Gastrectomy and Iron Absorption: Effects of Bleeding, Iron Loading and Ascorbic Acid in Rats. (31796)

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The incidence of iron deficiency anemia following gastrectomy led to numerous studies which failed to demonstrate significant intestinal blood loss from most anemic gastrectomized patients. Iron depletion was attributed to inadequate absorption. However, investigators showed normal absorption from test doses of iron salts and believed that the iron deficiency was caused by poor absorption of food iron(1-8).

Like man, gastrectomized rats develop a microcytic hypochromic anemia which responds to parenteral injections of large oral doses of iron. Whitehead and Bannerman showed that anemic gastrectomized and normal rats absorb similar quantities of iron(9). The etiology of iron deficiency in gastrectomized rats is the subject of this study.

Methods. Male albino rats of the WRCF strain, weighing 200 to 250 g, were used. Rats were housed in galvanized wire cages and fed a standard rat and mouse diet containing 15 mg of iron per 100 g of dry weight. It was estimated that rats consumed about 2 mg of iron daily on this diet(10). One-half the rats in this study were gastrectomized one month before iron absorption studies were initiated. Surgery was performed through a midline ventral incision; the stomach was excised and an end to end esophagoduodenostomy was constructed. Forty-eight rats of comparable weight were used as normal control animals. Rats were iron-loaded by intramuscular injection of 25 mg of iron as dextran-iron (Imferon), 7 to 14 days before iron absorption studies. Hematocrits were measured by the microhematocrit technique, the mean value for each group was greater than 36% and no selection was made upon this determination. Fecal collections were tested for hemoglobin by the benzidine method(11).

Iron absorption studies were performed by administration of test doses of radioiron through an olive-tipped 17 gauge endoesophageal tube. Test solutions contained 0.25 μc of ferric^{59} chloride and 0.25 mg of elemental iron as ferrous sulfate, brought to a volume of 1 ml with iron free distilled water. The pH of test doses was brought to 3.7 $\pm$ 0.1 by addition of either HCl or NaOH. Under these conditions most of the tracer dose is maintained in the ferrous state(12). Animals were fasted overnight and for 4 hours after dosing. Absorption of radioiron was measured with a small animal whole-body liquid scintillation detector (Packard ARMAC). Rats were placed in vented quart ice cream cartons and whole-body radioactivity was measured 3 hours and 7 days after dosing. Reference standards were prepared by adding a test dose of radioiron to 250 ml of water filled plastic bottles. The radioactivity in animals and standards was measured for two 100-second periods. Absorption of radioiron from test doses was calculated by the ratio of net counts 7 days after dosing (r_x) to those of the standard (s_x) and a similar ratio at 3 hours (r_o/s_o)(13).

$$\frac{r_x/s_x \times 100}{r_o/s_o} = \text{percentage absorbed}$$

Results. Gastrectomized rats developed an anemia which responded to parenteral doses of iron (Fig. 1). Untreated, the anemia becomes severe(9). Negative benzidine tests on fecal collections from 30 of 32 animals indicated that the anemia was not caused primarily by intestinal bleeding.

Unbled animals (Fig. 1). Normal animals absorbed significantly more radioiron from test doses of ferrous sulfate than gastrectomized rats (13 $\pm$ 3.8 *vs* 6.9 $\pm$ 2.4%; $p < 0.01$). Addition of 10 mg of ascorbic acid to test doses increased the amount of iron^{59} absorbed by both groups of animals but the differences became insignificant (17.5 $\pm$ 8.2% *vs* 12.7 $\pm$ 5.9%). Iron-loaded gastrectomized

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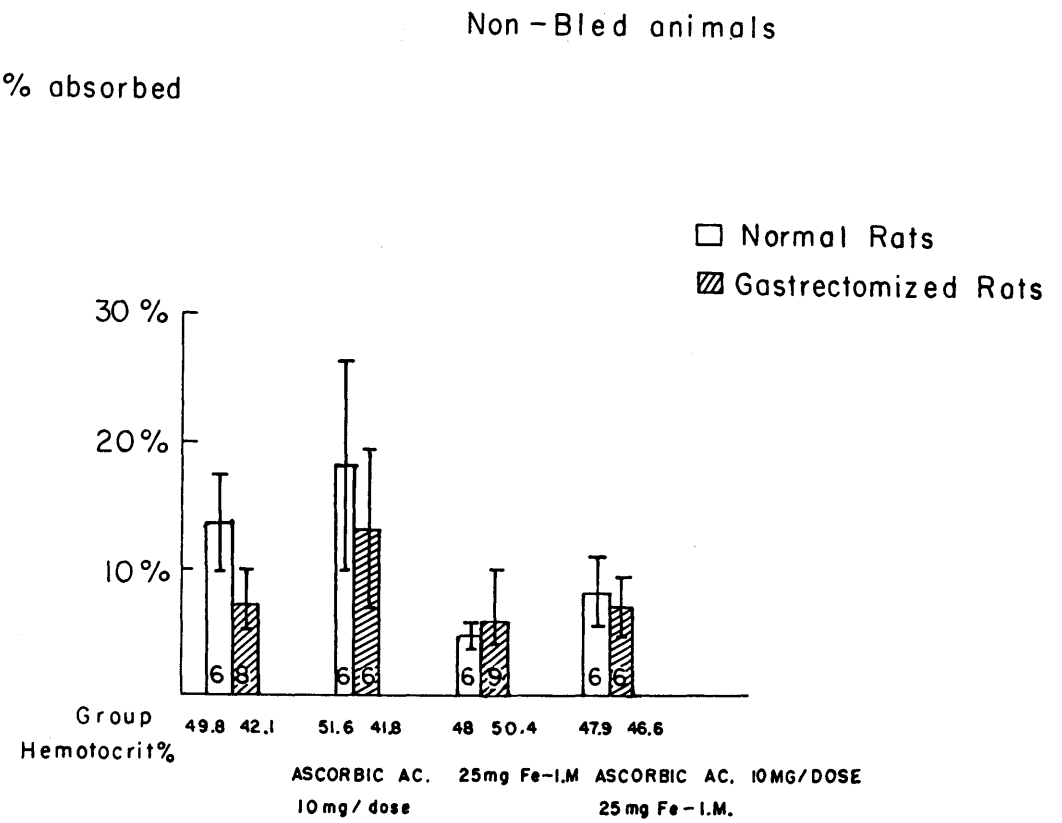

FIG. 1. Iron absorption in non-bled rats. Central bar indicates 1 standard deviation. Figure in columns shows number of animals in each experiment.

rats absorbed approximately the same amount of iron[59] from test doses as normal animals that received intramuscular injections of Imferon (5.6 ± 3.7% *vs* 4.2 ± 1.0%). Likewise, if iron-loaded rats were given test doses of radioiron containing ascorbic acid, both intact and gastrectomized rats absorbed similar quantities of iron (7.9 ± 2.6% *vs* 6.8 ± 2.2%).

Bled animals (Fig. 2). When similar studies were performed in rats that were bled and fed a milk diet, marked differences in iron absorption were observed between all groups of gastrectomized and control animals. Intact rats absorbed 59.1 ± 12.1% *vs* 17.7 ± 3.8% in the gastrectomized animals ($p<0.001$). Similar differences were observed if ascorbic acid was added to the test dose (55.1 ± 13.6% *vs* 19.8 ± 2%), if the animals had been iron-loaded before bleeding (57.5 ± 12.7% *vs* 12.4 ± 4.3%) or if the iron-loaded-phlebotomized rats were given oral doses of iron containing ascorbic acid (30.4 ± 14.1% *vs* 9.2 ± 5.1%). Even iron-loaded animals that were bled only 2.5 ml absorbed significantly greater amounts of iron[59] from test doses than similarly treated gastrectomized rats (24.4 ± 6.0% *vs* 7.1 ± 2.4%).

Discussion. The iron content of the mammalian body is small, about 60 parts per million(14). The concentration must remain within narrow limits to prevent the development of either siderosis or iron deficiency. The body's store of iron is primarily maintained by controlled absorption; the intestine responds to the body's requirement by increasing or decreasing the amount absorbed(10,15).

Like man, rats develop iron deficiency anemia after gastrectomy(1-8). Although previous studies showed no difference in the absorption of iron from test doses between normal and gastrectomized rats(9), our observations indicated that iron absorption was significantly decreased following gastrectomy. Dif-

ferences in the absorption of iron became statistically insignificant with the addition of ascorbic acid or after both groups of animals were iron-overloaded by the injection of Imferon. Contrariwise, bleeding the rats before measuring iron absorption accentuated differences between gastrectomized and intact animals. Loss of the stomach made rats incapable of responding appropriately to an increased requirement for iron.

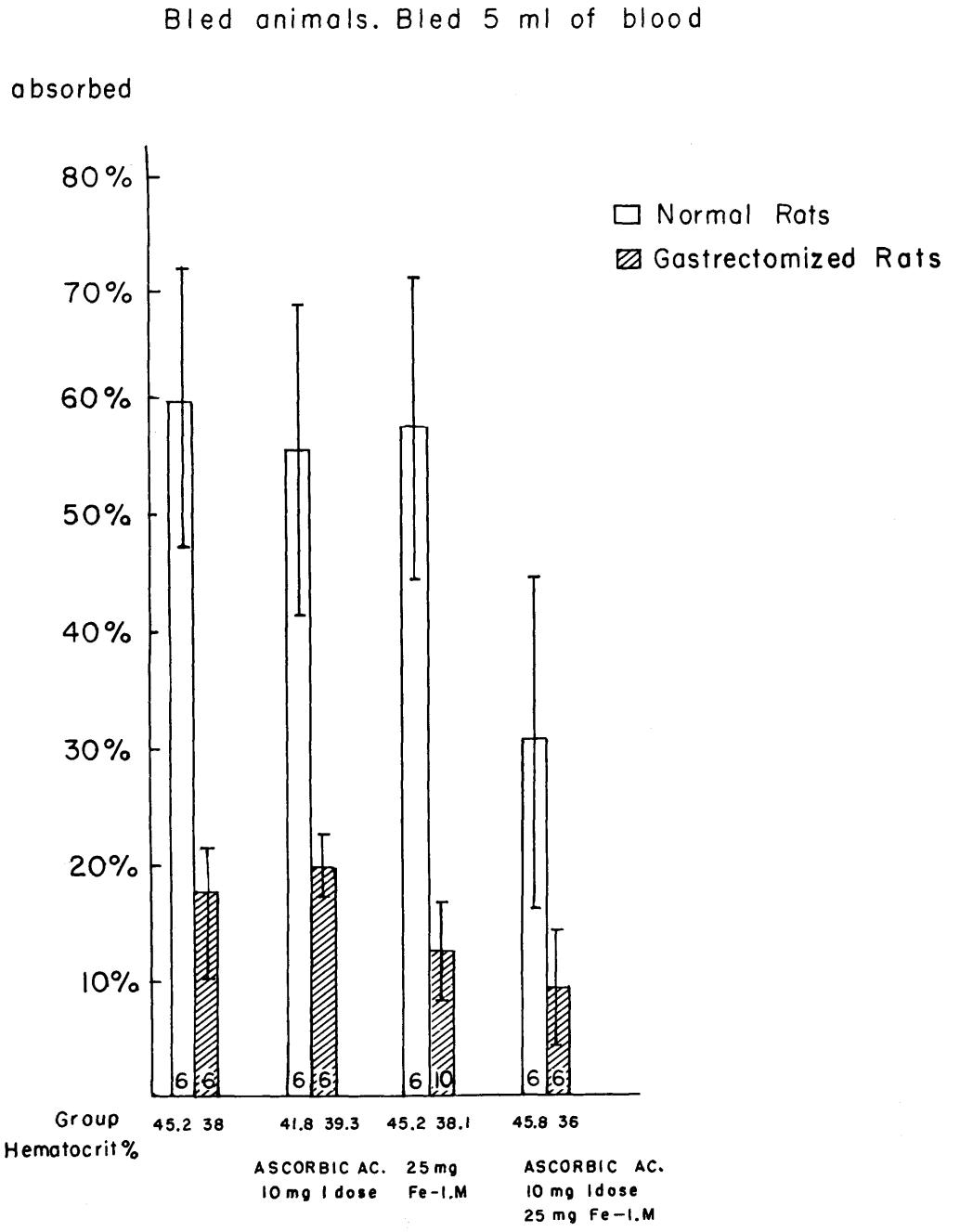

FIG. 2. Iron absorption in bled rats. Central bar indicates 1 standard deviation. Figure in columns shows number of animals in each experiment.

Summary. Gastrectomized rats developed iron deficiency because of decreased absorption of iron. Although these animals absorbed almost normal quantities of iron from test doses when they were iron replete, they were unable to increase absorption of iron appropriately in response to iron depletion. This suggested that iron deficiency occurred after gastrectomy because of an inability to adjust absorption in response to changes in the body requirement.

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Studies on Serological Relationships Between Strains of Adenovirus Types 3 and 7.* (31797)

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Adenovirus types 3 and 7 have many similar biological properties, but appear to be serologically unrelated when tested by neutralization or hemagglutination-inhibition (HI). Rowe *et al*(1,2) reported no cross-reactivity when types 3 and 7 were tested by neutralization with hyperimmune rabbit sera. Studies of reciprocal HI tests with rabbit antisera(3,4) substantiated the absence of serological relationship between the 2 types.

Types 3 and 7 have several biological properties in common including; similar neutralization rate kinetics with homotypic antiserum(5), quantity of infectious virus produced(5), length of latent period prior to multiplication(5), hemagglutination reaction (6), and type of cytopathic effect (CPE) elicited in monkey kidney tissue culture(7). Perhaps the single most important biological factor in which these 2 types resemble one another is the ability to cause tumors in newborn hamsters which contain indistinguishable "T" antigens(8).

With few exceptions the serological studies described above were carried out with prototype strains and antisera prepared to the same strains. In an attempt to better elucidate the serological relationship between strains of a given type and cross-reactions between types 3 and 7 we have studied 25 strains and their respective rabbit antisera by both neutralization and HI tests.

Methods and materials. Viruses. Strains were obtained from various sources. Most were obtained after relatively few passages; however, others had been carried extensively in the laboratory. Biographies of viruses used in this study are found in Table I.

Neutralization tests. Neutralization tests

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