Inhibition of Vitamin B-12 Absorption by Bile.* (26278)

P. C. JOHNSON,† T. B. DRISCOLL† AND W. L. HONSKA
Radioisotope Service, VA Hospital, and Dept. of Medicine, Public Health, University of Oklahoma, Oklahoma City

How intrinsic factor reacts to promote efficient absorption of B_{12} from the gastrointestinal tract is just now being fully elucidated(1). Since it has taken milligram amounts of purified intrinsic factor to catalyze the absorption of microgram amounts of B_{12}, it is possible that intrinsic factor may be destroyed by substances in the gastrointestinal tract or it may react with substances other than B_{12}(2). These substances would be inhibitors of B_{12} absorption and are likely produced by the intestinal mucosa since ingestion of B_{12} in the fasting state does little to improve absorption. Measuring liver concentration of absorbed B_{12} in rats, we have found that bile is a potent inhibitor of B_{12} absorption and this inhibition is corrected by whole gastric juice.

Methods. Rats weighing 160 g were given a 5% glucose in normal saline diet for 48 hours prior to operation. During ether anesthesia a silk ligature was placed around the duodenum one cm above or below the entrance of the bile duct. Following this, various solutions containing 0.03 μg Co^{60} labeled B_{12} were instilled into the duodenal lumen just distal to the ligature. The rats were deprived of food and water for 6 hours and were sacrificed at 6 hours. The liver was removed and washed in soapy water after which excess blood and water was expressed by blotting. The livers were then divided into 2 nearly equal parts, placed in test tubes which were then counted in a scintillation well detector. Radioactivity of each portion was summed to give total liver radioactivity. Amount of radioactivity in the liver was determined to a 5% average error. One of the following 4 solutions was instilled into the intestine: Control solution containing 0.03 μg of B_{12} alone; 0.5 ml of whole rat gastric juice and B_{12}; 0.5 ml rat bile and B_{12}; 0.5 ml of whole rat gastric juice, 0.5 ml of rat bile and B_{12}. All solutions were made up to a volume of 1 ml prior to instillation.

The rat gastric juice was obtained after the rats had been given a glucose and saline diet for 48 hours. The pylorus was ligated and 2 hours later the rat was sacrificed and the gastric juice harvested. The bile was obtained by cannulating the bile duct of a rat and allowing 12 hours for collection. The rat took in 5% glucose in normal saline *ad lib.* during bile collection. The gastric juice and bile were used within 24 hours of their collection. The pH of the bile was around 9 while the gastric juice had a pH of about 1. In the volumes used duodenal mucosa neutralized these solutions upon contact.

Results. Table I shows mean and standard error of mean liver concentration of Co^{60} B_{12} obtained from these animals under the various conditions of this experiment. In the control group a 50% decrease in liver Co^{60} B_{12} occurred in the animals when the ligature was placed above the entrance of the bile duct. This decrease in B_{12} absorption could be prevented by addition of 0.5 ml of whole gastric juice. Instillation of 0.5 ml bile reduced liver uptake of both sets of animals. Mixing whole gastric juice with the bile prior to instillation returned the absorption to control levels.

Discussion. These data show that, in the rat, bile is an inhibitor of B_{12} absorption since injection of bile or allowing it to enter the duodenum during absorption of B_{12} markedly decreases the amount of B_{12} absorbed by the intestine and concentrated by the liver. Addition of 0.5 ml of whole gastric juice is capable of correcting the absorptive defect produced by a similar volume of rat bile. In the absence of bile, gastric juice in the volume tested failed to improve B_{12} absorption. Rat bile and bile of other species contains B_{12}(3,4). The rat bile used in these studies was assayed for B_{12} content by

* This work was supported in part by N.I.H. grant.

† Present address: The Methodist Hospital, Houston, Texas.

TABLE I. Effect of Bile and Gastric Juice on Absorption and Liver Concentration of $Co^{60}B_{12}$ by the Intact Rat.

	No. of animals	Relationship of ligature to bile duct: Above	Below	No. of animals
Control	30	4.05 ± .33	7.50 ± .27	27
.5 ml gastric juice	20	6.65 ± .27	7.50 ± .36	20
.5 " bile	25	1.76 ± .42	1.74 ± .21	28
.5 " " + .5 ml gastric juice	20	4.98 ± .30	6.90 ± .60	25

Each figure is mean ± stand. error of mean in $\mu g \times 10^{-4}$ of B_{12}.

Dr. Paul S. Hagens using *Euglena gracilis*. His data showed that this lyophylized bile contained 95 $\mu\mu g$ of B_{12} per g of solids. This compares to a value of 4 $\mu\mu g/g$ in dog bile (5). Therefore, one ml of bile, 27 mg of solids, contains 8% as much B_{12} as was used in these experiments.

These experiments throw no light on why bile causes inhibition of B_{12} absorption in these animals; however, non-specific inhibitory binding of B_{12} would explain the results. It is known that bile will bind B_{12}(6). In these experiments, amount of intrinsic factor necessary to improve B_{12} absorption seems related to amount of bile present in the upper intestine. This effect would in part explain why isolation of potent pure intrinsic factor has been so difficult since minimal effective dose of intrinsic factor may be more related to the bile in the intestine than to amount of vitamin B_{12} ingested. It is possible that other intestinal secretions inhibit B_{12} absorption.

Summary. 1. In the intact rat the presence of bile in the small intestine inhibits B_{12} absorption. 2. The inhibitory effect of bile is prevented by simultaneous addition of rat gastric juice.

1. Cooper, B. A., Castle, W. B., *J. Clin. Invest.*, 1960, v39, 199.

2. Ellenbogen, L., Williams, W. L., *Biochem. Biophys. Res. Comm.*, 1960, v2, 340.

3. Grasbeck, R., Nyberg, W., Reizenstein, P., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 780.

4. Willigan, D. A., Cronkite, E. P., Meyer, L. M., Noto, S. L., *ibid.*,

5. Copperman, J. M., Luhby, A. L., Teller, D. W., Marley, J. F., *J. Biol. Chem.*, 1960, v235, 191.

6. Okuda, K., Grasbeck, R., Chow, B. F., *J. Lab. Clin. Med.*, 1958, v51, 17.

Received October 4, 1960. P.S.E.B.M., 1961, v106.

Gamma Globulin and Immune Mechanisms in the Chick Embryo.* (26279)

DONALD L. BALLANTYNE, JR. AND JOHN MARQUIS CONVERSE
(Introduced by C. A. Stetson, Jr.)
Institute of Reconstructive Plastic Surgery, New York University Medical Center and Dept. of Surgery, New York University School of Medicine

Green and Lorincz(1) stated that a serum protein with the properties of a natural antibody first appeared in the blood of chick embryos around the 17th day of embryonic development. This antibody, which is a part of the serum gamma globulin, seems to be responsible for destruction of the Krebs ascites tumor cells of mice injected intravenously into the chorio-allantois of chick embryos. Mouse tumor cells do not grow in hatched chicks. In connection with our findings(2) on the fate and behavior of mammalian skin heterografts transplanted to the chorio-allantois of the chick embryo, it was a natural sequence to learn whether gamma globulin placed directly on the embryonal membrane, or injected intravenously into the

* This work was supported by a grant from U.S.P.H.S.