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Effect of Different Intrinsic Factor Preparations on Co^{60} Vit. B_{12} Uptake in Rat Liver Slices. (24166)

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Miller and Hunter(1) reported that radioactive Vit. B_{12} is absorbed by rat liver slice, and that this absorption is increased markedly by addition of intrinsic factor rich extracts of hog stomach. This work has been confirmed (2,3). That this increase in binding is related to intrinsic factor present in the concentrate is suggested by the fact that it is abolished by boiling of intrinsic factor concentrate(1), and that proteins without known intrinsic factor activity fail to produce this increased binding. These proteins include gelatin, lysozyme, crystalline pepsin and pink protein(1). Addition of human gastric juice failed to enhance Cobalt-60 labeled Vit. B_{12} uptake by rat liver slice. Since this was similar to the results obtained with human gastric juice in gastrectomized rats, it seemed advisable to extend these observations to rat gastric juice, dog gastric juice, hog intrinsic factor concentrates and hog gastric juice to test the effect these intrinsic factor preparations have on liver slice technic.

Technic. Seventy-five to 100 mg slices were prepared from chilled rat livers and incubated for 30 min. at 37° in Warburg vessels containing Hastings' buffer(4) 15 mμg radioactive Vit. B_{12} and measured amounts of gastric juice. Prior to incubation the system was flushed 5 min. with 5% CO_2 and 95% O_2 mixture. After incubation, the slices were washed in fresh buffer. We found that the number of washes or length of time used in washing does not cause a change in radioactivity absorbed. The slices were transferred to glass test tubes and assayed in scintillation well type counter. The counts were corrected for background and expressed as specific activity (counts/min./100 mg). Various amounts of rat, dog, human and hog gastric juice were incubated with the liver slices. Rat gastric juice was obtained by placing a ligature over pylorus of rat stomach. Eight hours later the gastric juice was collected. Gastric juice was collected from dogs with Heidenhain pouches which had been stimulated by raw meat. Human gastric juice was obtained from fasting patients by gastric tube after insulin stimulation. Hog gastric juice was obtained by expressing gastric juice from surface of freshly slaughtered hog stomach. The hog intrinsic factor concentrate was furnished us.* In patients with pernicious anemia, this intrinsic factor concentrate is active at a dosage of 2-3 mg. The binding ability of various gastric juice fractions as tested by the method of Gregory and Holdsworth(5), *viz.* 0.61 μg of Vit. B_{12} containing 0.01 μc of Co^{60} were added to 5 mg of gastric juice. This mixture was dialyzed against tap water for 48 hours and the contents of dialysis tubing counted in scintillation well counter. These gastric juices were tested in a patient with pernicious anemia to prove that each had intrinsic factor activity. Twenty-five mg of

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TABLE I. Effect of Gastric Juice on Co^{60} Vit. B_{12} Liver Uptake in a Patient with Pernicious Anemia.

Control	.9%
25 mg rat gastric juice	3.5
" dog " "	.4
" human " "	5.4
" hog " "	5.0
Normal value = >4.7%	
.1 μc Co^{60}	.1 μg vit. B_{12}

gastric juice were added to 0.1 μg of Co^{60} labeled Vit. B_{12} and fed to a patient with pernicious anemia. The seventh day liver uptake of the Co^{60} was obtained by placing a 5" plastic scintillation detector over the liver area. The radioactivity measured was compared to a standard and is reported as per cent uptake(6).

Results. Table I shows per cent liver uptake of Co^{60} labeled Vit. B_{12} in pernicious anemia. The control value without added intrinsic factor was 0.9%. A normal patient will have a liver uptake greater than 4.7%. Addition of hog, rat and human gastric juice, increased this patient's liver uptake of Vit. B_{12}, thus proving that these preparations exhibit intrinsic factor activity. Dog gastric juice resulted in no increase.

Table II shows relative binding abilities of these gastric juice fractions. The greatest binding was exhibited by hog intrinsic factor. Binding ability of hog and rat gastric juice was approximately equal.

Fig. 1 shows the results obtained with rat liver slice. The abscissa is in net counts/min 100 mg of rat liver. Ordinate represents increasing amounts of added gastric juice fractions. The uptake of Co^{60} Vit. B_{12} increase when 30 μg of hog intrinsic factor concentrate was added. Adding 100 μg concentrate decreased uptake to the control level and adding over 150 μg inhibited this uptake. These results are nearly identical to those obtained by Miller and Hunter(1). Hog gastric juice produced the same degree of enhancement of the liver uptake as did the hog intrinsic factor concentrate. However, this occurred after 150 μg of gastric juice had been added instead of the 30 μg that produced maximal uptake using hog intrinsic factor concentrate.

TABLE II. Co^{60} Vit. B_{12} Binding Ability of Gastric Juice.

Source	μg
HIFC	.129
Human	.044
Dog	.030
Hog	.016
Rat	.015

μg non-dialyzable vit. B_{12}/mg gastric juice.

Dog gastric juice increased uptake to a greater degree than that produced by the hog intrinsic factor concentrate. At all concentrations, rat gastric juice slightly inhibited uptake of Co^{60} Vit. B_{12} by the rat liver slice.

Human gastric juice caused no enhancement of uptake. Inhibition was present even at the lowest concentrations, and increased with increasing dosages. Table III shows actual mean values obtained in these determinations.

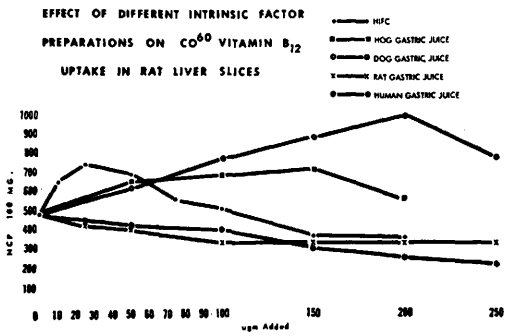

FIG. 1.

Discussion. With the exception of dog gastric juice, the gastric juices used in this experiment contained intrinsic factor since each one was capable of increasing Vit. B_{12} absorption in patients with pernicious anemia. Dog gastric juice was collected from Heidenhain gastric pouches, made up of part of the fundus and the cardia of the dog stomach. By measuring fecal excretion of Co^{60} Vit. B_{12} we have found that 25 mg doses of dog Heidenhain pouch juice were active in one of 4 other pernicious anemia patients. These data do not prove presence or absence of intrinsic factor in the pouch juice, since it may not be active in the human.

However, the effect on a rat liver slice was quite different. Preparations from the dog and hog increased Vit. B_{12} binding. Human

TABLE III. Co^{60} Vit. B_{12} Uptake by Rat Liver Slice.

μg added	Hog conc.	Hog	Dog	Human	Rat
10	641 ± 9*				
25	730 ± 19			474 ± 12	441 ± 14
50	656 ± 17	658 ± 19	640 ± 16	420 ± 9	425 ± 17
100	497 ± 16	687 ± 11	763 ± 7	393 ± 9	340 ± 20
150	361 ± 14	735 ± 11	888 ± 6	303 ± 13	333 ± 8
200	360 ± 14	558 ± 7	1018 ± 20	250 ± 16	342 ± 13
250			802 ± 25	216 ± 8	325 ± 22
	Control 485 ± 19		Net counts/min./100 mg liver		

* Mean ± S.E. of mean.

gastric juice markedly inhibited while rat gastric juice only slightly inhibited the binding. If this method is actually measuring intrinsic factor activity, we must conclude that there is a species difference in the mechanism of action of intrinsic factor.

It is probable that the gastric juices used here have different concentration ratios of intrinsic factor and non-specific Vit. B_{12} binding proteins. Three of the 4 gastric juices used were capable of correcting the specific absorptive defect in a patient with pernicious anemia and all 4 gastric juices would presumably correct the Vit. B_{12} absorptive defect in gastrectomized animals of the same species. Therefore if the failure of human and rat gastric juice to enhance Vit. B_{12} uptake by the rat liver slice were due to an excessive amount of non-specific Vit. B_{12} binding protein, we must conclude that the liver slice is more sensitive to these ratios than is the gastrointestinal tract. That this may be true is suggested by the work of Herbert(3). By sequential incubation of the liver slice, he was able to show an increased uptake using human gastric juice from patients with Sprue and Vit. B_{12} dietary deficiency states.

An alternate explanation for the findings obtained here is that liver slice uptake may not be related to intrinsic factor activity. It has long been known that gastric juice will bind Vit. B_{12}, making it non-dialyzable and unavailable to some micro-organisms. That this binding phenomenon is not synonymous with intrinsic factor has been confirmed(7,8). They have shown that degree of Vit. B_{12} binding is not well correlated with intrinsic factor activity and that gastric fractions are obtainable that will bind Vit. B_{12} but which have no intrinsic factor activity. Relative binding potency of the gastric fractions used in this experiment did not correlate with their intrinsic factor activity in a pernicious anemia patient or their uptake by the liver slice. Whether the binding of Vit. B_{12} to proteins other than intrinsic factor is related to the uptake phenomenon obtainable with the liver slice is not known. Miller(9) has shown that binding of Vit. B_{12} to human serum proteins is enhanced by addition of gastric juice obtained from normals and pernicious anemia patients who presumably have little intrinsic factor. There is a good possibility that these 2 reactions of gastric juice with serum proteins and with the liver slice may be similar, since each is inhibited by pseudo-Vit. B_{12}. If this is so, the rat liver slice technic is not measuring intrinsic factor activity but is measuring another type of binding of Vit. B_{12} which, at the present time, is without known physiological significance.

Summary. (1) We have confirmed the work of Miller and Hunter and have shown that addition of hog intrinsic factor to rat liver slice will increase uptake of $Cobalt^{60}$ labeled Vit. B_{12}. (2) Gastric juices with intrinsic factor activity from other animal sources have different effects on this uptake and this is not correlated with intrinsic factor activity in the human or their ability to make Vit. B_{12} nondialyzable.

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Tissue Distribution of C^{14}-Labeled Bacterial Polysaccharide in Guinea Pig, Rat, Mouse and Rabbit.*† (24167)

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In the guinea pig the adrenal gland has the highest tissue concentration of C^{14} after intravenous injection of labeled *K. pneumoniae* polysaccharide - complex(1-3). C^{14} - labeled polysaccharide recovered by extraction of liver, spleen and adrenal partially retains the haptenic properties of the original polysaccharide(3). Apparently the bacterial polysaccharide(3), like C^{14}-labeled dextran in dogs (4), gradually disappears from animal tissues. Stark(5), however, studying mice injected with C^{14}-labeled pneumococcal polysaccharide, found the C^{14} content of splenic extracts almost unaltered for one year, while the antigenic property of such extracts was markedly reduced. The present study of distribution of *K. pneumoniae* polysaccharide in 4 common laboratory animals is pertinent to the species variation in physiologic responses and antibody formation following injection of bacterial products.

Materials and methods. The C^{14}-labeled polysaccharide-complex was extracted with 0.01 N KOH from *K. pneumoniae*, type B, which had grown at 37°C for 16 hours upon agar media containing 1-C^{14} acetate(3). The polysaccharide-complex had an isotopic activity of 0.678 μc/mg, was non-antigenic, but haptenic, and had no lethal toxicity. The polysaccharide was prepared as a 1% solution in 0.85% NaCl and sterilized by autoclaving. A single intravenous injection of the polysaccharide was given to guinea pigs (200 to 250 g body weight), Sprague-Dawley rats (100 to 130 g), Swiss albino mice (30 to 32 g) and New Zealand rabbits (2 to 2.5 kg). The rabbits received 0.5 mg/100 g body weight and the other species were given 1 mg/100 g body weight. Animals were sacrificed at 1, 3, 7, 14 and 30 days. Additional groups of guinea pigs and rats were given a single injection of the same quantity of polysaccharide by subcutaneous, intraperitoneal and intravenous routes and killed at 1, 3, 6, 12 and 24 hours and at 7 days. Determination of the C^{14} in plasma and tissue was carried out as previously described(3). C^{14} content is given as percent of injected dose and per gram dry tissue for liver, spleen and adrenals. The pooled mesenteric lymph nodes and pooled aliquots of liver of rats given a single intravenous injection of polysaccharide and killed at 7 days were ground in a Potter homogenizer and separated into insoluble and soluble portions by centrifugation. The polysaccharide fractions of lymph nodes and of liver were then removed from the soluble aqueous portion by the same technic used for extraction of the polysaccharide from *K. pneumoniae* and from liver, spleen and adrenal of the guinea pig(3). Precipitin tests were performed with heat-inactivated rabbit anti-Klebsiella serum and quantities of lymph node and liver extracts with C^{14} content equivalent to original bacterial polysaccharide/ml of antiserum in 1:1 dilution with 0.85% sodium chloride solution. C^{14} content of both precipitate and supernatant was determined(3). Controls

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