Effect of Intrinsic Factor on Vit. B_{12} Uptake by Rat Intestine *in vitro*.* (24303)

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Since the original postulation of Castle(1) concerning factors involved in the etiology of pernicious anemia, many clinical studies have been performed to elucidate the mechanism by which the intrinsic factor stimulates intestinal absorption of B_{12}. More recently, the rat has been useful as experimental animal in study of the problem of B_{12} absorption. A number of attempts to simplify the experimental conditions still further by studying segments of intestine *in vitro* have been unsatisfactory(2,3). A recent report indicated a 2-fold stimulation of B_{12} uptake into isolated rat gut by hog intrinsic factor(4).

The present study deals with an application of the everted sac technic(5) to this problem. Rat gastric juice causes a marked stimulation of B_{12} uptake by rat intestine in this preparation.

Methods. Segments of washed intestine were everted, filled with fluid by means of blunt needle attached to syringe, and tied at both ends as previously described(5). The solution on the serosal side (about 1 ml) consisted of Krebs-Henseleit bicarbonate-saline containing 200 mg% glucose. The filled sac was placed in a 50 ml Erlenmeyer flask with 4 ml of bicarbonate-saline containing the following: 1 ml of neutralized gastric juice (pH 7.4), 1 ml containing 0.005 μg Co^{60} labeled B_{12} (1 μc/μg), in 0.15 M NaCl and 2 ml of a solution such that final concentration of ions was that of Krebs-Henseleit bicarbonate-saline and the final concentration of glucose was 200 mg%. The flasks were gassed with 5% CO_2 and 95% O_2 and incubated with shaking for 1 hr at 30 or 37°C. Both incubation temperatures were found satisfactory although somewhat larger B_{12} uptake was observed at 37°C. The sacs were then removed from the flasks and washed carefully with 3 separate 50 ml volumes of saline, taking care to remove any adhering mucus. The sac contents were removed by needle attached to syringe. The tissue was slit open, blotted on filter paper and weighed. Digestion of the tissue was carried out in 2 ml of concentrated H_2SO_4 at 100°C for 30 minutes and the counting performed in a well-type scintillation counter (with a background of about 100 counts/minute).

Results. Table I shows results of experiment in which 2 sacs of everted rat intestine were incubated for 1 hour with B_{12} on the mucosal side, one in the presence of neutralized gastric juice, the other in the presence of boiled gastric juice (10 minutes at 100°C). Gastric juice stimulated B_{12} uptake into the gut wall by a factor of 11-fold compared with the control. Concentration of B_{12} in the intestinal wall (195 cpm/100 mg tissue) was more than twice that in the final mucosal solution (73 cpm/0.1 ml). Despite the fact that both fluid and glucose moved across the full thickness of the tissue, no B_{12} appeared on the serosal side of the intestinal sac. Over 40 such experiments have been performed with similar results, maximum stimulation of B_{12} uptake by rat gastric juice being 25 fold.

The effect of various locations along the small intestine on B_{12} uptake with and without gastric juice was studied in a further series of experiments. Table II shows that the mid-portion of the rat intestine is susceptible to the greatest stimulation of B_{12} uptake in the presence of intrinsic factor.

Experiments were devised to test the possibility that intrinsic factor was adsorbed or bound in some manner to the intestinal epithelium prior to its reaction with Vitamin B_{12}. One such experiment is given in Table III. One sac of rat intestine was incubated with gastric juice for 45 minutes, washed care-

* This work was supported in part by grant from Nat. Inst. of Arthritis and Metabolic Diseases.

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TABLE I. Effect of Intrinsic Factor on B_{12} Uptake by Sacs of Rat Intestine.

	Intrinsic factor (gastric juice) Sac 1	Control (boiled gastric juice) Sac 2
B_{12} in gut wall (cpm/100 mg tissue)	195	17
B_{12} in serosal solution	0	0
Glucose transport (mg/100 mg tissue)	.95	.99
Fluid movement (ml/100 mg tissue)	.09	.15
Tissue wt (mg)	340	406

Initial B_{12} activity on mucosal side = 85 cpm/0.1 ml sol.
Incubation for 1 hr at 37°C.

fully in saline, and reincubated in a B_{12} solution for a further 60 minute period. Very little B_{12} uptake was observed. A second sac was treated similarly except that the second incubation solution contained intrinsic factor as well as B_{12}. A good uptake of B_{12} was obtained in this second segment of intestine. The pair of sacs incubated in saline acted as controls to demonstrate that incubation followed by washing does not alter the behavior of the tissue. A final sac was incubated first in B_{12} and then, following a saline wash, in intrinsic factor. Little uptake was observed.

Discussion. In the experiments with 1 hour incubation times no B_{12} appeared inside the sac (serosal side) although both fluid and glucose moved across the intestinal wall. This behavior is consistent with the time lag between uptake in the epithelium and appearance in the blood and other organs which occurs *in vivo*(6). Apparently an hour or more is required for passage across the epithelium in the rat. Another feature of this study which agrees with *in vivo* observations is the site in the small intestine of B_{12} absorption. In rats given B_{12} by mouth the midportion of the small intestine takes up considerably larger amounts of B_{12} than either high jejunum or low ileum(6,7). The experiment in Table III clearly shows that intrinsic factor was not adsorbed to the intestinal epithelium as one rinse completely removes its activity from the tissue. Only when B_{12} and intrinsic factor are present simultaneously is there uptake of B_{12} by the intestinal wall.

TABLE II. Effect of Intrinsic Factor (Rat Gastric Juice) on B_{12} Uptake by Various Segments along Rat Small Intestine.

Location		Stimulation of B_{12} uptake by intrinsic factor (% above control)*
High jejunum	(Sac 1)	0
Mid "	2	500
Low "	3	550
High ileum	4	1200
Mid "	5	20
Low "	6	0

* At each location an adjacent sac was used as control, 1 ml 0.9% NaCl substituted for 1 ml gastric juice. Incubation 1 hr at 30°C.

Summary. Sacs of everted small intestine of the rat incubated in bicarbonate-saline containing glucose and B_{12} showed only a very small B_{12} uptake in the absence of a source of intrinsic factor. In the presence of neutralized rat gastric juice the stimulation of B_{12} uptake was 10- to 25-fold. No B_{12} appeared on the serosal side of the preparation. Stimulation of B_{12} uptake was most marked in segments of intestine taken from the midportion of the small intestine. Evidence is presented that neither B_{12} nor intrinsic factor when added alone are adsorbed to the intestinal wall but must be present simultaneously to give B_{12} uptake.

TABLE III. Effect of Prior Incubation of Intestine in Intrinsic Factor on Subsequent B_{12} Uptake.

1st incubation (followed by saline wash)	2nd incubation	B_{12} uptake by intestine (cpm/100 mg tissue)
I.F.	B_{12}	12
I.F.	B_{12} + I.F.	104
Saline	B_{12}	7
"	B_{12} + I.F.	100
B_{12}	I.F.	8

Sacs prepared from consecutive segments of midportion of single rat intestine. I.F. = 1 ml of neutralized rat gastric juice. 1st incubation 45 min.; 2nd, 60 min. 37°.

One of us (T.H.W.) wishes to thank Dr. Eric G. Ball for discussions which led to the initiation of this project. The helpful advice of Dr. Frank H. Gardner is gratefully acknowledged.

1. Castle, W. B., *Am. J. Med. Sci.*, 1929, v178, 748.

2. Latner, A. L., Raine, L., *First European Symposium on Vit. B_{12} and Intrinsic Factor,* 1957, p243 Enke, Stuttgart.

3. Wolff, R., Weis, J., *ibid.,* 247.

4. Herbert, V., *Fed. Proc.,* 1958, v17, 440.

5. Wilson, T. H., Wiseman, G., *J. Physiol.,* 1954, v123, 116.

6. Booth, C. C., Chanarin, I., Anderson, B. B., Mollin, D. L., *Brit. J. Haematol.,* 1957, v3, 253.

7. Reynell, P. C., Spray, G. H., Taylor, K. B., *Clin. Sci.,* 1957, v16, 663.

Received July 16, 1958. P.S.E.B.M., 1958, v99.

Relationship of Circulating Antigen-Antibody Complexes, Antigen Elimination, and Complement Fixation in Serum Sickness.*† (24304)

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The symptoms and lesions of both clinical and experimental serum sickness usually develop during rapid immune elimination of antigen(1-5). In rabbits, this immune elimination of antigen is associated with circulating antigen-antibody complexes(6,7) and with a fall in serum complement(8,9). The present serologic and histologic studies were undertaken to find whether the level and persistence of the complexes, in the circulation, and/or magnitude of the fall in complement could be correlated with extent of the associated morphologic manifestations of serum sickness.

Materials and methods. Bovine serum albumin (BSA), Armour and Co., Lot No. P67908 was trace-labelled with I^{131} (I*) according to the method described by Talmage *et al.*(10). Twenty-six albino rabbits, weighing approximately 3 kg, were injected intravenously with 250 mg of I*BSA/kg and bled (2.5-3.0 ml sera) every 1 to 2 days starting on either the first or third day following the injection. The sera were analyzed for total I*BSA, I*BSA bound to globulin, and complement. Total I*BSA activity was determined by counting the protein-bound I* in aliquots of sera, following trichloroacetic acid precipitation. These counts were converted to % of injected I*BSA remaining in the total blood volume(10). All samples contained sufficient counts to insure a maximum counting error of less than 5%. I*BSA present as a circulating I*BSA-anti BSA complex was determined by the ammonium sulfate technic of Farr(11). Aliquots of sera were diluted with equal volumes of 0.15 M NaCl and the globulin of these mixtures was precipitated at 37°C with equal volumes of saturated ammonium sulfate. The precipitates were washed twice with 50% saturated ammonium sulfate and were counted for I*BSA activity. The counts were converted to % of injected BSA which was bound to the total serum globulin. The hemolytic activity of the sera (CH_{50}units) was determined by the method of Osler *et al.*(12). All animals which showed an immune elimination of the I* antigen before the sixteenth day following injection were sacrificed 1 day after antigen was eliminated. With 2 exceptions, all other animals were sacrificed on the 16th or 17th day. One rabbit which showed no immune elimination of antigen was sacrificed on the 14th day following injection of the antigen. Another rabbit which did not show an immune elimination until the 18th day was sacrificed one day later. Tissues obtained at autopsy from all animals were fixed in formalin, embedded in paraffin, cut at 5 μ and stained with hematoxylin and eosin for microscopic examination. From every animal the tissues taken for section in-

* Supported by Atomic Energy Comm. Contract AT(30-1)-1205. Authors are indebted to Miss Dorothea Williamson and Miss Jane Dickenson for very capable technical assistance.

† Publication No. 153 of Department of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

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