ment, antagonize or not alter the chick comb and rat accessory sex organ growth stimulation induced by a more potent androgen such as testosterone. Augmentation or antagonism may result depending on the dose relationship between the weaker and stronger androgens. In the chick comb studies the administration of relatively large amounts of the weak androgens induced an augmentation or additive effect when the quantity of the more potent androgen was relatively small. When the stronger androgen was administered in relatively large dosages the weaker androgens inhibited the growth response, probably by competitive action at the reactive tissue level.

Summary. A-nortestosterone and C-nor-D-homo-17α-epitestosterone acetate were very weak androgens in the chick comb assay. In addition A-nortestosterone was weakly androgenic in the immature castrate rat assay. Both compounds antagonized testosterone-induced chick comb growth, the antagonism being more pronounced when the dose of testosterone was relatively large. At the doses employed C-nor-D-homo-17α-epitestosterone acetate was anti-androgenic in the immature castrate male rat while A-nortestosterone was not.

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Role of Gastric Secretion in Iron Absorption.* (29081)

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The search for the controlling mechanism in iron absorption has been a long one. Iron is poorly excreted and poorly absorbed. Since ionic iron in high concentration is injurious to cells, it seems reasonable to suspect that prior to absorption from the stomach or intestine, the iron might be coupled to some compound. Moreover, since the presence of anemia promotes iron absorption, it might be assumed that increased amounts of such a substance would be found in anemic animals. A study of the kinetics of iron absorption in normal dogs also suggested the possibility of some substance in limited amounts which controls iron absorption(1). There is clinical evidence that gastrectomy reduces the ability to absorb iron. Hobbs has reported that iron deficiency anemia developed in half of the male patients and nearly all of the female patients who underwent gastrectomy, either subtotal or total(2). In only about one-third of the 247 patients studied was there evidence of possible blood loss or low dietary iron intake. Defective iron absorption was presumed to have accounted for anemia in the remaining patients.

The experiments reported here provide evidence that the gastric juice of anemic dogs contains a substance that increases iron absorption.

Methods. A. *In vivo experiments.* Mongrel dogs, both male and female, weighing 10-15 kg were made anemic by repeated phlebotomy (6 dogs), or by repeated injections of acetylphenylhydrazine (one dog). The iron deficient dogs were maintained on an iron

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deficient diet. The dog with hemolytic anemia was maintained on a commercial dog food. After 2 to 4 months (during which time hemoglobin levels were maintained below 10 g/100 ml) the following experiment was carried out. Fifty milligrams of ferric chloride solution, tagged with approximately 250 μc of $Fe^{59}Cl_3$ of high specific activity, was given via gastric tube to the "donor" dog. After 10 minutes, the stomach was aspirated as completely as possible. An aliquot of the aspirated juice was taken for a standard, and the remaining material was given via gastric tube to a normal non-anemia "recipient" dog. Whole blood samples were drawn at one, two and three weeks and the percent of the ingested dose or iron incorporated into circulating erythrocytes was determined. All samples were electroplated and counted in a gas-flow detector fitted with a Mylar window of less than 150 $\mu g/cm^2$ average density. Appropriate control experiments were carried out in a similar fashion differing only in that the donor dog was non-anemic.

A single experiment was done in which the dose of oral iron tagged with $Fe^{59}Cl_3$ was given with porcine intrinsic factor (Lilly). The control experiment utilized a similar dose of oral iron given to the same dog, but without intrinsic factor.

B. *In vitro experiments.* The binding of iron by gastric juice (or a fraction thereof) was investigated in two ways. In several experiments the percent of radioactive iron precipitated by trichloracetic acid (30% w/v) was measured by adding one milliliter of trichloracetic acid to one milliliter of crude gastric aspirate. The aspirates were obtained 10 minutes after tagged iron was given via stomach tube.

The gastric aspirate was also examined by fractionation on Sephadex G-25 gel. Two grams of gel, suspended in 0.07 M phosphate buffer (pH 7.35) were poured into a 10 mm $\times$ 30 cm chromatography column. Flow rates of about 0.5 ml per minute were obtained. The void volume of the columns varied around 6 ml. Corollary studies were done in which porcine intrinsic factor tagged with both $Fe^{59}Cl_3$ and $Co^{57}B_{12}$ was chromatographed on a similar Sephadex G-25 column.

TABLE I. Red Cell Utilization of Oral Iron.

No.	Control %	Exp %	Diff	Control %/ Exp %
1	17.9	24.9	+ 7.1	.71
2	7.2	33.1	+25.9	.22
3	8.6	15.4	+ 6.8	.56
4	2.9	6.9	+ 4.0	.42
5	1.0	8.3	+ 7.3	.12
6	24.6	44.8	+20.2	.55
7*	20.1	27.9	+ 7.8	.72
t_6			3.77	6.58
p			<.01	<.01

* Hemolytic anemia.

TABLE II. Trichloracetic Acid Precipitation of Crude Gastric Juice.

Gastric juice samples	pH	Bound radioactivity (%)
Normal dogs	1.72	1
	1.44	1
	1.28	4
	1.35	1
Anemic "	2.15	42
	1.93	19
	1.82	18

Results. The amounts of iron absorbed as determined by the erythrocyte utilization of iron are shown in Table I. In each set of experiments there was a significant rise in absorption of iron from the control level to that absorbed with gastric juice from anemic dogs. Statistical analysis of the data revealed the changes to be significant at the 1% level. The analysis shows that the differences themselves as well as the ratio of "control percent utilization" to "experimental percent utilization" were highly significant.†

Table II shows the percentages of "bound" iron in several gastric juice samples from normal and also anemic animals. Increased binding of iron to material precipitated by trichloracetic acid was evident in the anemic gastric juice.

Fractionation of the gastric juice was carried out on Sephadex G-25. The large molecular (c 4500 molecular weight) fraction which was found seems to bind iron preferen-

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tially. The specific activity of this fraction was approximately 14 times that of a second well defined fraction. Further studies to characterize more adequately and identify these fractions are necessary.

Fractionation of porcine intrinsic factor was also done on Sephadex G-25. The iron-binding fraction was again defined and was detected at a volume similar to that noted in the gastric juice fractionations. On the other hand two B_{12} binding proteins were found. Chosy and Shilling also noted 2 binding proteins for this vitamin, the second fraction having a higher specific activity and apparently representing intrinsic factor(3).

Discussion. The experiments provide evidence for a factor in gastric juice from anemic dogs which significantly increases the absorption of iron from the gastrointestinal tract. In each pair of experiments this increase was apparent.

The fact that gastrectomy is associated with pernicious anemia as well as iron deficiency anemia leads us to postulate that the mechanism of iron absorption might well be similar to that of Vit. B_{12}. Thus, it is hypothesized that the gastric mucosa secretes a factor which combines with iron and renders it "absorbable" by the mucosa of the gastrointestinal tract. A single pair of experiments has been done to test the effect of porcine intrinsic factor (Lilly) on iron absorption. The control iron utilization was 16.4%. The experimental utilization with intrinsic factor was 27.9%. However, Sephadex fractionation of intrinsic factor tagged with both $Co^{57}B_{12}$ and $Fe^{59}Cl_3$ indicates that the iron-binding factor is different from B_{12} binding factor.

Preliminary studies indicate that the iron-binding factor of gastric juice has an appreciable molecular weight (c 4500). It is most likely a protein since absorption at 280 mμ was appreciable. Histochemical studies on the fractions in question further suggest that the factor is a mucoprotein or glycoprotein.

If, indeed, the proposed mechanism of iron absorption is correct, it would nicely explain many of the problems of iron absorption. The comparatively small percentage of iron normally absorbed could be due to secretions of limited amounts of this factor. In anemia, increased amounts of this substance may be secreted and thus larger amounts of iron could be absorbed. In non-iron-deficient anemias increased amounts of this factor would also be produced and, hence, iron absorption enhanced. The common clinical finding of iron-deficiency anemia following gastrectomy would also be easily explained if this hypothesis is correct.

Summary. Experimental evidence is presented for the existence of a substance in gastric juice from anemic dogs which facilitates iron absorption from the gastro-intestinal tract of normal dogs. These data would be consistent with the theory that iron may be absorbed in a manner similar to Vit. B_{12}. An intrinsic substance, secreted by the stomach, may be necessary for absorption of iron.

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