the responsible organisms nor the site of their action is known. Comparison of germfree and conventional animals, however, has revealed differences in the metabolic activity of neurons of Auerbach's plexus(11), in the intestinal complement of pharmacologically active amines(12), in the luminal content of certain musculo-active substances(13), and most recently, in the threshold of response of cecal smooth muscle to chemical stimulation(14). At present, in our laboratory, the *in vitro* neuromuscular activity of various isolated segments of germfree and conventional gastrointestinal tract is being studied in an attempt to clarify the possible role of these factors.

Summary. The rate of propulsion of gastrointestinal contents was compared in germfree and conventional mice using a non-absorbable radioactive substance, yttrium 91. Following intragastric administration, the marker was propelled much more rapidly through the gastrointestinal tract of conventional as compared to germfree animals, demonstrating that "normal" propulsive activity is determined to a significant degree by the presence of the normal flora. This function of the flora has important implications for host defense. The precise mechanism of action of the flora is obscure.

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Starch and Iron Absorption. (32431)

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The habitual ingestion of large amounts of laundry starch is a common practice among Negro females in certain geographic areas of the United States(1). The amount ingested may exceed two pounds of starch daily and is frequently associated with marked iron deficiency(2). The severity of "starch eaters anemia" seems greater than expected from an iron deficient diet alone(3) and led us to postulate that starch interfered with iron absorption. The present investigation was performed to study this hypothesis and reports the acute and chronic effects of starch ingestion upon iron absorption in rats.

Materials and methods. Male albino rats, Walter Reed Carworth Farms strain, weighing 140 to 200 g were obtained and measurements of iron absorption and kinetics were performed when animals on a normal high protein diet weighed 170 to 200 g. The principles of laboratory animal care as promulgated by the National Society for Medical Research, were observed. The standard laboratory diet contained 25% protein and 9 mg of iron per 100 g dry weight. Special diets were made with starch (Argo), sucrose (USP), casein (USP) and corn oil (Mazola) and were supplemented with an iron-free commercial salt mixture and vitamins. Sufficient ferrous sulfate was added to certain of the prepared diets

to bring the iron content to 9 mg per 100 g. Diets were assayed for iron content by a modification of the method of Lorber(4). Animals were fed special diets for 2 weeks before study.

Iron absorption studies were performed in rats fasted for 16 hours. Oral test doses containing 0.5 μc of ferrous^{59} citrate (Abbott 0.6 μg/mc) and 0.50 mg of iron as commercial ferrous sulfate (Vitarine Corp.) were injected into the stomach through a 17 gauge olive tipped endoesophageal needle. Whole body retention of the radioiron 7 days later was considered equivalent to absorption. Whole body radioactivity was measured in a small animal wholebody liquid scintillation detector (Packard ARMAC), as previously described(5).

Blood for microhematocrit determinations and measurements of the serum iron and iron binding capacity was obtained from the retro-orbital venous plexus with heparinized capillary tubes. The serum iron concentration and the unsaturated iron binding capacity were determined by the "one-tube method" (6).

Plasma clearance studies were performed following the intravenous injection of iron^{59} labeled rat serum for 15 minutes at 37°C before injection of 0.25 ml into the dorsal vein of the penis. The iron binding capacity of the incubated serum was not exceeded. At 10, 20, 30 and 40 minutes following injection, 0.02 ml of whole blood was obtained from the tail vein of the rat with a calibrated hemoglobin pipette. The blood was diluted into 2 ml of water and counted in a well-type crystal scintillation detector (PACKARD Auto Gamma Spectrometer, Model 410 A). The plasma iron clearance (T/2) was determined from the best fitted plot of these points on semilogarithmic graph paper. The whole blood volume was estimated to be 5% of the animal body weight. The plasma volume was calculated from the hematocrit and estimated blood volume. Daily iron turnover was calculated from the serum iron concentration, plasma volume and plasma iron clearance studies(7,8). The incorporation of iron^{59} into red blood cells was determined by measurement of the radioactivity in blood specimens obtained 20 hours after intravenous injection of labeled plasma(8,9). The non-heme iron concentration was measured in homogenates of weighed aliquots of liver(10).

Results. Fasted rats, raised on a normal iron-replete diet, were given 5 ml oral doses of labeled iron (10.5 mg) containing either 1 g of starch, 1 g of sucrose or no additive to ascertain if starch had a direct intraluminal effect upon iron absorption. Normal control animals absorbed 15.6% (SE 1.3%) of the test dose of iron. Rats dosed with the starch

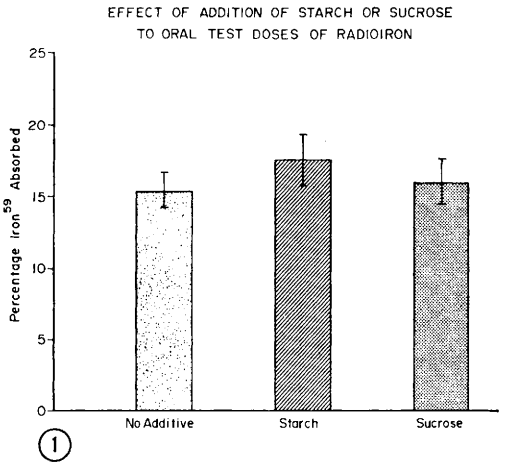

FIG. 1. Addition of either 1 g of starch or sucrose to oral test doses of iron^{59} had no significant effect upon iron absorption.

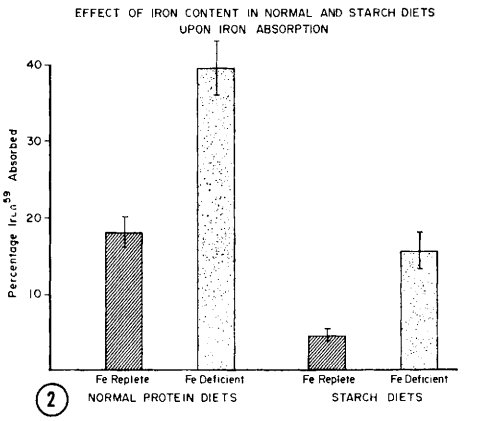

FIG. 2. Rats were fed either a normal high protein or starch diet that contained either 5 or 90 μg of iron per gram of food. Animals ate these diets for 2 weeks before receiving an oral test dose of radioiron. Rats fed an iron replete high protein diet absorbed 18% of the test dose of iron. Iron absorption by animals maintained on other diets was 39% (iron deficient normal diet), 4% (iron replete starch diet) and 15% (iron deficient starch diet).

suspension and the sucrose solution absorbed 18.3% (SE, 2.0%) and 16.4% (SE, 1.8%) of the test dose of radioiron, respectively (Fig. 1).

Rats fed a protein depleted starch diet for 14 days lost body weight (1 g/day); whereas, animals that consumed a normal high protein diet gained 2 to 3 g daily. Comparative studies of iron absorption showed no significant difference between rats fed the iron-replete high protein diet and animals that ate an iron deficient starch diet. However, studies of rats maintained on iron deficient protein replete diet and animals fed a protein deficient diet with iron added showed that protein depletion caused a relative inability to absorb appropriate quantities of iron from oral test doses (Fig. 2).

Rats weighing 150 g were fed diets containing various quantities of protein and either starch or sucrose for 2 weeks before study. Animals fed a high protein diet consumed 15 g of food and gained more than 2.5 g of body weight each day. In contrast, rats fed protein depleted starch or sucrose diets ate only 8 to 9 g of food and lost approximately 1 g of body weight daily. Protein depletion and weight loss were accompanied by significant decreases in iron absorption, red cell incorporation of radioiron, iron turnover and total iron-binding capacity with prolongation of the plasma $iron^{59}$ clearance and an increased serum iron concentration. The addition of small amounts of casein (5 g/100 ml) to the starch diet made it more acceptable for consumption; the rats gained weight (1 g/day), absorbed 7.5% of the oral test dose of iron and had improvement of iron kinetic studies. To ascertain if this partial correction of iron metabolism was caused by weight gain or dietary protein, rats were fed limited quantities of a normal high protein diet (7.5 g/day). These animals sustained a weight loss comparable to rats on a protein depleted starch or sucrose diet. However, iron absorption and kinetic studies were similar to those observed in rats gaining weight on a 5% casein diet. (Table I).

Discussion. The normal rat grows continuously throughout life. Fed a normal high protein diet, these animals gain 2 to 3 g of body weight and must absorb at least 120 μg of iron daily to maintain a normal body iron concentration of 60 parts per million(11,12). Erythropoiesis keeps pace with growth to maintain a normal hemoglobin concentration in the circulating blood. Conversely, iron absorption and erythropoiesis must be diminished with a retarded growth rate or marked siderosis and polycythemia would occur; normally, iron excretion and hemolysis are limited(9,13,14).

Previous investigation showed that both the rate of erythropoiesis and the quantity of iron in body tissues seem to influence iron absorption(8). Starvation and protein depletion increase the concentration of iron in various body organs and cause marked decreases in the rate of erythropoiesis and iron absorption(5,15,16).

In the rat, starch and sucrose diets seem to produce a combination of the effects of starvation and protein depletion. These animals eat poorly and lose weight to produce relative iron-over loading, a reduction in iron requirements and decreased iron absorption. Experiments with rats fed limited quantities of a high protein diet show enhanced erythropoiesis and absorption of more iron than rats maintained on protein-free food.

In starch eating humans iron overloading can not be a major factor because starch diets are iron deficient and weight loss is not a prominent clinical feature in adult females with this syndrome. However, protein deficiency may contribute to the severity of iron deficiency by decreasing erythropoiesis because of a relative unavailability of essential substrates from dietary protein for hemoglobin synthesis(16).

Summary. Decreased iron absorption was observed in rats fed protein deficient starch and sucrose diets. This was not caused by a direct intraluminal effect of starch or sucrose upon iron absorption. The abnormality was attributed to both a retarded rate of growth and a relative deficiency of protein for hemoglobin synthesis. We postulated that "starch eaters anemia" is caused by a lack of dietary iron and may be complicated by a decreased capability to absorb iron because of protein

TABLE 1. *Effect of Dietary Composition upon Iron Absorption and Kinetics*

		Normal diet, unrestricted intake	Normal diet, unrestricted intake	Normal diet, restricted intake	Starch diet, unrestricted intake	Sucrose diet, unrestricted intake	Starch casein, unrestricted intake
Additives to diet (g/100 g)	Casein	27	27	27			5
	Starch	60		60	86	86	81
	Sucrose		60				
	Corn oil	10	10	10	10	10	10
Dietary composition (g/100 g)	Protein	25	25	25	1	0	5
	Chos.	53	55	53	85	86	71
	Fat	10	10	10	10	10	10
	Iron	.009	.009	.009	.009	.009	.009
Food consumption (g/day/rat)		15.2	15.4	7.5	8.3	8.7	11.6
Mean wt of rats (g)		188	184	134	136	131	167
Iron absorption (%)		17.5 (2.1)	18.3 (1.8)	6.6 (1.2)	2.7 (.3)	2.2 (.4)	7.6 (1.3)
RBC incorporation of radioiron (%)		41 (1.8)	40 (2.4)	24 (2.9)	5 (.4)	6 (.5)	28 (1.7)
Serum iron (μg/100 ml)		128 (8.2)	119 (5.6)	149 (12.7)	168 (11.4)	175 (14.3)	142 (9.7)
Total iron binding capacity (μg/100 ml)		472 (18.9)	465 (20.4)	418 (13.4)	382 (14.3)	376 (17.3)	411 (12.0)
Plasma iron^{59} clearance (T/2)		67 (5.1)		89 (8.2)	122 (8.9)		94 (7.2)
Daily iron turnover (μg)		103 (8.3)		65 (5.1)	54 (4.9)		73 (7.8)
Liver iron concentration (μg/g)		105 (8.7)		173 (10.7)	270 (12.6)		158 (14.2)

deprivation and decreased erythropoiesis.

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Light Induced Exophthalmos in the Domestic Fowl.*† (32432)

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Eye enlargement in chickens exposed to continuous light during the growing period has been described(2,3,4). This condition has been characterized by elongation of the eyelids, increased size and weight of the eye (mainly the vitrous body), decreased lens size, and decreased corneal curvature. Pathologically, this condition has shown an increase in the number of eye lesions and a subsequent blindness at arount 2 years after commencement of treatment. Histological examination of the enlarged eyes revealed a thinning of the nerve fiber and rod and cone layers of the retina. Retinal damage has also been found in rats exposed to short periods of excessive light(1,5,6).

In this laboratory, an eye enlargement was also observed in birds subjected to low intensity light (0.01 foot-candles) by Lauber (unpublished). This eye abnormality has the feature of exophthalmia as opposed to the buphthalmia produced in continuous "bright" light (1-10 foot-candles). This paper is concerned with changes occurring in the eyes of birds exposed to low intensity specific spectra light.

Materials and methods. Day-old White Leghorn chicks were randomly assigned to 4 light-control pens. Feed and water were supplied *ad libitum*. Feed used was chick starter for 8 weeks, then chick developer for 10 weeks, and finally, a breeder hen diet for the remainder of the experiment.

Pens were assigned light treatments of clear (controls), blue, green, and red light. Clear light was supplied by plexiglass $CuSO_4$ filtered incandescent light. The colored light was produced by filtering incandescent light through a $CuSO_4$ bath and filters obtained from the Carolina Biological Supply House. These filters transmit narrow, non-overlapping spectra of equivalent energy (microwatts/cm^2/sec.). The blue, green, and red filtered light had a maximum intensity of .006-.009 microwatts/cm^2/millimicron, while the intensity of the clear filtered light was around 0.96 microwatts/cm^2/millimicron, which corresponds to an intensity of 5.5 foot-candles.

At 20 weeks of age, half the birds from each light treatment were transferred to normal

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