trophils and on the total plasma ascorbic acid in the blood of the rat, indicates that the amount of total plasma ascorbic acid rises temporarily in these animals following this type of stress and that it is a reliable index of the severity of such stress and possibly of ACTH production following such stimulation.

1. Selye, H., *Canad. M. A. J.*, 1944, v50, 426.
2. Stewart, C. P., Horn, D. B., and Robson, J. S., *Biochem. J.*, 1953, v53, 254.
3. Goldie, H., Jones, A. M., Ryan, H., and Simpson, M., *Science*, 1954, v119, 353.
4. Consolazio, C. F., Johnson, R. E., and Marek, E., *Metabolic Methods*, 1st ed., 1951, C. V. Mosby, Saint Louis, pp. 198-199.
5. Dougherty, T. F., and White, A., *Endocrinology*, 1944, v35, 1.

Received June 10, 1955. P.S.E.B.M., 1955, v90.

Effect of Intrinsic Factor Concentrate on Vit. B_{12} Absorption by Gastrectomized Rats. (22009)

BACON F. CHOW, JULIAN K. QUATTLEBAUM, JR., AND CHARLES ROSENBLUM.

From the Departments of Surgery, The Medical School, of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., and Research Laboratories, Merck & Co., Rahway, N. J.

A recent attempt(1) to use normal rats to test the potency of an intrinsic factor preparation revealed only an inhibitory effect on the absorption of oral vit. B_{12} by this test animal. Normal rats had been employed for this study which involved doses of 4.2 and 0.42 μg of radioactive vitamin labeled with cobalt 60.

Normal rats do not develop a macrocytic anemia comparable to pernicious anemia, and may thus be considered *a priori* unsuitable for the evaluation of intrinsic factor potency. In humans, however, gastrectomized individuals (2,3) have been employed successfully to demonstrate intrinsic factor despite the absence of anemia or megaloblastic marrow. This suggested the possibility that gastrectomized rats would serve as appropriate test animals. Accordingly studies were undertaken to examine the effect of potent intrinsic factor concentrates upon the extent of absorption of radioactive vit. B_{12} by gastrectomized rats. Furthermore, physiological doses of 200 mμg and 50 mμg of vitamin, and 2 different intrinsic factor concentrates, were investigated.

Methods. Adult rats of different strains weighing 200-300 g were used. Upon open ether anesthesia total gastrectomy was performed utilizing an end to end esophagoduodenostomy to restore intestinal continuity. Fine silk was used for sutures and ligatures throughout. Total removal of the stomach was verified in each instance by histological preparation of a saggital section through the removed organ. In general at least 10 days were allowed for recovery from the operation before transfer of the animals to the nutrition laboratory. During the first 10 days after operation, the animals were fed on a milk and bread diet and lost about 10% of pre-operative body weight. Subsequently, they were offered a basal soybean diet, and during test periods were given 10 g of this diet daily. *Materials.* Two commercially available intrinsic factor concentrates were employed. Preparation I was used only in preliminary study (Series A) with low specific activity vit. B_{12}-Co^{60}. Its clinical potency was not retested. Preparation II was of proven potency at 40 mg, as tested hematologically and by the Schilling method(4), and at 20 mg by the latter procedure. Its binding power was determined by the tracer-dialysis method(5) to be 250 mμg/mg. The vit. B_{12} labeled with Co^{60} employed in the preliminary study had a specific activity of $\simeq$ 55 μc/mg; and the activity of the second preparation, used in 3 additional definitive studies, was $\simeq$ 200 μc/mg.

Method. Four series of experiments (A, B, C and D) were performed in such manner

TABLE I. Summary of Fecal Excretion Results—Gastrectomized Rats vs Controls.

Series	mμg B_{12}	mg IF	Phase	Duration (days)	No. of animals Gastrectomized	No. of animals Control	Group avg % of dose excreted Gastrectomized	Group avg % of dose excreted Controls
A	200	0	I	5	1	2	107	71 ± 6
		10	II	4	1	2	109	95 ± 3
B	200	0	I	7	4	4	102.0 ± 5.8	84.9 ± 5.1
		3	II	2	4	4	96.4 ± 2.6	>82.9 ± 6.4
C	50	0	I	8	4	4	101.7 ± 4.1	62.4 ± 14.5
		15	II	4	4	4	100.5 ± 2.9	97.5 ± 2.8
D	50	0	I	6	6	6	99.4 ± 5.0	50.0 ± 13.6
		.2	II & III	6	5*	6	104.6 ± 3.0	71.0 ± 17.2
		1	III & II	6	4*	6	99.9 ± 4.7	98.6 ± 3.5

* One animal was found dead before conclusion of this phase.

that each rat served as its own control. After a 24-hour basal collection period, each animal received a 50-200 mμg of Co^{60} vit. B_{12} by stomach syringe, and feces collected daily until fecal radioactivity was negligible (Phase I). Then the same dose of tracer was administered simultaneously with a fixed amount of intrinsic factor concentrate, and collections continued as before (Phase II). In one series (D) a third phase involving administration of additional intrinsic factor was introduced. In this series, control and gastrectomized rats were each divided into 2 groups for Phase II, one group receiving 0.2 mg of concentrate, the second group receiving 1 mg. During Phase III, these dosages were reversed. In Series A, animals were fed milk to flush out their bowels at the conclusion of Phase I. Radioactivity measurements by gamma ray scintillation counting(1,5) were performed on feces homogenates in water suspension. Although stools were collected daily, several daily collections were combined for measurement in certain instances. Excretion was essentially complete in 3-4 days. No radiometric examination was made of rat organs.

Results of excretion measurements were calculated as "% of Dose." A large value signifies negligible absorption, while a low excretion indicates absorption. Basal periods are not reported since activities were zero. Basal corrections for Phases II and III were based upon the average activity (usually negligible) excreted for the 2 days immediately preceding these phases.

Group average excretion values (± average deviation from the mean) are compiled in Table I for each of the 4 series. Also shown are the doses of vit. B_{12} and intrinsic factor administered, the duration of each phase and the number of animals employed. Series D excretions listed for phases II and III actually represent averages for 0.2 and 1.0 mg intrinsic factor fed in both phases.

Discussion. In none of the 4 series did the gastrectomized rats absorb significant amounts of vit. B_{12} within our experimental error, regardless of the presence of intrinsic factor; and in Series A, C and D, intrinsic factor reduced absorption by the control rats to essentially zero. This inhibitory effect of intrinsic factor is not shown in Series B control animals, in part because fecal collections during the second phase (plus intrinsic factor) was discontinued after the second day. Undoubtedly further excretion of radioactivity would have been noted had the experiment been continued. Furthermore, it is questionable whether complete inhibition could be attained in this experiment in view of the low ratio (3.75) of intrinsic factor binding capacity to B_{12} dose.

Series C and D were performed with 50 mμg of radioactive vitamin since the percentage absorption would be greater than at the 200 mμg level, and because 50 mμg represents a physiological dose equivalent weight-wise to 10-12 μg per adult human. In these experiments it was established unequivocally that gastrectomized rats absorb only a negligible fraction of the oral vitamin, whether or not intrinsic factor is administered, and that intrinsic factor inhibits absorption of the vitamin by the controls.

In Series C, the quantity of intrinsic factor concentrate employed (15 mg) was disproportionately large, but was reduced in Series D to 1.0 mg and 0.2 mg, the latter amount being calculated just to bind completely the oral vit. B_{12} dose (*i.e.* binding power 250 mμg/mg or 50 mμg/0.2 mg). Complete inhibition of absorption occurs even with 1 mg of concentrate; and at 0.2 mg inhibition is still evident though to a reduced extent. It appears that complete inhibition of vit. B_{12} absorption occurs when the ratio (IF binding capacity)/(vit. B_{12} dose) is approximately 5. Until such an excess capacity is established, inhibition increases gradually with increasing quantity of intrinsic factor.

Obviously, the rat, gastrectomized or normal, is not a suitable animal for testing intrinsic factor activity, which causes only a diminished absorption in normal rats and which has no effect on gastrectomized animals. It is possible that the rat can utilize only endogenous intrinsic factor and that intrinsic factor of foreign origin is ineffective and inhibitory. The behavior of the gastrectomized animals may also indicate that the stomach is the major seat of vit. B_{12} absorption by the rat. These points are to be tested by experiments with rat stomach concentrate. If this concentrate proves to be effective, it will demonstrate species specificity in the action of intrinsic factor.

The livers of gastrectomized rats contain an abnormally low vit. B_{12} content, as would be expected from their inability to absorb appreciable quantities of the vitamin orally.

On a weight basis, the rat appears capable of absorbing greater quantities than the human. Thus, in the absence of intrinsic factor, absorption from an oral dose of 50 mμg amounts to 20-25 mμg; and from 200 mμg, absorption is 30 mμg. The equivalent dose figures for a 60 kg human would be 10 μg and 40 μg, and the corresponding absorption would amount to 4-6 μg. Actually the human absorbs(6) only 1.5 μg from such oral levels.

Summary. 1. Gastrectomized rats and appropriate control animals were fed 50-200 mμg cobalt-60 labeled vit. B_{12} with and without intrinsic factor concentrates. Absorption was determined by scintillation counting of feces homogenates. 2. No absorption of vit. B_{12} by the gastrectomized animals was noted. 3. Absorption by control animals was inhibited by intrinsic factor concentrate, the extent of inhibition increasing with the quantity of concentrate administered.

The authors are grateful to Miss Hazel E. DeLisle of the Research Laboratories, Merck & Co., Inc. for her part in the performance of the radioactivity measurements.

1. Rosenblum, C., Woodbury, D. T., Gilfillan, E. W., and Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 268.

2. Swenseid, M. E., Halsted, J. A., and Libby, R. L., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 226.

3. Halsted, J. A., Gasster, M., and Drenick, E. J., *New England J. Med.*, 1954, v251, 161.

4. Schilling, R. F., *J. Lab. Clin. Med.*, 1953, v42, 860.

5. Rosenblum, C., Woodbury, D. T., and Reisner, E. H., Jr., *Proc. Second Radioisotope Conference,* Oxford, July 19-23, 1954, Butterworth Scientific Publications, London, 1954, v1, p287.

6. Glass, G. J. B., Boyd, L. J., and Stephanson, L., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 522.

Received June 28, 1955. P.S.E.B.M., 1955, v90.