

The Role of the Pancreas in Vitamin B₁₂ Absorption: Studies of Vitamin B₁₂ Absorption in Partially Pancreatectomized Rats

Philip P. Toskes, Julius J. Deren

J Clin Invest. 1972;51(2):216-223. <https://doi.org/10.1172/JCI106806>.

Research Article

The effect of partial pancreatectomy (80-90%) on vitamin B₁₂ absorption was studied in the rat. The absorption of 5 ng of ⁵⁷Co-labeled vitamin B₁₂ was significantly reduced from 70 ±2.5% (mean ±SE) in control and sham-operated rats to 32 ±2.6% in partially pancreatectomized rats. Hog pancreatic extract (0.17 g/kg) improved vitamin B₁₂ absorption from 30.0 to 61.0% in partially pancreatectomized rats but did not alter vitamin B₁₂ absorption in control rats. Chloramphenicol did not enhance vitamin B₁₂ absorption in partially pancreatectomized rats with pancreatic extract-improved vitamin B₁₂ malabsorption. The partially pancreatectomized rats with pancreatic extract-improved vitamin B₁₂ malabsorption were sacrificed and the stomach and small bowel studied *in vitro* to further define the pathogenesis of the vitamin B₁₂ malabsorption. Rat gastric intrinsic factor stimulated vitamin B₁₂ uptake by intestinal sacs prepared from partially pancreatectomized rats 3.1-fold. Gastric intrinsic factor prepared from partially pancreatectomized rats was as effective in promoting vitamin B₁₂ uptake by rat intestinal sacs as intrinsic factor prepared from control rats. These data indicate that partially pancreatectomized rats develop an abnormality in the absorption of labeled vitamin B₁₂ which can be corrected by pancreatic extract. The vitamin B₁₂ malabsorption is due to neither an alteration in gastric intrinsic factor activity nor an impairment of the intrinsic factor-vitamin B₁₂ receptor in the intestine. It is suggested that in the [...]

Find the latest version:

<https://jci.me/106806/pdf>



The Role of the Pancreas in Vitamin B₁₂ Absorption: Studies of Vitamin B₁₂ Absorption in Partially Pancreatectomized Rats

PHILIP P. TOSKES and JULIUS J. DEREN

From the Gastrointestinal Section of the Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104

ABSTRACT The effect of partial pancreatectomy (80–90%) on vitamin B₁₂ absorption was studied in the rat. The absorption of 5 ng of ⁵⁷Co-labeled vitamin B₁₂ was significantly reduced from 70 ± 2.5% (mean ± SE) in control and sham-operated rats to 32 ± 2.6% in partially pancreatectomized rats. Hog pancreatic extract (0.17 g/kg) improved vitamin B₁₂ absorption from 30.0 to 61.0% in partially pancreatectomized rats but did not alter vitamin B₁₂ absorption in control rats. Chloramphenicol did not enhance vitamin B₁₂ absorption in partially pancreatectomized rats with pancreatic extract-improved vitamin B₁₂ malabsorption. The partially pancreatectomized rats with pancreatic extract-improved vitamin B₁₂ malabsorption were sacrificed and the stomach and small bowel studied *in vitro* to further define the pathogenesis of the vitamin B₁₂ malabsorption. Rat gastric intrinsic factor stimulated vitamin B₁₂ uptake by intestinal sacs prepared from partially pancreatectomized rats 3.1-fold. Gastric intrinsic factor prepared from partially pancreatectomized rats was as effective in promoting vitamin B₁₂ uptake by rat intestinal sacs as intrinsic factor prepared from control rats. These data indicate that partially pancreatectomized rats develop an abnormality in the absorption of labeled vitamin B₁₂ which can be corrected by pancreatic extract. The vitamin B₁₂ malabsorption is due to neither an alteration in gastric intrinsic factor activity nor an impairment of the intrinsic factor–vitamin B₁₂ receptor in the intestine. It is suggested that in the partially pancreatectomized

rats the intrinsic factor–vitamin B₁₂ complex exists in a form which is not available for absorption.

INTRODUCTION

Although several patients with pancreatic exocrine insufficiency have been noted to poorly absorb vitamin B₁₂ the importance of normal pancreatic function for optimal vitamin B₁₂ absorption is generally not appreciated (1–5). Furthermore, the pathogenesis of the observed vitamin B₁₂ absorptive defect has not been clarified.

The relationship between the pancreas and the intestinal absorption of vitamin B₁₂ was studied by measuring the absorption of this vitamin in rats subjected to partial pancreatectomy. The results demonstrate (a) that the partially pancreatectomized rat has a defect in vitamin B₁₂ absorption, (b) that the administration of hog pancreatic extract restores vitamin B₁₂ absorption to normal, (c) that intrinsic factor extracted from the gastric mucosa of partially pancreatectomized rats stimulates vitamin B₁₂ absorption *in vitro*, and (d) that gastric intrinsic factor stimulates vitamin B₁₂ uptake in gut sacs prepared from partially pancreatectomized rats. These data indicate that partially pancreatectomized rats develop a defect in vitamin B₁₂ absorption which is due to neither an alteration in gastric intrinsic factor activity nor to an impairment of the intrinsic factor–vitamin B₁₂ receptor in the intestine.

METHODS

Animals. Male albino rats,¹ purchased in groups of 12, were delivered to our animal facilities weighing between 75 and 100 g and were allowed *ad lib.* stand-

¹Charles River Breeding Laboratories, Brookline, Mass.

Presented in part at a meeting of the American Federation for Clinical Research, Atlantic City, N. J., May 1970.

Dr. Toskes' present address is Walter Reed Institute of Army Research, Washington, D. C.

Received for publication 10 March 1971 and in revised form 20 August 1971.

ard lab chow² and water. When they reached a weight of approximately 130–150 g rats were selected at random for pancreatic surgery while others served as sham-operated or unoperated controls.

Surgery. Partial pancreatectomies were performed as described by Scow (6). In brief, rats were fasted for 18–20 hr and anesthetized by intraperitoneal pentobarbital (40 mg/kg). The abdomen was entered via a mid-line incision and the pancreas exposed. The rat pancreas consists of three major segments; the gastrosplenic segment comprises 70% of the total pancreatic mass, the duodenal segment nestled in the medial aspect of the duodenal loop contributes about 25%, and the small biliary segment surrounding the bile duct constitutes approximately 5% of the total pancreatic mass. The entire gastrosplenic and a variable portion of the duodenal segment were stripped by rolling small cotton swabs across the parenchyma but the biliary segment was allowed to remain intact; thus 80–90% of the pancreas was extirpated. Sham operations were performed in a similar manner except that the pancreas was manipulated rather than excised. A minimum period of 2 wk elapsed before absorption studies were performed. Operated rats lagged behind the growth rate of control animals for 5–7 days but by the beginning of the second postoperative week the absolute weight and rate of growth were comparable in both groups.

Vitamin B₁₂ absorption studies. 1 ml of B₁₂-⁵⁷Co³ containing 5 ng of vitamin B₁₂ (13–15 μCi/μg) was administered via a gastric tube to rats having access to only water for 12 hr. The rats were placed in restraining cages⁴ that allowed for complete separation of urine and feces. 2 hr after gastric intubation the rats were allowed free access to food and water. Stools were collected for 4–5 days at the end of which time less than 1% of the orally administered dose appeared in the stool per day.

Effect of pancreatic extract on vitamin B₁₂ absorption. Hog pancreatic extract⁵ (0.17 g/kg) was administered via gastric tube immediately after the labeled vitamin B₁₂.

Effect of chloramphenicol on vitamin B₁₂ absorption. Chloramphenicol⁶ was added to the drinking water for 5 days before and during the 5 days required to complete the vitamin B₁₂ absorption studies. Each rat ingested from 50 to 75 mg of chloramphenicol per day.

Vitamin B₁₂ uptake by everted rat gut sacs. Rats were sacrificed by decapitation and the small bowel from the ligament of Treitz to the ileocecal valve was perfused *in situ* with 0.9% NaCl at room temperature. The mesentery was removed and the gut was divided into four quarters beginning at the ligament of Treitz. All but the third quarter was discarded. This segment was everted on a metal probe and gut sacs were prepared as previously described (7). The sacs were filled with Krebs-Ringer bicarbonate (8) and incubated separately in Erlenmeyer flasks containing 2 ml Krebs-Ringer bicarbonate with 100 mg/100 ml D-glucose and 1 ng/ml B₁₂-⁵⁷Co. Rat intrinsic factor (IF) was prepared by scraping the mucosa of the glandular portion of the stomach and homogenizing in saline. 0.1 ml of homogenate (containing 5 mg wet weight of gastric mucosa) was

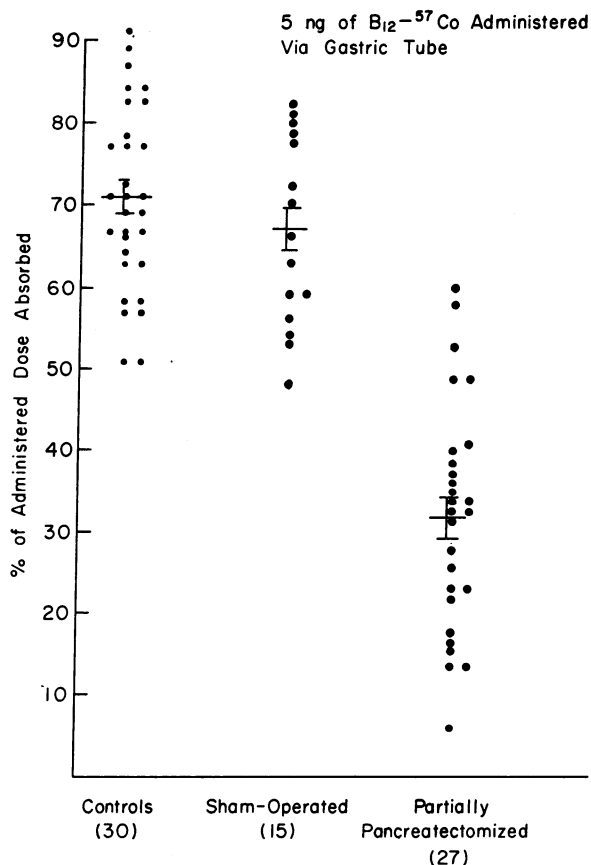


FIGURE 1 Vitamin B₁₂ absorption in control, sham-operated, and partially pancreatectomized rats, means ± SE.

added to alternate flasks. Incubation was carried out in a oscillating water bath for 1 hr at 37°C. The sacs were removed, drained, rinsed briefly in ice-cold saline, weighed, and counted. Results were expressed as nanograms of vitamin B₁₂ accumulated by the sac per gram wet weight per hour.

Fecal fat analyses. Partially pancreatectomized rats and control rats were placed in metabolic cages for 3–6 days. Quantitative measurement of the food intake and stool output were made. Stool and food were analyzed for fatty acid content by the method of Van de Kamer, Ten Bokkel Huinink, and Weyers et al. (9) and the results expressed as the coefficient of absorption.

Transit time. A dose of 1 ml of a solution containing sodium chromate⁷ was administered via a gastric tube to fasted rats. The rats were then placed in restraining cages and stool collected at 4-hr intervals. Results were expressed as the cumulative per cent excretion of the administered dose.

Counting procedure. Stools were brought to dry weight by incubating overnight at 95°C and counted with a well-type scintillation crystal with a gamma ray spectrometer and scalar⁷ under controlled geometric conditions at window settings appropriate for ⁵⁷Co or ⁵¹Cr.

Statistical analyses were performed using the Student's *t* test.

⁷ Picker Nuclear, White Plains, N. Y.

² Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.

³ Squibb Institute for Medical Research, New Brunswick, N. J.

⁴ Aerospace Industries, Garnerville, N. Y.

⁵ Viokase, VioBin Corp., Monticello, Ill.

⁶ Parke, Davis & Co., Detroit, Mich.

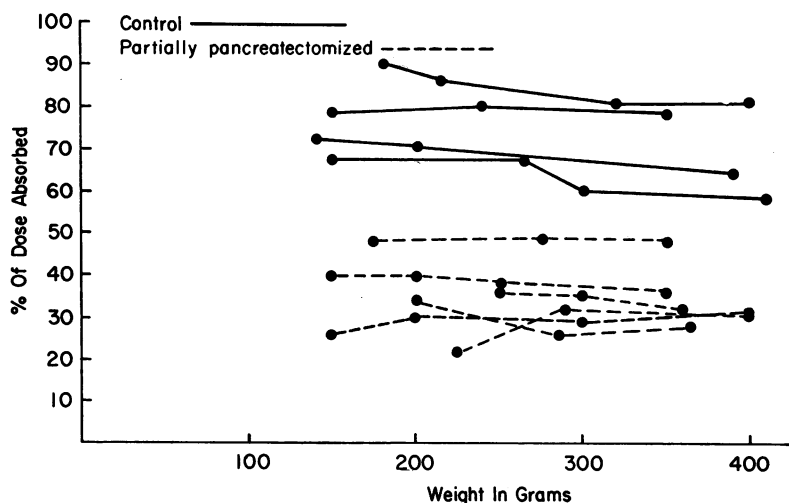


FIGURE 2 Results of repeated vitamin B₁₂ fecal excretion tests in control and partially pancreatectomized rats.

RESULTS

Vitamin B₁₂ absorption in control, sham-operated, and partially pancreatectomized rats As shown in Fig. 1, 30 control rats absorbed $71.0 \pm 2.0\%$ (mean \pm SEM) and

15 sham-operated rats absorbed $67.2 \pm 2.9\%$ of the administered vitamin B₁₂ ($P > 0.05$). By contrast, 27 partially pancreatectomized rats absorbed $32.6 \pm 2.6\%$ ($P < 0.01$ compared to both control and sham-operated rats). All control rats and all but one of the 15 sham-operated rats absorbed greater than 50% of the administered dose, whereas 24 of the 27 of the partially pancreatectomized rats absorbed less than 50%.

- B₁₂-⁵⁷Co Absorption Alone
- ▲ B₁₂-⁵⁷Co Absorption With Pancreatic Extract

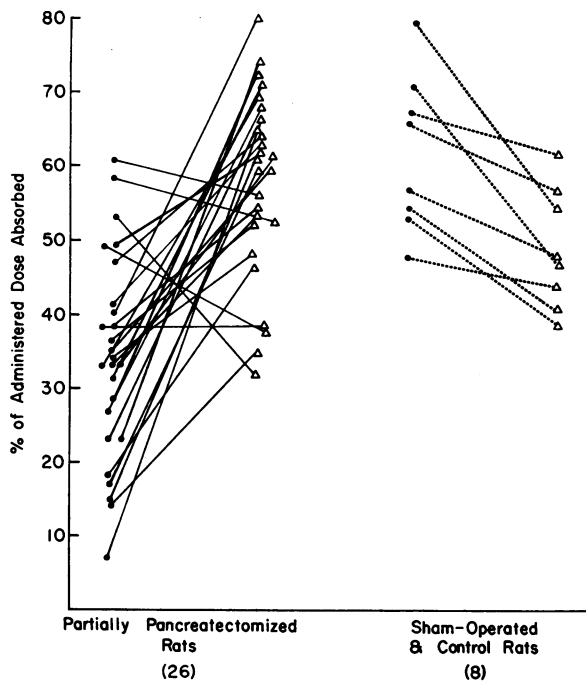


FIGURE 3 Effect of hog pancreatic extract (0.17 g/kg) on vitamin B₁₂ absorption in partially pancreatectomized, sham-operated, and control rats.

Results of repeated fecal excretion tests to assess the reproducibility of vitamin B₁₂ absorption. Vitamin B₁₂ absorption was assessed over a 5 month period during which time body weight increased from 100 to 400 g. As shown in Fig. 2, all four control rats absorbed greater than 50% of the administered dose while vitamin B₁₂ absorption was consistently less than 50% in the partially pancreatectomized rats. The ability to absorb vitamin B₁₂ in a given rat was quite reproducible during the study period. No value departed by more than 16% from the mean value obtained from averaging all studies performed in a given rat over a 5 month period.

Effect of pancreatic extract on vitamin B₁₂ absorption. Hog pancreatic extract (0.17 g/kg body weight) was administered concurrently with labeled vitamin B₁₂ to 26 partially pancreatectomized rats. Absorption increased from $33.2 \pm 2.6\%$ to $57.8 \pm 2.4\%$ of the administered dose ($P < 0.01$) (Fig. 3). Pancreatic extract increased vitamin B₁₂ absorption in 21 of the 26 partially pancreatectomized rats from a mean of $30.0 \pm 2.4\%$ to $61.0 \pm 2.3\%$ ($P < 0.01$). Vitamin B₁₂ absorption was restudied in 13 partially pancreatectomized rats with vitamin B₁₂ malabsorption ($29 \pm 3.3\%$) that previously responded to pancreatic extract ($62.5 \pm 3.7\%$). A value similar to that observed in the initial study period was obtained ($[32.4 \pm 2.7]$ data not shown). In one rat pan-

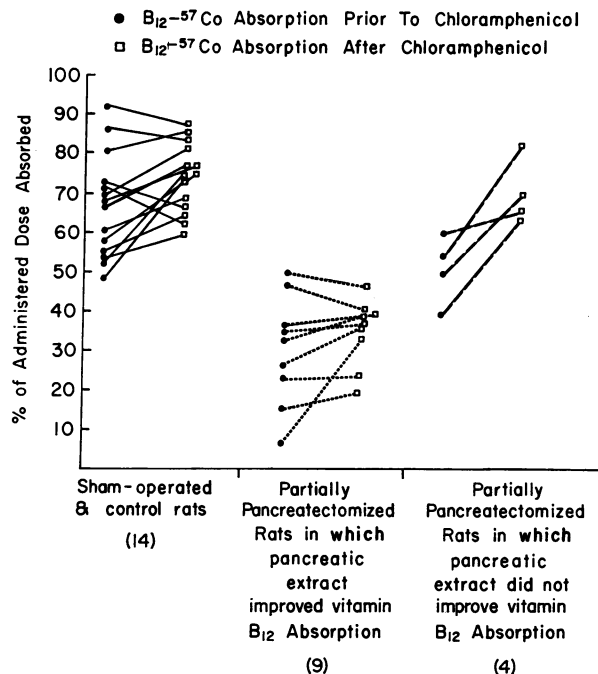


FIGURE 4 Effect of chloramphenicol (50–75 mg/day) on vitamin B₁₂ absorption in sham-operated, control, and partially pancreatectomized rats.

creatic extract failed to alter vitamin B₁₂ absorption. The four partially pancreatectomized rats that demonstrated the least impairment of vitamin B₁₂ absorption after surgery showed a further decrease (19%) in vitamin B₁₂ absorption when pancreatic extract was administered. Pancreatic extract administered to eight control and sham-operated rats decreased vitamin B₁₂ absorption by 14%.

Effect of chloramphenicol on vitamin B₁₂ absorption. The effect of chloramphenicol on vitamin B₁₂ absorption was studied in three groups of rats: (a) 14 control and sham-operated rats, (b) 9 partially pancreatectomized rats in which pancreatic extract had improved vitamin B₁₂ absorption 104%, and (c) 4 partially pancrea-

TABLE I
Fecal Fat Excretion in Control, Sham-Operated, and Partially Pancreatectomized Rats

	No. of rats	Coefficient of absorption*
Control and sham-operated rats	20	89.8 ± 0.79‡
Partially pancreatectomized rats	12	85.9 ± 1.62‡

* (Fatty acid intake—fatty acid output in stool) / (fatty acid intake) × 100.

‡ Values given are mean ± SE and were not significantly different, *P* > 0.05.

tectomized rats in which pancreatic extract failed to improve vitamin B₁₂ absorption (Fig. 4). In control and sham-operated rats chloramphenicol failed to significantly (*P* > 0.05) improve vitamin B₁₂ absorption (66.1 ± 3.5 to 71.8 ± 2.4). Similarly, chloramphenicol did not significantly (*P* > 0.05) alter vitamin B₁₂ absorption in partially pancreatectomized rats with pancreatic extract improved vitamin B₁₂ absorption (30.0 ± 4.3% to 34.6 ± 2.8%). The four partially pancreatectomized rats that failed to respond to pancreatic extract showed a 38% enhancement of vitamin B₁₂ absorption from a mean of 50 to 68.8% of the administered dose.

Fecal fat excretion in control and partially pancreatectomized rats. As shown on Table I, when fed a diet containing 5.3% fat, there was no significant difference in the coefficient of absorption between control and pancreatectomized rats.

Stomach to anus transit time in control and partially pancreatectomized rats. As shown in Fig. 5, the rate at which the labeled chromate appeared in the stool was comparable in partially pancreatectomized and control rats.

Ability of intestinal sacs from control and partially pancreatectomized rats to respond to gastric intrinsic factor *in vitro*. Four partially pancreatectomized rats with impaired vitamin B₁₂ absorption (mean of 29% of the administered dose) whose vitamin B₁₂ absorption was improved with pancreatic extract (to a mean of 65%) were sacrificed. Everted sacs were prepared from the mid-gut and incubated either with labeled vitamin B₁₂ or vitamin B₁₂ and rat gastric intrinsic factor (prepared from unoperated rats). As shown in Table II, gastric intrinsic factor stimulated vitamin B₁₂ uptake a mean of 3.1-fold as compared to 2.8-fold stimulation with sacs prepared on the same day from control rats of a similar size.

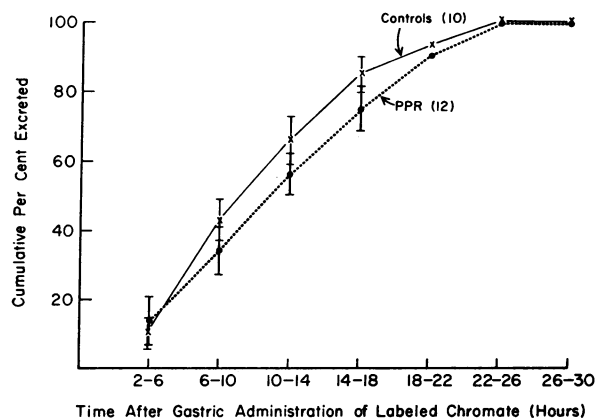


FIGURE 5 The stomach to anus transit time of labeled chromate in 10 control and 12 partially pancreatectomized rats (PPR), means ± SE.

TABLE II

Effect of Rat Gastric Intrinsic Factor Obtained from Control Rats on Vitamin B₁₂ Uptake by Intestinal Sacs Prepared from Partially Pancreatectomized Rats

	Weight at time of sacrifice	Vitamin B ₁₂ uptake		IF-mediated B ₁₂ uptake Non-IF-mediated B ₁₂ uptake
		B ₁₂	B ₁₂ + IF	
	g	ng/g wet weight per hr		
Partially pancreatectomized rats				
1	385	0.24 (4)	0.88 (4)	3.7
2	365	0.27 (4)	0.67 (5)	2.4
3	555	0.28 (3)	0.83 (4)	3.0
4	405	0.43 (4)	1.5 (3)	3.4
Control rats				
1	345	0.26 (4)	1.00 (4)	3.8
2	445	0.32 (4)	0.82 (5)	2.6
3	365	0.42 (8)	0.82 (8)	2.0

The number in parentheses denotes the number of sacs studied.

Comparison of gastric intrinsic factor prepared from unoperated rats with intrinsic factor prepared from partially pancreatectomized rats. When rat intrinsic factor obtained from gastric homogenates from partially pancreatectomized rats was added to intestinal sacs prepared from control rats, the stimulation of vitamin B₁₂ uptake was comparable to the enhancement of uptake obtained with intrinsic factor prepared from control rats (Table III).

DISCUSSION

The present schema for vitamin B₁₂ absorption envisages the ingestion of vitamin B₁₂ (actually coenzyme B₁₂ bound to protein [10-13]), release from its protein bond as a result of proteolytic digestion in an acid medium, binding to intrinsic factor in the stomach or upper portion of the small bowel (14-16), passage down the small bowel, attachment of the IF-B₁₂ complex to a specific receptor located in the brush border of the ileal epithelial cell (17-21), and eventual release of the vitamin into the portal circulation unaccompanied by IF (22, 23). In addition a factor elaborated by the pancreas may be an obligatory requirement for optimal vitamin B₁₂ absorption. Such a feature has been suggested by the observation that among patients with unexplained vitamin B₁₂ malabsorption (measured by the urinary excretion test [24]) were several with pancreatic insufficiency (1, 2). The pathogenesis of the observed vitamin B₁₂ absorptive defect has not been clarified, although the improvement in vitamin B₁₂ absorption after sodium bicarbonate administration suggested that an increased

hydrogen ion concentration in the ileal lumen accounted for the malabsorption (3). However, the improvement in vitamin B₁₂ absorption after the administration of pancreatic extract for several days (3) or as a single dose concomitant with the labeled B₁₂ (5) cannot be explained on the basis of altered luminal pH. Furthermore, the pH of the ileal contents of subjects with pancreatic insufficiency and vitamin B₁₂ malabsorption was similar to that of patients with pancreatic insufficiency and normal vitamin B₁₂ absorption and to that of normal volunteers (5). Calcium ions have also been demonstrated to be necessary for IF-mediated vitamin B₁₂ uptake in vitro (25, 26). It has been suggested that in severe fat malabsorption large amounts of calcium soaps may form to lower the intraluminal concentration of ionic calcium to levels too low to allow for the vitamin B₁₂-IF complex to attach to the ileal receptors (27-29). However, the total calcium concentration in the ileal aspirates was similar in subjects with pancreatic insufficiency with normal or abnormal vitamin B₁₂ absorption or in normal volunteers (5).

The data in this study indicate that rats subjected to 80-90% pancreatectomy absorbed significantly less orally administered labeled vitamin B₁₂ compared to control and sham-operated animals. The partially pancreatectomized rats appeared healthy, had a growth rate comparable to control rats (data not shown), and in accord with the results of previous authors showed no evidence of fat malabsorption (30). Since the rate at which a non-absorbable marker (sodium chromate) appeared in the stool after intragastric instillation was similar in partially pancreatectomized rats and control rats, differences in the rate of movement down the small bowel would appear to be an unlikely explanation for the vita-

TABLE III
Comparison of Gastric Intrinsic Factor Obtained from Control and Partially Pancreatectomized Rats on Vitamin B₁₂ Uptake by Rat Intestinal Sacs Prepared from Control Rats

Incubation conditions	No. of sacs studied	B ₁₂ uptake*
		ng/g wet weight per hr
B ₁₂ alone	24	0.33 ± 0.024
B ₁₂ + intrinsic factor prepared from control rats	13	0.83‡ ± 0.047
B ₁₂ + intrinsic factor prepared from partially pancreatectomized rats	12	0.83‡ ± 0.055

* Values given are mean values ± SE.

‡ Values are significantly different from that observed with vitamin B₁₂ alone.

min B₁₂ malabsorption. It is possible, however, that the measurement of stomach to anus transit time may not adequately reflect the transit time in a localized area of the gastrointestinal tract (31).

To determine whether the observed defect in vitamin B₁₂ absorption was related to a specific deficiency of a pancreatic factor or to bacterial overgrowth consequent to small bowel manipulation, the effect of pancreatic extract and a broad spectrum antibiotic (chloramphenicol) on vitamin B₁₂ absorption were compared. When pancreatic extract was administered to 26 partially pancreatectomized rats, vitamin B₁₂ absorption was noted to increase in 21 (from a mean of 30 to 61% of the administered dose) and remained unchanged or decreased in five rats. Nine randomly selected rats with pancreatic extract-improved vitamin B₁₂ absorption were placed on chloramphenicol. Vitamin B₁₂ absorption increased slightly (although not significantly at the 5% level). Control and sham-operated rats also exhibited a slight although statistically insignificant increase in vitamin B₁₂ absorption when chloramphenicol was added to their drinking water. Improvement in vitamin B₁₂ absorption in rats after chloramphenicol administration has been observed by others (32). When chloramphenicol was administered to four partially pancreatectomized rats that failed to show improvement in vitamin B₁₂ absorption with pancreatic extract, the absorption of this vitamin improved 38% (from a mean of 50 to 68.8%). The latter value is in the range for vitamin B₁₂ absorption observed in unoperated rats. It is of interest that the partially pancreatectomized rats that responded to pancreatic extract had a more severe impairment of vitamin B₁₂ absorption (30%) as compared to partially pancreatectomized rats that failed to improve with pancreatic extract (50%). Thus, rats subjected to a 80–90% pancreatectomy consistently absorbed vitamin B₁₂ poorly and could be segregated into two distinct groups based on their responses to pancreatic extract and chloramphenicol. One group consisted of a small number of rats that had a modest impairment of vitamin B₁₂ absorption. Vitamin B₁₂ absorption in this group failed to improve with pancreatic extract but was restored to values observed in control rats after chloramphenicol administration. Presumably, the mechanism of vitamin B₁₂ malabsorption in this group was related to bacterial overgrowth secondary to surgery. Most rats subjected to a partial pancreatectomy fell into a second category characterized by a more severe defect in vitamin B₁₂ absorption that improved with pancreatic extract but was not altered by the administration of a broad spectrum antibiotic. In these rats vitamin B₁₂ malabsorption was apparently secondary to a deficiency of a factor elaborated by the pancreas.

There are a number of possible mechanisms by which a pancreatic factor may influence vitamin B₁₂ absorption. Studies of gastric intrinsic factor and intestinal sacs from partially pancreatectomized rats with pancreatic extract-improved vitamin B₁₂ malabsorption allowed for further definition of the pathogenesis of the vitamin B₁₂ absorptive defect. First, a pancreatic factor may be required to interreact with either the vitamin B₁₂ binding site of intrinsic factor or the site on the intrinsic factor molecule that attaches to the receptor in the brush border of the ileal epithelial cell. Evidence against the pancreatic factor acting directly upon intrinsic factor was obtained from the observation that vitamin B₁₂ uptake in intestinal sacs prepared from control rats was stimulated by rat gastric intrinsic factor obtained from partially pancreatectomized rats. Second, a pancreatic factor may be required to modify the intestinal receptor in order for it to combine with the intrinsic factor–B₁₂ complex or the receptor may be synthesized by the pancreas and secondarily attached to the brush border (33). This also seems unlikely since vitamin B₁₂ uptake by intestinal sacs from partially pancreatectomized rats with vitamin B₁₂ malabsorption was readily stimulated by intrinsic factor prepared from gastric homogenates of control rats. Third, there is evidence that vitamin B₁₂ may form complexes in the gastrointestinal tract (34–37), which may not be readily absorbed. The malabsorption observed in partially pancreatectomized rats may be explained by the failure to maintain the intrinsic factor–B₁₂ complex in a form that is available for absorption (37). Alternatively, an inhibitor to intrinsic factor-mediated vitamin B₁₂ absorption (38) may be present whose activity is maintained at a minimal level by a normal supply of a component of the pancreatic secretion. In the partially pancreatectomized rats, the activity of this inhibitor may suffice to impair vitamin B₁₂ absorption. Finally, the surgical procedure may have altered the enterohepatic circulation of vitamin B₁₂ (39) leading to an expansion of the vitamin B₁₂ pool in the upper gastrointestinal tract of partially pancreatectomized rats. The orally administered labeled cyanocobalamin would therefore be diluted in a larger pool of nonradioactive vitamin B₁₂ in partially pancreatectomized rats as compared to controls and would lead to the observed differences in the absorption of labeled vitamin B₁₂.

These results indicate that partial pancreatectomy in the rat induces a defect in the absorption of labeled vitamin B₁₂ which was corrected by the administration of exogenous pancreatic extract. This animal model should prove useful in determining the pathophysiology of the vitamin B₁₂ malabsorption (as measured by the urinary excretion test) associated with pancreatic exocrine insufficiency in the human.

ACKNOWLEDGMENTS

We would like to acknowledge the encouragement and careful review of the manuscript by Doctors William B. Castle and Sidney Cohen, the technical assistance of Mrs. Scriostea-Rogers and Miss Nina Shopa, and the secretarial assistance of Mrs. Simone Tischhauser.

B_{12} - ^{57}Co was kindly provided by the Squibb Institute for Medical Research, New Brunswick, N. J. The work was supported in part by Training Grant T-01-AM-5462, National Institutes of Health.

REFERENCES

- McIntyre, P. A., M. V. Sachs, J. R. Krevans, and C. L. Conley. 1956. Symposium on Hematology: Pathogenesis and treatment of macrocytic anemia. *Arch. Intern. Med.* **95**: 541.
- Perman, G., R. Gullberg, P. G. Reizenstein, B. Snellman, and L. G. Allgen. 1960. Study of absorption patterns in malabsorption syndromes. *Acta Med. Scand.* **168**: 117.
- Veeger, W., J. Abels, N. Hellemans, and H. O. Nieweg. 1962. Effect of sodium bicarbonate and pancreatin on the absorption of vitamin B_{12} and fat in pancreatic insufficiency. *N. Engl. J. Med.* **267**: 1341.
- LeBauer, E., K. Smith, and N. J. Greenberger. 1968. Pancreatic insufficiency and vitamin B_{12} malabsorption. *Arch. Intern. Med.* **122**: 423.
- Toskes, P. P., J. Hansell, J. Cerda, and J. J. Deren. 1971. Vitamin B_{12} malabsorption in chronic pancreatic insufficiency: studies suggesting the presence of a pancreatic "intrinsic factor." *N. Engl. J. Med.* **284**: 627.
- Scow, R. O. 1957. Total pancreatectomy in the rat: operation, effects and post operative care. *Endocrinology.* **60**: 359.
- Wilson, T. H., and G. Wiseman. 1954. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol. (London).* **123**: 116.
- Krebs, H. A., and K. Henseleit. 1932. Untersuchungen ueber die Harnstoffbildung im Tierkoerper. *Hoppe-Seyler's Z. Physiol. Chem.* **210**: 33.
- Van de Kamer, J. H., H. Ten Bokkel Huinink, and H. A. Weyers. 1949. Rapid method for the determination of fat in feces. *J. Biol. Chem.* **177**: 347.
- Hedbom, A. 1955. A native vitamin B_{12} polypeptide complex. *Biochim. Biophys. Acta.* **17**: 447.
- Rosenblum, C., D. A. Willigan, H. T. Meriwether, and E. P. Cronkite. 1960. Stability of injected vitamin B_{12} CO^{60} and vitamin B_{12} content of dog liver. *Proc. Soc. Exp. Biol. Med.* **105**: 142.
- Robinson, F. A., M. E. H. Fitzgerald, K. Fehr, and J. J. Grimshaw. 1954. Vitamin B_{12} in crude liver extracts. *Nature (London).* **174**: 558.
- Toohey, J. I., and H. A. Barker. 1961. Isolation of coenzyme B_{12} from liver. *J. Biol. Chem.* **236**: 560.
- Abels, J., and R. F. Schilling. 1964. Protection of intrinsic factor (IF) by vitamin B_{12} . *J. Lab. Clin. Med.* **64**: 375.
- Okuda, K., and K. Sasayama. 1965. Intestinal distribution of intrinsic factor and vitamin B_{12} absorption. *Amer. J. Physiol.* **208**: 14.
- Highley, D. R., and L. Ellenbogen. 1962. Studies on the mechanism of vitamin B_{12} absorption: *in vivo* transfer of vitamin B_{12} to intrinsic factor. *Arch. Biochem. Biophys.* **99**: 126.
- Rothenberg, S. P. 1968. Identification of a macromolecular factor in the ileum which binds intrinsic factor and immunologic identification of intrinsic factor in ileal extracts. *J. Clin. Invest.* **47**: 913.
- MacKenzie, I. L., R. M. Donaldson, Jr., W. L. Kopp, and J. S. Trier. 1968. Antibodies to intestinal microvillous membranes. II. Inhibition of intrinsic factor mediated attachment of vitamin B_{12} to hamster brush borders. *J. Exp. Med.* **128**: 375.
- Kopp, W. L., J. S. Trier, I. L. MacKenzie, and R. M. Donaldson, Jr. 1968. Antibodies to intestinal microvillous membranes. I. Characterization and morphologic localization. *J. Exp. Med.* **128**: 357.
- Donaldson, R. M. Jr., I. L. MacKenzie, and J. S. Trier. 1967. Intrinsic factor-mediated attachment of vitamin B_{12} to brush borders and microvillous membranes of hamster intestine. *J. Clin. Invest.* **46**: 1215.
- Boass, A., and T. H. Wilson. 1964. Intestinal absorption of intrinsic factor and B_{12} -intrinsic factor complex. *Amer. J. Physiol.* **207**: 27.
- Cooper, B. A., T. Yates, and J. J. White. 1966. Studies with antibody to human intrinsic factor indicating absence of IF from human portal blood during B_{12} absorption. *Clin. Res.* **14**: 314. (Abstr.)
- Reizenstein, P. G., E. P. Cronkite, L. M. Meyer, and E. A. Usenik. 1960. Lymphatics in intestinal absorption of vitamin B_{12} and iron. *Proc. Soc. Exp. Biol. Med.* **105**: 233.
- Schilling, R. F. 1953. Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12} . *J. Lab. Clin. Med.* **42**: 860.
- Herbert, V., and W. B. Castle. 1961. Divalent cation and pH dependence of rat intrinsic factor action in everted sacs and mucosal homogenates of rat small intestine. *J. Clin. Invest.* **40**: 1978.
- Cooper, B. A., and W. B. Castle. 1960. Sequential mechanisms in the enhanced absorption of vitamin B_{12} by intrinsic factor in the rats. *J. Clin. Invest.* **39**: 199.
- Grasbeck, R., and W. Nyberg. 1958. Inhibition of radio-vitamin B_{12} . Absorption by ethylenediaminetetraacetate (EDTA) and its reversal by calcium ions. *Scand. J. Clin. Lab. Invest.* **10**: 448.
- Grasbeck, R., I. Kantero, and M. Siurala. 1959. Influence of calcium ions on vitamin B_{12} absorption in steatorrhea and pernicious anemia. *Lancet.* **1**: 234.
- Rodriguez-Rosado, A. L., and T. W. Sheehy. 1961. The role of calcium on the intestinal absorption of vitamin B_{12} in tropical sprue. *Amer. J. Med. Sci.* **242**: 548.
- Uram, J. A., L. Friedman, and O. L. Kline. 1960. Relation of pancreatic exocrine to nutrition of the rat. *Amer. J. Physiol.* **199**: 387.
- Summers, R. W., T. H. Kent, and J. W. Osborne. 1970. Effects of drugs, ileal obstruction and irradiation on rat gastrointestinal propulsion. *Gastroenterology.* **59**: 731.
- Nygaard, K. 1968. Nutrition and absorption after resections and bypass-operations on the small intestine in rats. *Acta Chir. Scand.* **134**: 63.
- Ugolev, A. M. 1965. Membrane (contact) digestion. *Physiol. Rev.* **45**: 555.

34. Okuda, K., and K. Zashima. 1968. Absorption of methyl-enzimidazolyl cobamide coenzyme. Its chemical behavior in the intestine, role of intrinsic factor and pancreatic digestion. *Proc. Int. Union Physiol. Sci.* 7: 328. (Abstr.)
35. Okuda, K., and T. Fujii. 1968. Inactivation of vitamin B₁₂ in the intestine and a new role of intrinsic factor. Proceedings of the 8th International Congress of Gastroenterology, Prague. 752. (Abstr.)
36. Okuda, K. 1968. Intestinal absorption of vitamin B₁₂ and pancreatic digestion. Proceedings of the 22nd Congress, International Society of Hematology, N. Y., 98. (Abstr.)
37. Okuda, K., T. Kitazaki, and M. Takamatsu. 1971. Inactivation of vitamin B₁₂ by a binder in rat intestine and the role of intrinsic factor. *Digestion.* 4: 1.
38. Temperley, I. J., D. G. Weir, D. Collery, and J. M. Scott. 1969. Inhibition of free intrinsic factor activity by duodenal juice. *Gastroenterology.* 57: 273.
39. Grasbeck, R., and P. Reizenstein. 1958. Biliary and fecal vitamin B₁₂ excretion in man. An isotope study. *Proc. Soc. Exp. Biol. Med.* 97: 780.