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Received March 15, 1962. P.S.E.B.M., 1962, v110.

The Site of Vitamin B_{12}-Intrinsic Factor Complex "Releasing Factor" Activity in the Rat Small Intestine. (27502)

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It has recently been reported(1) that an aqueous extract prepared from rat whole small intestine will break the bond between much of the radioactive vit. B_{12} bound to rat stomach extract rendering the vitamin dialyzable. However, less of the radioactivity bound to human gastric juice, and none of that bound to a hog intrinsic factor concentrate was rendered dialyzable during incubation at pH 7 and 37° C. The effect was minimal below pH 4.0. Subsequently, it was determined (2) that small intestinal extract prepared from human ileum mucosa would release vit. B_{12} preferentially from human intrinsic factor at pH 7.4. It was speculated that this apparently species-related "releasing factor" may play a role in the physiologic mechanism for absorption of vit. B_{12}, by releasing vit B_{12} from intrinsic factor at or within the intestinal mucosal wall.

A seemingly less species-related intestinal extract "releasing factor" has also been found by others(3) in homogenates obtained from rat small intestine. This factor was equally active in splitting the bond between vit. B_{12} and intrinsic factor preparations from human, rat or hog sources. It is possible that the apparent lack of specificity of this particular rat small intestine homogenate was due to its concentration being so high that species specificity might have been obscured. Still other workers(4) have demonstrated that extracts from human small intestine, kidney, liver, thyroid, heart muscle, brain, spleen and lung were all capable of splitting off vit. B_{12} previously bound to human gastric juice, serum or plasma. The identity or lack thereof of these various releasing factors remains to be determined.

The present study was undertaken to define the site of releasing factor activity in the rat small intestine by means of extracts prepared by our previous method(1).

Materials and Methods. Adult Sprague-Dawley rats were sacrificed. The pylorus was transected and the small intestine washed through with 0.9% NaCl. It was then stripped free from its mesenteric attachment, cut into 10 pieces of approximately equal length, each of which was put into a separate beaker containing 10 ml of 0.9% NaCl. The contents of each beaker were minced, homogenized for 50 seconds in a Waring blendor and centrifuged for 10 minutes at 3,000 r.p.m. The supernatant fluid was then aspirated and frozen at −17° until used as a source of "releasing factor."

Rat intrinsic factor concentrate (RIFC) was prepared as previously described(1). Two ml of RIFC resulted from each stomach. The

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TABLE I. "Releasing Factor" Activity of Homogenate Supernates Prepared from Different Portions of Rat Small Intestines.

Portion of small intestine*	Distribution of radioactivity (c/m/ml) after incubation using extracts from: 1 small intestine‡ In bag	1 small intestine‡ In tube†	3 small intestines In bag	3 small intestines In tube†	4 small intestines§ In bag	4 small intestines§ In tube†	1 gastrectomized rat small intestine‡ In bag	1 gastrectomized rat small intestine‡ In tube†
No. 1	244	1077	3,018	7,980	981	5920	137	370
2	2	1203	66	15,220	46	7250	8	580
3	4	1137	66	15,502	50	6820	8	559
4	6	1190	65	15,570	45	6820	7	569
5	5	1136	65	15,032	51	7370	6	542
6	5	1234	61	17,421	46	7420	4	555
7	4	1203	53	15,870	49	7250	4	568
8	3	1250	49	15,390	39	7200	4	575
9	2	1122	65	15,390	45	7370	4	502
10	4	1177	77	18,806	57	6520	7	547
None	6	1150	55	15,610	56	7130	0	548

* Approximately equal portions of 1/10 of small intestine numbered serially from its proximal end.

† All radioactivity was in the test tube prior to incubation.

‡ Counts determined by pulse-height analysis.

§ Incubation carried out in unbuffered 0.85 % NaCl.

NB: Discrepancy in total counts of bag plus tube content in any given experiment is due to radioactive B_{12} trapped in the wall of the dialysis bag itself. This trapping was maximal when diffusion was maximal.

protocol for determining the presence of "releasing factor" activity was essentially that previously described(1) with minor modifications. A knot was tied in one end of a piece of Visking casing, 8 mm in diameter and 20 cm in length. Four ml of Krebs-Ringer Tris pH 7.4 (KRT)(5) was pipetted into the casing and a knot tied in the other end, at a distance of 15 cm. Excess casing beyond the knots was cut away. This casing bag, functioning as a semi-permeable membrane, was folded in half, with an air bubble in each limb, and inserted into a screw-capped test tube containing 0.5 ml of a premixed solution of 0.25 ml RIFC and 0.25 ml of water containing 2,500 $\mu\mu$g radioactive vit. B_{12}† plus an additional 0.25 ml of "releasing factor" (rat small intestine homogenate supernate), plus sufficient KRT to make a total volume of 4 ml. The screw-capped tubes were then rotated end over end at the rate of 10 r.p.m. in an incubator in room light at pH 7.4 and 37°C for 5 hours. After this incubation, the radioactivity of the solutions in the test tube and in the dialysis bag, respectively, were determined in a well-type scintillation detector.

† Co^{57} and Co^{60}, B_{12}, kindly provided by Dr. Elmer Alpert, Merck, Sharp & Dohme Research Laboratories, West Point, Pa.

Results. As indicated in Table I by the gain in radioactivity within the dialysis bag, releasing factor activity appears to be confined to the proximal 10% (portion #1) of the rat small intestine. This was true whether "releasing factor" was prepared from corresponding intestinal segments of 1, 3 or 4 rats or, in one case, from a rat gastrectomized 2 years earlier and whether incubation was carried out in KRT at pH 7.4 or in unbuffered 0.85% NaCl. A homogenate prepared from freshly obtained rat pancreases had no "releasing factor" activity at pH 7.4, Table II. When both dialysis mediums consist of saline, the $Co^{60}B_{12}$ becomes about equally distributed between bag and test tube by the end of 5 hours(1).

Discussion. The present finding that "releasing factor" activity is confined under our

TABLE II. Failure of Rat Pancreas Homogenate Supernate to Provide "Releasing Factor" Activity.

Source of "releasing factor"	Distribution of radioactivity (c/m/ml) after incubation: In bag	In tube
Proximal 10% of 3 rat small intestines	774	5711
3 rat pancreases	147	7587
None	135	7594

experimental conditions to the proximal 10% of the rat small intestine is difficult to reconcile with a physiologic role of "releasing factor" in absorption of vit. B_{12} in the rat. This is so because the major site of adsorption of vit. B_{12} in the rat is not the first portion but rather the middle of the small intestine(6,7, 8,9). However, "releasing factor," like intrinsic factor, might normally be secreted into the lumen of the bowel proximal to the locus of its function in promoting assimilation of intrinsic factor-bound vit. B_{12}. Thus, although vit. B_{12} is normally absorbed in the human ileum, the defective vit. B_{12} assimilation in tropical sprue and idiopathic steatorrhea(10, 11,12) may be related in part to a reduction in "releasing factor" output by the atrophic jejunal mucosa. At any rate, the species-related activity of this "releasing factor" for the rat suggests that it does play a physiologic role, as does the finding of similar species-related "releasing factor" activity in human ileal mucosa extract(2). The fish tapeworm, *Diphyllobothrium latum,* also appears to be a source of a material which will release vit. B_{12} from either human or hog intrinsic factor(13, 14) presumably to the advantage of the parasite. What, if any, relation there may be between this "releasing factor" and that in rat or human small intestine is undetermined.

Summary. Vitamin B_{12} bound to rat intrinsic factor concentrate was made dialyzable by the supernate of the homogenate of only the proximal 10% of the rat small intestine. Since the major site of absorption of vit. B_{12} in the rat is the mid-ileum, the present finding throws doubt upon, but does not exclude, the possible physiologic role of such "releasing factor" in absorption of vit. B_{12} by the rat.

The authors are indebted to Brenda Conti and Nancy Cunneen for technical assistance.

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Received March 28, 1962. P.S.E.B.M., 1962, v110.

Comparative Physiology of Sweat.* (27503)

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Abundant evidence is available that human sweat is hypotonic to plasma(1). The concentrations of sodium and chloride in normal human sweat usually do not exceed 60 meq/l and potassium concentrations are usually between 10 and 15 meq/l. There is little difference in the electrolyte composition of human palmar and human body eccrine sweat(2). Morphologically, the body eccrine and palmar eccrine sweat glands have similar secretory

* These studies were supported by USPHS grant.
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