patients with idiopathic hypercalciuria was supersaturated with respect to brushite at all pH. However, the urine of normocalciuric subjects with stone was supersaturated only at high urinary pH.

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An Effect of Intestinal Motility on Iron Absorption (33649)

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Iron absorption is influenced by numerous intraluminal, mucosal and corporeal factors (1). Most manipulations affecting iron absorption do not produce measurable effects for several days after their initiation (2). Endotoxin is exceptional because it markedly diminishes iron absorption within hours after injection and seems to act by decreasing the transfer of dietary iron from the intestinal absorptive cells into the body (3, 4). The rapid effect of endotoxin upon iron absorption suggested that it triggered a basic regulator of iron absorption and that the response was not mediated by changes in erythropoiesis or the body iron stores. To study this phenomenon, we measured iron absorption in rats dosed with drugs that altered various manifestations of the endotoxin response. Preliminary studies showed that antihistaminics and corticosteroids produced no significant change in iron absorption by either normal or endotoxin treated rats. On the contrary, phenoxybenzamine (Dibenzyline), an alpha adrenergic blocking agent, increased iron absorption in both normal and endotoxin-treated animals. The present paper reports investigations of the effect of phenoxybenzamine upon iron absorption.

Methods. Male albino rats of the Walter Reed Carworth Farm strain, weighing 200–250 g, were used. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Rats were raised in a pathogen-free environment and housed in galvanized wire cages. The animals were fed a standard laboratory diet containing 25% protein and 9 mg of iron/100 g of dry weight.

Drugs were administered by intraperitoneal injection. These included: phenoxybenzamine hydrochloride, 3 mg (Smith, Kline & French Laboratories); endotoxin as *E. coli* lipopolysaccharide, 0.1 mg (Difco Laboratories); and atropine sulfate, 30 mg.

Iron absorption studies were performed with a test dose containing 0.5 μCi of ferrous^{59} citrate and 0.25 mg of elemental iron as ferrous sulfate in 0.5 ml of distilled water. The test dose was administered to rats fasted for 16 hr by injection into the stomach with an olive-tipped 17-gauge endoesophageal needle. Whole-body radioactivity (0.8 MeV-∞) was measured in a small animal whole-body liquid scintillation detector (ARMAC, Packard Instrument Co.) 3 hr and 7 days after dosing to determine the percentage of the

TABLE I. Effect of Endotoxin and Blocking Agents upon Iron Absorption.

	Whole-body retention of ^{59}Fe at 7 days (%)	
	Mean	SE
Control	10.9	1.1
Endotoxin	4.8	0.5
Phenoxybenzamine	18.6	1.9
Phenoxybenzamine and endotoxin	11.5	1.5
Atropine	15.2	1.9
Atropine and endotoxin	9.7	2.0

oral test dose absorbed by the rats (5). In certain studies, additional whole body measurements of radioactivity were made to determine the rate of transit of iron^{59} through the intestinal tract. In other studies, whole body radioactivity was measured before and after excision of the unopened intestinal tract. Intestinal loops were isolated by ligatures of umbilical tape at the gastroduodenal junction and at the ligament of Treitz. Duodenal doses of iron were administered by inserting a 20-gauge hypodermic needle into the stomach and through the pylorus; the ligature of umbilical tape was tightened around the pylorus before the injection of the test dose.

The iron content of the proximal quarter of the small intestine was measured in weighed specimens excised from rats fasted for 16 hr. The excised intestinal segments were opened lengthwise, washed thoroughly in several changes of alkalinized iron-free distilled water and homogenized in a Virtis tissue grinder. The nonheme iron content of the homogenates was measured by a modification of the method of Brückman and Zondek (6)

Ferrokinetic studies were performed by the injection of iron^{59} labeled serum into the dorsal vein of the penis. Plasma iron clearance was determined by measurements of radioactivity in whole blood specimens obtained 10, 20, 30, and 40 min after injection of labeled serum. Plasma iron turnover was calculated from the serum iron concentration and plasma iron clearance rate. The incorporation of iron^{59} into red blood cells was determined by measurement of the radioactivity in blood specimens 20 hr after the intravenous injection of radiolabeled serum (1, 7).

Results. The parenteral administration of either phenoxybenzamine (3 mg) or atropine (30 mg) to rats 1 hr before an oral test dose of radioiron markedly increased iron absorption in normal rats and in animals that received 0.1 mg of *E. coli* endotoxin 12 hr previously (Table I). The duration of effectiveness of a single dose of phenoxybenzamine was studied by varying the interval between injection and administration of the oral test dose of iron. Increased amounts of iron were absorbed from oral test doses administered from 1 to 24 hr after a 3 mg injection of phenoxybenzamine (Fig. 1).

To determine the mechanism by which phenoxybenzamine affected iron absorption, we measured various factors that are usually altered with changes in iron absorption (Table II). There was a significant decrease in the serum iron concentration and increased rate of clearance of iron^{59} from plasma of

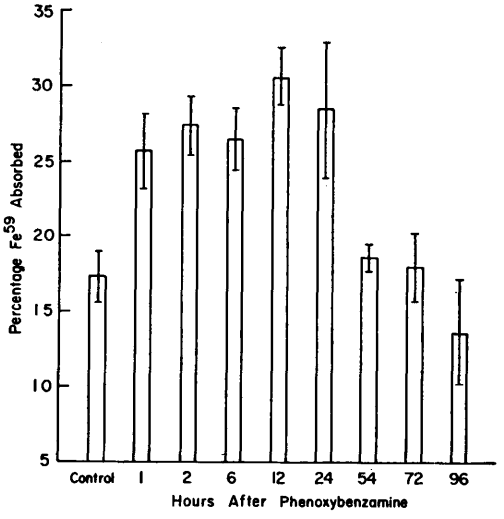

FIG. 1. Iron absorption at intervals after phenoxybenzamine: phenoxybenzamine was injected intraperitoneally into rats at time intervals preceding administration of the oral dose of ^{59}Fe. These time intervals are indicated on the horizontal axis; the bars indicate the percentage of ^{59}Fe absorbed when measured 7 days after dosing.

TABLE II. Effect of Phenoxybenzamine at Intervals after Injection.

Hours after phenoxybenzamine:	Control	2	6	12	24	48
Iron absorption (%)	17.2 ± 1.7	25.7 ± 2.5	27.3 ± 1.8	30.6 ± 1.8	28.5 ± 4.5	18.7 ± 0.8
Serum iron (μg/100 ml)	138 ±10	126 ± 1.8	109 ±12	87 ±10	107 ± 9	166 ±16
Plasma iron clearance (T ½ min)	74 ± 5	60 ± 3	49 ± 4	48 ± 3	52 ± 4	
RBC ^{59}iron incorporation	41.5 ± 1.7	42.0 ± 2.4	40.9 ± 1.4	42.0 ± 1.4	40.8 ± 2.2	
Intestinal iron concentration (μg/g)	15.4 ± 1.4	16.4 ± 0.8	16.4 ± 0.6	16.6 ± 1.5	18.7 ± 1.0	

phenoxybenzamine treated animals. These changes were proportionate to each other so that there was no consistent change in the calculated plasma iron turnover. During the 24-hr period after an injection of phenoxybenzamine, there was no significant alteration in the red blood cell incorporation of radioiron or the intestinal iron concentration. This paucity of changes in both ferrokinetic and mucosal iron measurements led us to scrutinize other factors which might affect iron absorption.

Rats given an oral dose of radioiron 1 hr after an injection of phenoxybenzamine were killed at intervals. The entire gut was excised and radioactivity in the carcass was quantified. Similar amounts of radioiron were detected in the carcasses of both normal and phenoxybenzamine treated rats until 6 hr after dosing. Measurements of body radioactivity at 24 and 48 hr after dosing showed that phenoxybenzamine treated animals had absorbed more iron (Fig. 2). To ascertain if the delayed absorption of increased amounts of iron by phenoxybenzamine treated rats might be caused by slowed intestinal motility, we measured the whole-body retention of iron^{59} in live animals at intervals after an oral dose of iron^{59}. There was a delayed transit of iron^{59} through the intestinal tract of phenoxybenzamine treated animals (Fig. 3). Atropine produced a similar effect and there also was a delayed transit of nonabsorbable chromic^{51} chloride through the gut of phenoxybenzamine treated animals. Additional evidence that phenoxybenzamine increased iron absorption by its effect upon intestinal motility was obtained by studies which showed similar absorption of iron from the isolated duodenal segments of both normal and phenoxybenzamine treated rats. In contrast, endotoxin treated animals had decreased iron absorption from isolated intestinal loops (Table III).

Discussion. Phenoxybenzamine is a long acting alpha adrenergic blocking agent which was found to increase iron absorption in

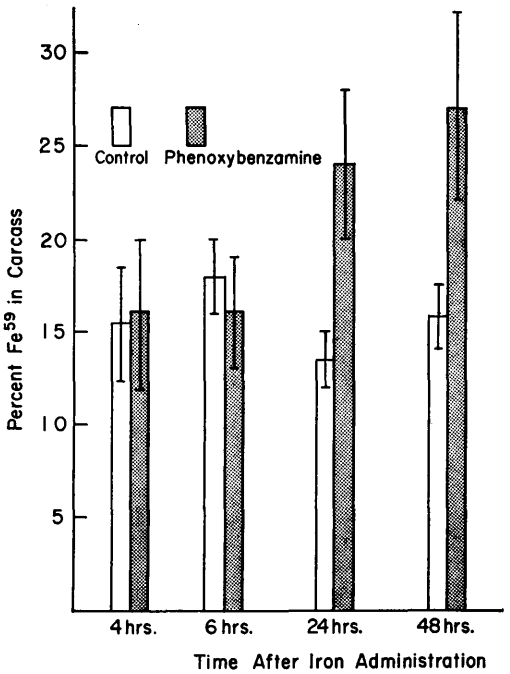

FIG. 2. Carcass uptake of ^{59}Fe at intervals after oral dose of iron: phenoxybenzamine was given intraperitoneally to rats 1 hr prior to the oral administration of ^{59}Fe. At time intervals after dosing with ^{59}Fe the guts of the animals were excised, and the percentage of ^{59}Fe retained in the carcasses was determined.

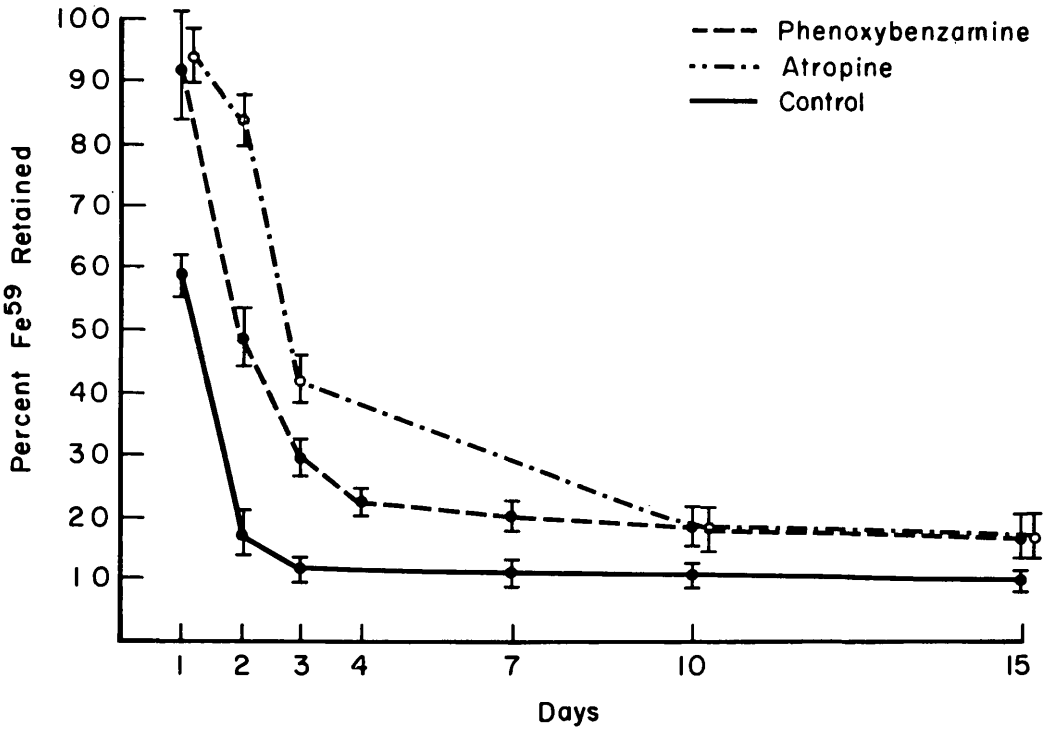

FIG. 3. Percentage of ^{59}Fe in whole body at intervals after oral dose of iron: rats were injected with phenoxybenzamine or atropine. The rats were then given an oral dose of ^{59}Fe and at intervals thereafter the percentage of ^{59}Fe remaining in the whole animal was determined.

both normal and endotoxin treated animals (8). Although phenoxybenzamine caused significant changes in the serum iron concentration and rate of plasma iron clearance, there was no effect upon the plasma iron turnover, the red blood cell incorporation of iron^{59} or the iron concentration in the duodenal mucosa. Similar to atropine, phenoxybenzamine seemed to enhance iron absorption by slowing intestinal motility and increasing the duration of exposure of luminal iron to absorptive cells and not by an effect upon ferrokinetics or the mucosal block. Confirmation of this hypothesis was obtained by measurement of iron absorption in isolated intestinal loop experiments and at intervals after the administration of a test dose of iron.

TABLE III. Absorption of Radioiron from Isolated Duodenal Loops.

	Mean	SE
Control	4.5	0.5
Endotoxin (12 hr previously)	2.4	0.3
Phenoxybenzamine (1 hr previously)	4.3	0.5

Previous investigators reported that phenoxybenzamine had little effect upon intestinal motility (8). On the contrary, the present studies showed a marked slowing in the transit of radiolabeled compounds through the intestinal tract. Although it has been presumed that the rate of intestinal transit played a role in the absorption of dietary iron, documented studies of this effect have not been reported previously.

Summary. The rate of intestinal transit affects the absorption of dietary iron. The parenteral administration of alpha adrenergic blocking agents or parasympathetic blocking agents significantly increases the absorption of iron by decreasing intestinal motility. That the intestinal transit of iron can play an important role in iron absorption is suggested by effects observed in both normal animals and animals with a decreased capability to absorb iron.

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Protein Evaluation of Two Species of Cucurbita Seeds* (33650)

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Protein malnutrition is recognized to occur in many areas throughout the world (1, 2). Efforts to develop suitable vegetable protein sources have been investigated as a means of supplementing current dietary protein supplies (3, 4). Surveys have indicated that about two-thirds of the total caloric intakes of the population of underdeveloped countries are derived from cereals and only about 5% from animal protein.

Since information concerning the nutritive value of the protein and the presence of other factors which may affect the protein quality in plants of the Sonora Desert is sparse, we chose to study the nutritive value of native Cucurbita seeds. These data concerning gourd seeds become important since certain species of Cucurbita are xerophytic and can be grown as a food crop in the Sonora Desert region. Two of these species, *C. digitata* gray and *C. foetidissima* HBK, were studied.

Experimental Methods. White mice of weanling age were housed in stainless steel cages with raised wire floors. Twelve mice were used per dietary treatment (equal number of each sex) and were housed two of the same sex per cage. The mice were maintained at 25 ± 1° with feed and water supplied *ad libitum*. The study consisted of seven dietary treatments (Table I). The protein sources employed were dried whole egg, *C. digitata* seeds and *C. foetidissima* seeds. The *C. digitata* and *C. foetidissima* seeds were dried and prepared in the following manner: (i) ground, (ii) ground and fat extracted, and (iii) autoclaved for 0.5 hr at 120° (15 lb pressure). The *C. digitata* seeds had the following composition: 20% protein and 20.2% fat. The *C. foetidissima* seeds had 32.3% protein and 30.4% fat. All diets were calculated to be isocaloric and each protein source was fed at a level calculated to supply 10% protein.

The mice were weighed and sacrificed at the end of the third week. Livers were extracted from all surviving mice and immediately frozen for glutamic-oxalacetic and glutamic-pyruvic transaminase activity estimations (6, 7). The feed and feces were collected and analyzed for Cr_2O_3 marker, nitrogen, energy (8), and amino acids.

Results and Discussion. The protein sources used in the experiment were analyzed for amino acid composition by a microbiological method (9) (Table II). These results showed the seeds for *C. foetidissima* to be deficient in several amino acids, while the whole egg met the listed NRC amino acid requirements

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