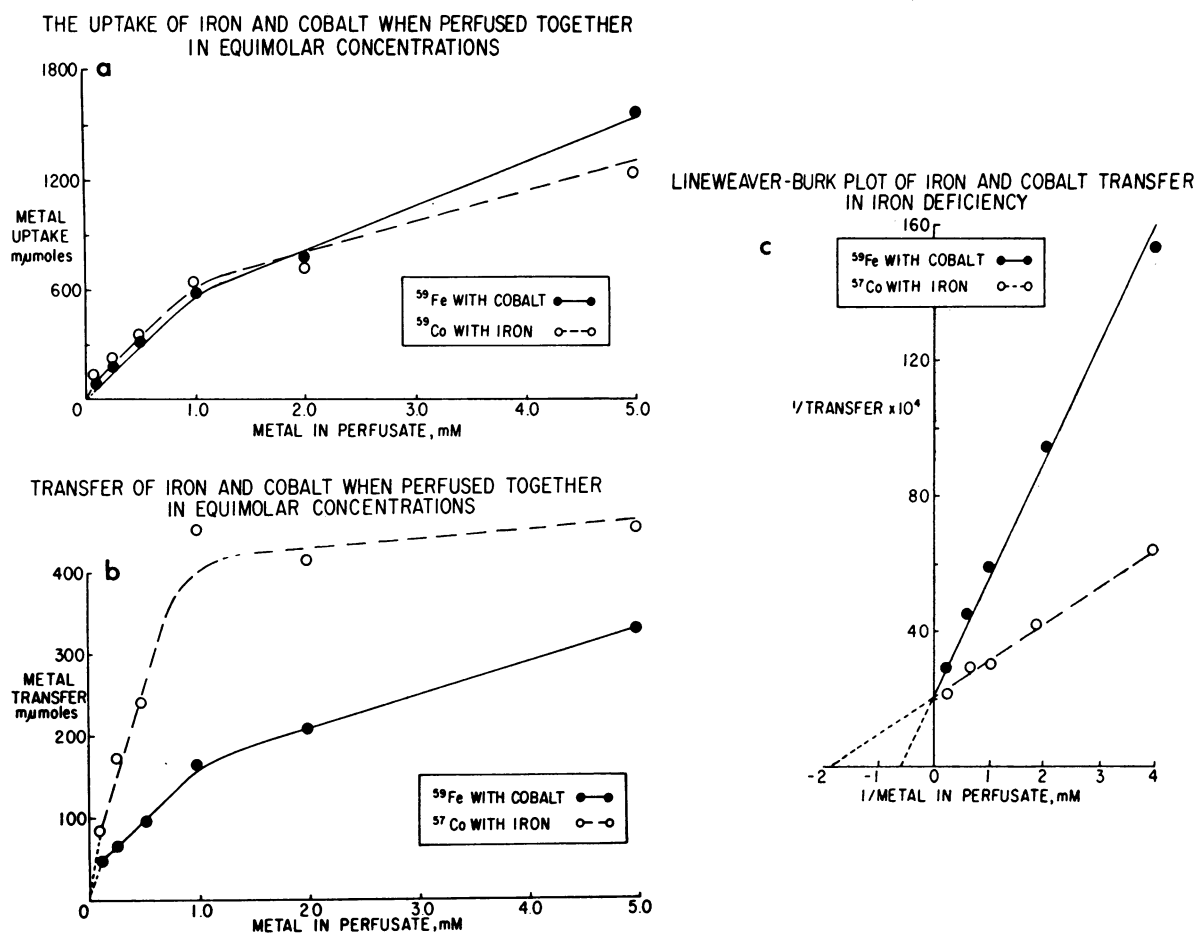


FIGURE 6 Intestinal uptake and transfer of equimolar concentrations of iron and cobalt with a 30 min perfusion of the duodenum in iron deficiency. The uptake of iron and cobalt is shown in (a), and the transfer of these metals is given in (b). The equations for the regression of uptake or transfer against the concentration of metal in mM ( $x$ ) of cobalt with equimolar amounts of iron (CoFe), or iron with equimolar amounts of cobalt (FeCo) are given. The variance ratio ( $F$ ) describes the significance of the curvilinearity of the regression; the  $R^2$  value represents the proportion of the sum of the squared deviations accounted for by the curvilinear regression. Uptake CoFe =  $116.8 + 437.4 x - 42.5 x^2$ ,  $F = 24.0$  ( $P < 0.01$ ),  $R^2 = 0.880$ ; uptake FeCo =  $3.1 + 476.8 x - 25.8 x^2$ ,  $F = 1117$  ( $P < 0.01$ ),  $R^2 = 0.995$ . Transfer CoFe =  $68.0 + 333.7 x - 51.7 x^2$ ,  $F = 59.5$  ( $P < 0.01$ ),  $R^2 = 0.735$ ; transfer FeCo =  $23.7 - 127.2 x - 12.0 x^2$ ,  $F = 14.8$  ( $P < 0.01$ ),  $R^2 = 0.660$ . Note the equations describing the uptake of these metals at high concentrations. A Lineweaver-Burk plot of the transfer of iron and cobalt in iron deficiency is depicted in (c). The  $V_{max}$  for iron,  $426 \pm 82$  (SD)  $\mu\text{moles}$ , and the  $V_{max}$  for cobalt,  $505 \pm 39$   $\mu\text{moles}$ , was similar, but the  $K_m$  for each metal was different ( $1.7 \pm 0.7$  mM, and  $0.4 \pm 0.1$  mM, respectively).

### Retention of iron and cobalt in the intestinal mucosa

30 min after the oral administration of a test solution containing iron and cobalt in equivalent amounts, twice as much iron as cobalt was present in the intestinal mucosa (Fig. 7). After this, there was a decline in the activity of both metals. At the end of 24 hr, 6% of the iron remained, one-half of which was in the proximal

intestine, whereas only 0.3% of the cobalt was present throughout the entire intestine.

### DISCUSSION

In iron-deficient animals, the decline in the rate of cobalt absorption with either continued perfusion or with increasing concentrations in the perfusate suggests saturation of binding sites for cobalt transport in the mu-



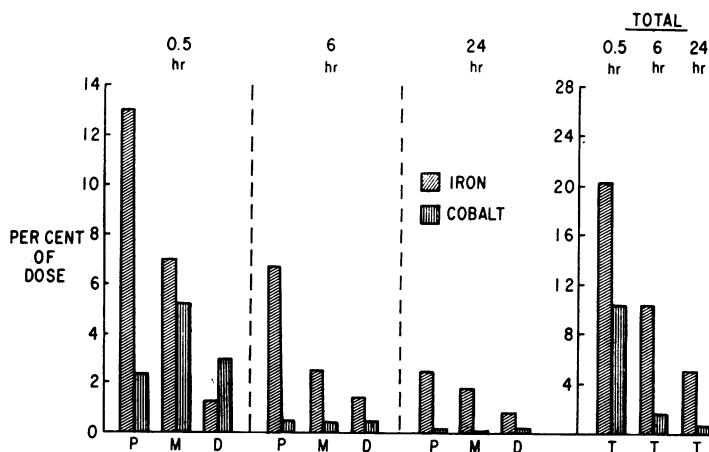


FIGURE 7 Retention of an oral dose of iron and cobalt in the intestinal mucosa of rats with normal iron stores. The values for iron or cobalt in the proximal third of the intestine are represented as P, from the middle third of the intestine as M, and from the distal of the intestine as D. The retention of these metals in the total small intestine is represented as T; note the different scale used for the T values.

cosa (Figs. 2 *b* and 4 *a*). Comparable results were obtained with iron (Figs. 2 *a*, and 4 *b*). The dose-response lines for both cobalt and iron approached but did not achieve a plateau (Fig. 4 *a* and 4 *b*). This implies that an additional mechanism is operative in which the amount of metal in the perfusate is an important determining factor. A similar conclusion was reached by Bannerman, O'Brien, and Witts (16), and Gitlin and Cruchaud for iron absorption in the mouse (17).

The inhibitory effect of iron on cobalt absorption, and cobalt on iron absorption, and the close similarity between the maximum transport velocity ( $V_{max}$ ) of the two metals in iron deficiency suggests that they compete for analogous binding sites in the transport system. The small  $K_m$  for cobalt, 0.4 mM, in comparison with iron, 1.7 mM, indicates that the affinity of the transport system for cobalt is greater than iron. This would explain why cobalt reduced iron absorption to a greater extent than iron reduced cobalt absorption, both in this study and in the investigation of Schade, Felsher, Bernier, and Conrad (6).

Cobalt was absorbed in only small amounts in iron-loaded animals (Figs. 1 *b* and 3 *a*). Except at the lower concentrations, its absorption was proportional to the size of the dose, suggesting a process, such as diffusion, in which the limiting factor was the amount of metal in the intestinal lumen. At the lower concentrations, however, a greater proportion of the dose was absorbed, suggesting that an additional process was also operative, one in which binding sites became saturated. The magnitude of this second component was small, and cobalt absorption appeared to be predominantly dose-dependent

in iron-loaded animals. The results of the dose-response study with iron in these animals were similar to cobalt.

With continued perfusion of cobalt in iron-loaded animals, saturation of binding sites was not observed, whereas this was found with iron (Fig. 1 *a*). The reason for this difference is unknown. It may be due to the sequestration of iron in an intracellular pool(s). The addition of iron to the cobalt perfusate had no appreciable effect on cobalt absorption, whereas cobalt employed in larger doses than in a previous study (5) inhibited iron transport. In the presence of cobalt, iron absorption was closely dependent on dose, suggesting that absorption under these conditions was due largely to diffusion (Fig. 3 *c*). Schade, Felsher, Glader, and Conrad (18) have made a similar observation in iron-normal animals. The absence of an effect of iron on cobalt absorption in the iron-replete animals may have been due to the greater affinity of cobalt for binding sites; it might also be explained on the basis that the predominant diffusion of cobalt masked any effect which iron might have had on the saturable transport system for cobalt.

In spite of the many similarities between the absorption of iron and cobalt in iron-deficient and iron-loaded animals, there were a number of differences. Firstly, more iron than cobalt accumulated in the intestinal mucosa in both the dose-response and time-response studies. Secondly, in contrast to cobalt, the intestinal uptake of iron continued to increase even though the transfer of iron out of the cell approached a steady state (Fig. 4 *b*). Thirdly, in the iron-loaded animals, cobalt transfer was greater than iron transfer at 60 and 90 min. Fourthly, significant amounts of iron were retained in the mucosa

for at least 24 hr whereas cobalt declined to negligible levels (Fig. 7). These differences are probably due to diversion of a portion of the uptake of iron but not of cobalt into a mucosal storage pool(s). The chemical nature of storage iron in the mucosa has not been established with certainty, but a proportion of it is in the form of ferritin (20). Schade et al., observed that cobalt does not bind to ferritin *in vitro*, nor does it stimulate ferritin synthesis (6, 22). Thus, one mechanism for the storage of iron in the mucosa is not available to cobalt. Brown and Rother (21) found three times more iron than cobalt in the TCA-precipitable (trichloroacetic acid) fraction of the soluble portion of homogenized rat duodenal mucosa 30 min after the uptake of similar amounts of each metal. Thus a greater portion of iron in the mucosa is protein bound.

While it is evident that binding sites are implicated in the transfer of cobalt and iron from the mucosa to the body, it is not clear whether binding sites are also involved in the uptake of metal from the lumen. Certain pieces of evidence suggest that they are involved: firstly, the rate of uptake of both metals was initially rapid but declined as the perfusion continued, and it also decreased with the administration of progressively increasing doses; secondly, each metal inhibited the uptake of the other; thirdly, because uptake from the lumen was proportionately greater than transfer from the cell to the body, more metal isotope accumulated in the mucosa of the iron-deficient than in the iron-loaded animals; and fourthly, Schade et al. (18) found cobalt, in a dose 12 times greater than that used in the study, inhibited iron uptake. Some of our observations may be explained by factors other than saturation of binding sites. Firstly, the change in the rate of uptake with time makes a valid estimate of the effect of dose on uptake difficult (19), and the change in uptake may be accounted for equally well by the rapid passage of metal into the mucosa followed by a decline as the intracellular pool(s) fills (20, 21). Secondly, part of the inhibitory effect of one metal on the uptake of the other could be secondary to reduction in transfer of metal from the cell to the body. Thirdly, the radioactivity in the mucosa may not be representative of the actual size of either the transit or storage pools, because neither the specific activity of metal was determined nor unloading of the mucosal cells followed. Hence, binding sites may be involved in the uptake step, but this has not been established with certainty.

The evidence is consistent with the following model (Fig. 8). The absorption of cobalt and iron is mediated by a transport system in which two processes operate simultaneously; the first, diffusion, is limited largely by the concentration of available metal in the lumen of the intestine, whereas the second depends upon the activity of a mechanism which displays saturation kinetics and com-

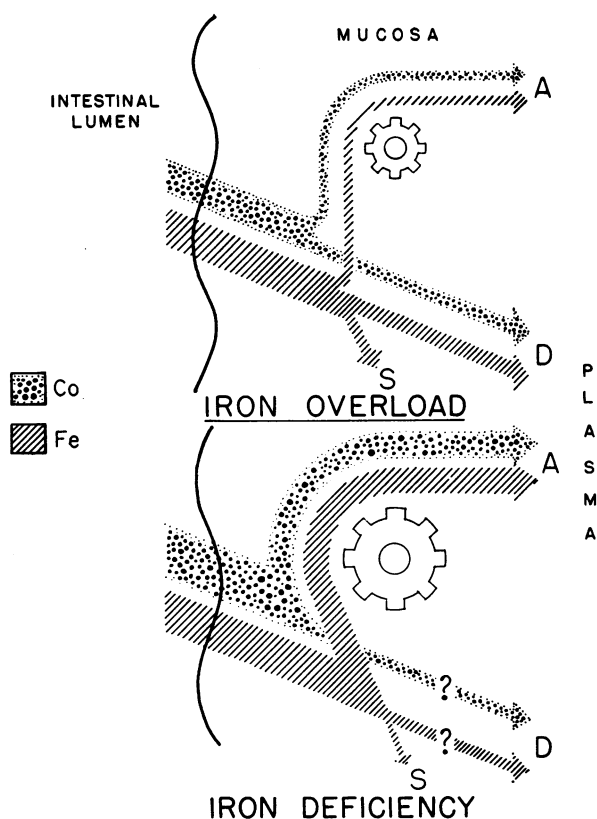


FIGURE 8 Proposed model for the intestinal transport of iron and cobalt in the rat. *A* represents the "active" transport process(es) for the absorption of iron and cobalt; its location(s) in the mucosal cell is unknown. *D* is the "passive" process that is dependent on the concentration of metal in the lumen. *S* represents metal that is sequestered in the mucosa and subsequently lost with desquamation of the epithelium (10). The size of the arrows depicts the relative magnitude of the various components in the iron-replete and the iron-deficient states except the magnitude of *D* in iron deficiency is unknown. In iron repletion, both iron and cobalt are absorbed predominantly by diffusion. A proportion of the iron but not of the cobalt is sequestered in the cell and is subsequently lost with desquamation of the epithelium. In iron deficiency, iron and cobalt absorption is increased by the enhanced activity of a common transport system which has a greater affinity for cobalt. The absorption of iron and cobalt is predominantly by this mechanism in iron deficiency. Sequestration of iron in the mucosa is negligible in iron deficiency because of the rapid movement of iron from the mucosal cell to the plasma. The size of the "active" component in animals with "normal" iron stores lies between the two extremes of iron overload and iron deficiency shown above; its magnitude depends upon the need for iron. The regulation of iron absorption resides with the control of the activity of the "active" component of the transport system.

petitive inhibition. The former process prevails when iron stores are replete, whereas the latter predominates when there is a need for iron, such as in iron deficiency.

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