PHYSIOLOGY

Active Transport of Iron by Intestine: Effect of Erythropoiesis stimulated by Phenylhydrazine

Intestinal absorption of radio-iron in intact rats was increased during the enhanced erythropoiesis which followed haemolysis induced by phenylhydrazine[1]. Similarly, erythropoiesis stimulated by acetylphenylhydrazine in dogs also increased iron absorption *in vivo*[2]. This report describes experiments designed to study the effects of increased erythropoiesis in the rat on iron transport across everted duodenal gut sacs *in vitro*. The *in vitro* technique has defined an active transport mechanism for iron absorption[3,4] which varies adaptively with dietary iron and pregnancy[5] and, as will be shown, the cellular mechanism is also involved in the response to increased erythropoiesis.

Male, albino, Sherman strain rats, 80–140 g, were injected subcutaneously with 100 mg/kg body-weight of phenylhydrazine-HCl (recrystallized from boiling water) dissolved in 0·9 per cent sodium chloride, and controls received saline alone. Seven days later rats were killed, the proximal 10 cm of small intestine excised and two consecutive everted gut sacs prepared[4], each 5 cm long. Mean wet weights of the proximal and distal sacs were approximately 770 and 500 mg, respectively. Sacs were incubated in Warburg vessels under oxygen, at 37° C, for 2·5 h in the ambient medium previously described[4], containing 0·1 mM freshly prepared ferrous sulphate, 0·8 mM sodium ascorbate, and sufficient $^{59}FeSO_4$ (Abbott Laboratories, Oak Ridge) to give about 10,000 c.p.m./ml. in a well-type scintillation counter. After incubation sacs were drained, and iron-59 and iron in the mucosal and serosal media were estimated as previously described[4]. Heparinized capillary tubes were used for haematocrit determinations.

Following phenylhydrazine injection the initial mean haematocrit, 42 per cent, decreased to 29 per cent at 3 days and returned to the initial control value at 7 days. Everted gut sacs were then prepared from thirty-one control and forty phenylhydrazine-treated rats and tested *in vitro* (Table 1). Phenylhydrazine treatment increased net transfer of iron (estimated chemically) to the serosal surface by 19·7 per cent ($P < 0·05$) and 54·7 per cent ($P < 0·01$), respectively, in the proximal and adjacent distal segments. Final iron-59 concentration ratios serosal/mucosal were increased correspondingly by 24·4 per cent ($P < 0·02$) and 54·8 per cent ($P < 0·001$) in the proximal and distal segments, respectively. Gut sacs prepared from intestine more than 10 cm distal from the pylorus showed no clear effect of prior phenylhydrazine treatment.

Table 1. Effect of Prior Phenylhydrazine on Iron Transport by Everted Duodenal Gut Sacs *in vitro*

Group	No.	Net Fe transfer to serosal medium mμmolcs/gut sac		Iron-59 concentration ratio serosal/mucosal	
		Proximal sac*	Distal sac	Proximal sac	Distal sac
Control	31	31·4 ± 10·5†	12·8 ± 6·6	4·5 ± 1·4	2·0 ± 1·1
Phenyl-hydrazine	40	37·6 ± 12·0	19·8 ± 10·3	5·6 ± 2·1	3·1 ± 1·4
P value		< 0·05	< 0·01	< 0·02	< 0·001

* Two gut sacs were prepared from the proximal 10 cm of rat small intestine and results are shown for the more proximal and adjacent distal sac.
† Mean ± *S.D.*

The results indicate that the mucosal mechanism for active transport of iron is responsible, in part at least, for enhanced absorption observed after phenylhydrazine treatment. Treated rats were not anaemic at the time of the experiment, and the stimulus for increased iron transport was apparently increased erythropoiesis following haemolysis[1]. Response of the iron transfer mechanism observed *in vitro* was limited to the proximal 10 cm of small intestine and distinctly less in magnitude than the more than two-fold increase observed in intact rats[1]. Thus factors other than the mucosal mechanism studied here may participate in the response to increased erythropoiesis *in vivo*. Increased iron transport observed in the present experiments also differs from that following a low iron diet[5]. Dietary restriction yielded much greater increases in transport *in vitro*, and the most proximal segment showed the greatest effect.

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Increased Active Transport of Glucose through the Intestine during Pregnancy

A well-known aspect of the physiological adaptation to pregnancy is the increase in ingestion of food as a result of stimulation of the lateral nuclei of the hypothalamus.

From a teleological point of view, it might be thought that an increase in the absorptive capacity of the intestine would be associated with this increase in ingestion. It is, in fact, known that iron is absorbed more rapidly during pregnancy, in proportion to the development of the haematopoietic organs of the foetus[1], but few other investigations of intestinal absorption in pregnancy have been carried out.

We have therefore investigated the effect of pregnancy on the absorption of *d*-glucose—one of the most actively transported substances. Other experiments dealing with the absorption of glycine are in progress. The Sols y Ponz technique of successive absorptions *in vivo* was used, as in previous investigations[2].

Table 1. Influence of Pregnancy on the Absorption Rate of *d*-Glucose through the Intestine of Rats *in vivo*

No. of rats	Glucose (mmole)	Intestinal* absorption (μmole/cm)
Not pregnant		
8	300	43·3 ± 0·8
9	150	20·0 ± 0·05
12	75	13·0 ± 0·02
7	20	5·0 ± 0·09
Pregnant		
9	300	52·1 ± 0·5
10	150	26·1 ± 0·7
10	75	16·8 ± 0·2
7	20	7·0 ± 0·4

* Time of absorption, 30 min

Thirty-six white Wistar rats, weighing from 180 to 200 g and 12–15 days pregnant, and a control group of thirty-six rats of similar characteristics but not pregnant, were perfused with solutions of 300, 150, 75 and 20 mM glucose at a pressure of 12 cm of water for 30 min. In each animal, four successive absorptions were carried out.

In Table 1 the average values for both types of experiments are indicated with their corresponding standard errors. The results show a very significant increase ($P < 0·01$) in the absorption of glucose in the pregnant rats over that in the controls. This increase is observed with all the concentrations of glucose, amounting to 21 per cent for 300 mM, and 40 per cent for 20 mM.

Similar results have been found for glycine[2]. The observed increase in the absorption rate is another example