Assay of Intrinsic Factor Activity on Guinea Pig Intestinal Mucosa Homogenate.* (28149)

ZAIDA CASTRO-CUREL AND GEORGE B. JERZY GLASS
Gastroenterology Research Laboratory, Section of Gastroenterology, Department of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals, New York City

Several reliable *in vivo* tests are used to measure intestinal absorption of labeled B_{12} in man and to assay the intrinsic factor (IF) activity of materials of unknown potency. Despite this, there is a definite need for an *in vitro* test for IF activity to make the assay independent of available patients with pernicious anemia. Attempts to this end have met with various degrees of success.

The *in vitro* uptake of labeled B_{12} by rat liver slices(1,2), or rat liver homogenates(3, 4) did not correlate well with the isotope tests in humans(5,6) and raised theoretical objections(7). Electrophoretic quantitation of the IF-related B_{12} binder in hog gastric mucosa extracts(8) and in human gastric juice (9) is an indirect assay only. Measurement of absorption of labeled B_{12} in the everted rat intestinal sacs by Wilson and Strauss(10) and Herbert(11), on rat intestinal mucosa scrapings by Coates and Holdsworth(12), or on rat intestinal mucosa homogenized scrapings by Herbert and Castle(13), represents a good *in vitro* assay only for rat IF. Because of species differences, it cannot be applied with equal sensitivity to the assay of human and hog IF.

Wilson *et al.*(14) demonstrated that the guinea pig intestine was suitable not only for the guinea pig IF assay, but also for that of the human, hog, rat, rabbit, and hamster IF. Consequently, Wolff and Nabet(15) and Cooper *et al.*(16) used guinea pig everted intestinal sacs for assay of IF activity of human gastric juice. Since then, however, the technic of rat intestinal mucosa homogenates has proved to be easier and better duplicated than the method of rat everted intestinal sacs (13). Herbert and Castle, therefore, recently mentioned the possibility of using guinea pig intestinal mucosa homogenate for IF assay (13). Sullivan *et al.*(17) and Castro and Glass(18) have presented data on this subject. A more detailed report on the use of guinea pig intestinal mucosa homogenate for assay of IF activity follows.

Materials. Human native and lyophilized gastric juices were obtained from normal individuals, patients with duodenal ulcer and gastric hypersecretion, and pernicious anemia cases. Diagnoses were confirmed by hematological and isotope technics. The juices were immediately neutralized, centrifuged, filtered, and kept at 4°C until used, or dialyzed extensively and lyophilized. *Human gastric mucosa extracts* were prepared from the fundus and body of human stomachs removed by subtotal gastrectomy from patients with duodenal ulcer. 0.5 M glycine buffer of pH 9.0 was instilled into the lumen of the stomach before and during the operation. The gastric segment to be resected was clamped at both ends. Together with the buffer contained therein, it was immediately frozen and kept in the freezer until it was processed further. In the cold room the specimens were thawed, the intraluminal content was discarded, and the mucosa scraped off. The scrapings were immersed in a beaker containing neutral 0.9% NaCl, homogenized with neutral saline solution (0.9% or 2%) at the ratio of 1 g in 2 ml in a Waring blendor for 1 minute, then centrifuged at 6,000 $\times$ *g* for 30 minutes. The supernatants were decanted and the residue homogenized once more (1:1 w/v) and centrifuged at the same speed. Both supernatants were put together and referred to as "extracts." *Rat whole stomach extracts* were prepared from Sprague-Dawley rats as described by Nieweg *et al.*(19). *Rat gastric mucosa extracts* were prepared from the glandular portion of the stomachs of Sprague-Dawley rats kept on a sugar and water diet for 48 hours. Two hours after injection of atropine sulfate (20 mg/100 g weight)(20), the animals were sacrificed by

* Supported by Grant-in-Aid from Inst. of Arthritis and Metab. Dis., N.I.H., P.H.S.

a blow to the head; the abdomen was opened, the cardiac and pyloric ends of the stomach were ligated, and the stomachs were resected and immersed in cold saline. The glandular portion of the stomach was cut off, weighed, and homogenized twice in a Waring blendor for 1 minute with neutral 0.9% NaCl. After centrifugation, the supernatants were pooled and stored in the freezer.

Methods. Guinea pig intestinal mucosa homogenates (GPIMH) were prepared by the same method as the rat intestinal mucosa homogenates of Herbert and Castle(13), with the following modifications: 1) The guinea pig intestine was transected not at the pyloric, but at the cecal end, since Wilson *et al.*(14) and Wolff and Nabet(15) have shown that the most active Vit. B_{12} absorption is localized in the distal part of the ileum in the guinea pig. Only the distal 3 feet were, therefore, used for the mucosal scrapings. 2) The scrapings of the mucosa were done according to the method of Borgstrom and Dahlquist(21) who used the homogenized scrapings of hog intestinal mucosa for the study of enzymes. 3) We omitted the step of manual shaking of the suspension of scrapings to avoid foaming and introduction of individual variations in a standardized procedure. Furthermore, we did not use both the particulate floating material and the sediment for the assays, as Herbert and Castle did with the rat intestine, but used only the sediment, since the floating fatty layer was not always present. Finally, we adjusted the pH of saline to pH 7.0. The pH of the available 0.9% NaCl is often below 6.0, and this can lower the final pH of the buffered system.

With these modifications, we prepared the GPIMH as follows:

Fresh GPIMH: Non-fasting animals were killed by a blow to the head. The small intestine was transected at the cecal end of the ileum and freed from the mesentery by pulling out the lower transected end. A 3-foot segment of intestine was then cut off and placed in cold, neutralized physiological saline solution. It was cut in half and everted with a glass rod. The intestinal content was removed by dipping into 3 changes of neutral, cold physiological saline, following which the intestine was cut into segments approximately 5 cm long. Each of the segments was placed on a glass tray and scraped with the aid of 2 glass slides. All the scrapings were then immediately placed into a beaker containing 20 ml neutral physiological saline, and transferred with saline rinsing into a Waring blendor. They were homogenized for 1 minute at the maximal blendor's speed, and transferred into plastic centrifuge tubes, following which they were centrifuged at 1300 $\times$ *g* for 10 minutes. A fatty floating layer occasionally appeared, which was skimmed off and discarded. The supernatant was decanted, and only the precipitate was used as "fresh GPIMH." It was resuspended in neutral physiological saline to make up a volume of 20 ml for each distal 3 feet of 2 guinea pig intestines so that 1 ml of this suspension used for the assay corresponded to 1/20 of the homogenized scrapings of the distal 3 feet of 2 animals. Thus, 20 assays were possible with the homogenized scrapings of 2 guinea pigs.

Lyophilized homogenates were prepared from a pool of fresh scrapings from the distal 3 feet of the small intestine of 20 guinea pigs, processed on the same day, extensively dialyzed against cold running water for 16 hours, lyophilized, and stored in a desiccator at 4°C. An aliquot, corresponding to the distal 3 feet of the small intestine of one guinea pig, resuspended in neutral 0.9% saline was employed for 10 assays.

Assay on GPIMH: To a 25 ml beaker containing 5 ml of Krebs-Henseleit bicarbonate glucose medium, the following were added in the order listed: 1) 1 ml of the dilution of IF containing material in saline; 2) 1 ml of $Co^{57}B_{12}$ solution containing 500-5000 $\mu\mu$g of labeled B_{12}, the specific activity of which was to provide 7-10 cpm per 1 $\mu\mu$g when counted in the heavily shielded well counter attached to the gamma-analyzer; and 3) 1 ml of a suspension of fresh or of lyophilized GPIMH in 0.9% NaCl solution, which corresponded to 1/10 of the distal 3 feet of the small intestine of one guinea pig. The mixtures were incubated in a Dubnoff shaking incubator in the air, at 37°C, for

TABLE I. Uptake of $Co^{57}B_{12}$ by 10 Samples of Dialyzed and Lyophilized Homogenates of Guinea Pig Small Intestinal Mucosa Incubated with 2,500 $\mu\mu$g $Co^{57}B_{12}$ and 0.05 ml of a Pool of Normal Native Human Gastric Juice.

Specimen	Uptake of $Co^{57}B_{12}$ ($\mu\mu$g)
1	104
2	117
3	146
4	134
5	135
6	146
7	139
8	138
9	153
10	172
Mean	140.0
Standard deviation	14.5
Confidence range (mean ± 2 st. dev.)	111-169.

one hour. After incubation, the samples were quantitatively transferred to Lusteroid plastic tubes and centrifuged at 1300 × *g* for 10 minutes. The supernatants, containing an excess of unbound $Co^{57}B_{12}$ were decanted and the residues were washed twice with 7 ml of physiologic saline, containing 10 mM $CaCl_2$. The upper part of the plastic tubes was cut off and the tubes were placed in a heavily shielded well counter. The counts were recorded for 5 minutes at an optimal setting of the gamma-analyzer for Co^{57}; this permitted us to obtain a background as low as 11-18 cpm, with counts of the samples ranging from 280 to 3040 cpm. The counts were corrected for background and physical decay, and were recalculated into $\mu\mu$g of $Co^{57}B_{12}$, using $Co^{57}B_{12}$ standards.

Each GPIMH assay was carried out in duplicate, and mean values of the uptake were calculated from 10 identical assays in the dialyzed and lyophilized GPIMH system listed in Table I. The same level of $Co^{57}B_{12}$ and the same amount of a pool of native human gastric juices were used. Mean uptake under these conditions, calculated with standard deviations, was 140 ± 14.5 $\mu\mu$g and standard error of the method was 4.8%. It appeared convenient to calculate the enhancement of $Co^{57}B_{12}$ uptake produced by the IF-containing materials in the GPIMH system in per cent over the control base line, obtained with saline, thus: E in % = (U − C) × 100/C, in which U is uptake in presence of IF, and C control uptake with saline in absence of IF. Any positive value means enhancement in %; any negative value, inhibition of uptake. We do not confer any significance to enhancement of inhibition below 50% when fresh GPIMH homogenates were employed, and below 30% when lyophilized homogenates were used.

Results. The guinea pig intestine takes up a certain amount of $Co^{57}B_{12}$ in this GPIMH assay, without any IF added from extraneous sources. Individual variations in the rate of this uptake were determined for the fresh GPIMH (Table II). *Uptake of $Co^{57}B_{12}$ by freshly processed homogenates* prepared by the method described from 20 pairs of guinea pigs and analyzed in duplicate averaged 146 ± 42 $\mu\mu$g, calculated with standard deviation when incubated with 2,500 $\mu\mu$g $Co^{57}B_{12}$ in saline. The 95% confidence range of the uptake by GPIMH under these circumstances was from 62-230 $\mu\mu$g of $Co^{57}B_{12}$. *Uptake of $Co^{57}B_{12}$ by the dialyzed and lyophilized pool of GPIMH,* prepared from 10 pairs of guinea pigs was, as mentioned above, 140 ± 14 $\mu\mu$g, when calculated as a mean with standard deviation, the confidence range being 111-168 $\mu\mu$g. The uniformity of the lyophilized GPIMH pool appears to be thus

TABLE II. Uptake of $Co^{57}B_{12}$ in $\mu\mu$g by Fresh GPIMH's.

Assay	Sample 1	Sample 2	Mean
1	138	127	133
2	55	53	54
3	101	124	113
4	99	147	123
5	162	205	184
6	158	153	156
7	183	176	181
8	127	134	131
9	124	129	127
10	172	178	175
11	219	208	214
12	230	232	231
13	106	118	109
14	180	180	180
15	174	178	176
16	136	119	128
17	130	143	137
18	130	137	134
19	126	137	132
20	96	93	95
Mean			146
Standard deviation			42
Confid. range (mean ± 2 st. dev.)			62-230

TABLE III. $Co^{57}B_{12}$ Uptake by Fresh GPIMH's Incubated Alone and with IF-Containing Materials from Human and Rat Stomachs, at Various Levels of $Co^{57}B_{12}$.

IF material			$Co^{57}B_{12}$ in $\mu\mu$g		Uptake of $Co^{57}B_{12}$ above control in %
Preparation	#	Amt	Added	Taken up	
Native pooled normal human gastric juice	a*	0 ml	500	115	0
		.05 "		95	−19
		.10 "		77	−35
	b	0 "	1000	101	0
		.05 "		158	57
		.10 "		154	52
	c	0 "	1250	107	0
		.01 "		140	31
		.025 "		174	65
		.05 "		210	96
	d	0 "	2500	174	0
		.05 "		306	75
		.10 "		307	76
	e	0 "	5000	293	0
		.01 "		301	3
		.025 "		324	11
		.05 "		386	32
		.1 "		414	41
		.25 "		362	26
Extract from human gastric mucosa #5 + #6	a	0 g	500	60	0
		.02 "		93	38
	b	0 "	1250	69	0
		.025 "		231	235
	c	0 "	2500	131	0
		.025 "		308	135
	d	0 "	5000	166	0
		.12 "		235	42
Rat whole stomach extract	a	0 "	1250	107	0
		.005 "		159	48
		.01 "		182	70
		.02 "		182	70
	b	0 "	1500	115	0
		.01 "		193	59
		.02 "		170	47
	c	0 "	2000	104	0
		.01 "		201	93
		.02 "		199	91
	d	0 "	2500	114	0
		.01 "		227	99
		.02 "		238	108

* Letters refer to various assays on the same material.

greater than that of freshly prepared homogenates.

The dependence of B_{12} uptake by GPIMH from *the ratio of IF-containing material to the level of* $Co^{57}B_{12}$ *used,* was determined by increasing concentrations of normal gastric juice and whole stomach extracts, and adding them to various levels of $Co^{57}B_{12}$. The assays were done on the same day and on the same batch of GPIMH. Results obtained are listed in Table III; 2,500 $\mu\mu$g $Co^{57}B_{12}$ in the GPIMH system represents the best compromise figure to assure the saturation of B_{12} binding capacity of the human and rat IF, and to provide high vit. B_{12} uptake by the intestinal mucosa. At 1250 $\mu\mu$g, the increase of uptake is sometimes higher in percentage than with 2,500 $\mu\mu$g, but the net increase in terms of cpm is lower than with 2,500 $\mu\mu$g dose. At 5,000 $\mu\mu$g dose there is a decrease in percentage in the enhancing effect of the IF upon B_{12} uptake by the GPIMH. Too little $Co^{57}B_{12}$ in the system (500 $\mu\mu$g) makes it inefficient, and reveals no enhancement of uptake, even in the presence of active IF materials. Overloading of the system with excessive amounts of IF decreases the efficiency of the assay

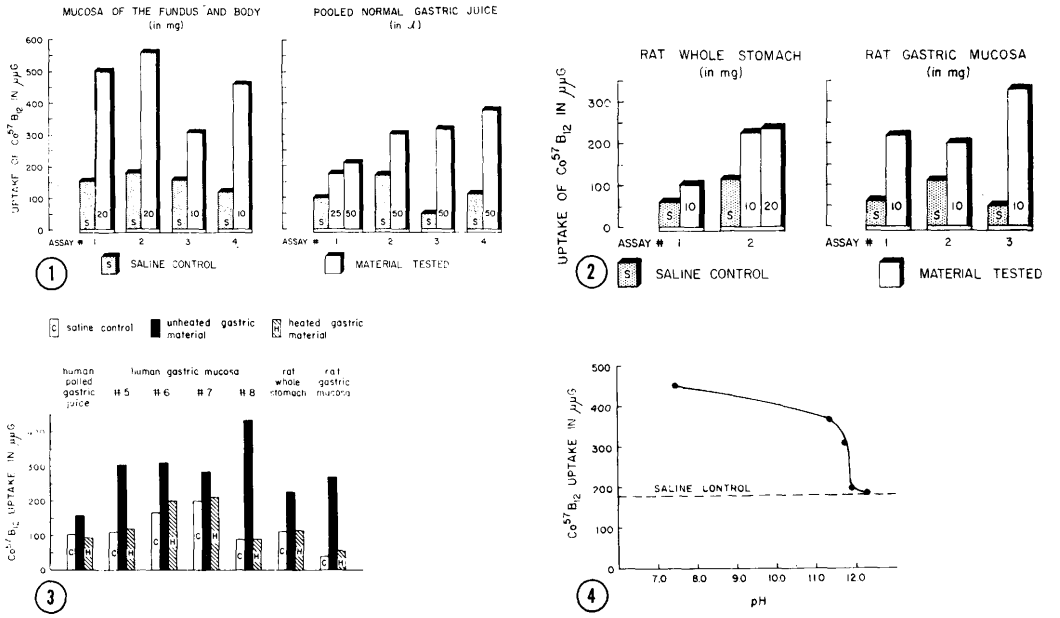

FIG. 1. Uptake of $Co^{57}B_{12}$ by guinea pig intestinal mucosa homogenate. Figures in columns of mucosa of fundus and body refer to mg of wet wt original mucosa from which extract was prepared. In columns of gastric juice, figures refer to μl (λ) of gastric juice used for assay. Each assay number refers to individual samples from different materials.

FIG. 2. Uptake of $Co^{57}B_{12}$ by guinea pig intestinal mucosa homogenate. Figures in columns refer to mg of wet wt of rat whole stomach or rat gastric mucosa, respectively, used for assay. The rat whole stomach extract, as well as rat gastric mucosa extract were prepared from a pool of several rat stomachs.

FIG. 3. Effect of heating gastric materials on $Co^{57}B_{12}$ uptake by GPIMH.

FIG. 4. Effect of alkalinization of human gastric mucosa extract on uptake of $Co^{57}B_{12}$ by GPIMH.

and tends to reduce the differences in uptake in the presence and absence of added sources of IF (Table III). The optimal amounts of IF-containing materials, to promote highest $Co^{57}B_{12}$ uptake by the fresh GPIMH at 2,500 μμg $Co^{57}B_{12}$ level, are apparent from Table III.

The results obtained with various *dialyzed and lyophilized IF materials from human and animal sources* are graphically shown in Fig. 1 and 2. A 2-5-fold enhancement of uptake was obtained by a) dialyzed and lyophilized human gastric mucosa, b) 3 pools of lyophilized human gastric juices from normals or patients with gastric hypersecretion, c) rat whole stomachs, and d) rat gastric mucosa extract. One of the 5 extracts prepared from the fundal mucosa of the stomachs of patients with duodenal ulcer was also assayed for IF activity by Schilling test at the dose equivalent of 20 g of wet mucosa on a patient with pernicious anemia in remission. This was done to determine whether the technic used for extraction did not impair IF activity of these materials, which are known to represent a potent source of IF. This material, when tested at the dose of the extract equivalent to 20 g wet weight of gastric mucosa, gave 23.7% urinary excretion in 48 hours on Schilling test when given together with 0.5 μg $Co^{57}B_{12}$. This proved that the technic used for extraction and lyophilization had preserved potent IF activity of fundal gastric mucosa. Some of the gastric juices collected from normals or patients with duodenal ulcer after Histalog (25 mg subcut.), were dialyzed and lyophilized within a few days after collection. Because they were stored in the refrigerator for a few days after collection, we ascertained whether their IF activity was lost after the process of dialysis and lyophilization. One of these juices was tested for its IF activity by Schilling test on a patient with pernicious

TABLE IV. Comparison of *in vivo* and *in vitro* IF Activity of Various Materials from Human Stomach.

Materials tested		Uptake of $Co^{57}B_{12}$ on GPIMH		Schilling test			Hepatic uptake
IF source	Dose	($\mu\mu$g)	(% above control)	PA patient	Dose of tested material	Urinary excretion (%)	
Human gastric mucosa extract	0	51	0	A.L.	20 g*	23.7	
	.005 g	194	280				
	.01 "	258	405				
	.02 "	298	484				
	.04 "	240	370				
	.10 "	154	201				
Fresh native human g.j.'s collected from 5 patients with peptic ulcer and normals	0	314	0	G.S.	30 ml	9.9	
	.025 ml	731	133				
	.05 "	920	192				
	.10 "	1038	230				
	0	218	0	M.L.	30 "	6.2	
	.025 "	521	139				
	.05 "	740	239				
	.10 "	1090	400				
	0	218	0	G.S.	30 "	17.7	
	.025 "	536	145				
	.05 "	687	216				
	.10 "	823	277				
	0	498	0	G.S.	30 "	18.4	
	.025 "	1137	128				
	.05 "	1096	120				
	.10 "	1061	113				
	0	369	0	G.S.	30 "	29.1	
	.025 "	950	156				
	.05 "	1128	205				
	.10 "	990	167				
6-mo-old g.j. collected after histamine from atrophic gastritis patient	0	359	0	G.S.	30 "	1.6	
	.025 "	418	13				
	.05 "	384	8				
	.10 "	318	−11				
Dial. and lyophilized g.j. from patient with gastric hypersecretion	0	168	0	G.S.	30 mg	15.7	
	.05 mg	170	150				
	.10 "	186	173				
Dial. and lyophilized g.j.'s from 2 PA patients	0	116	0	—	—	—	negative (but corrected by HIFC)
	.05 "	165	42				
	.10 "	184	58				
	0	116	0	G.S.	30 "	1.85	
	.05 "	154	32				
	.10 "	142	29				

* Wet weight of original mucosa.

anemia. Its IF activity was potent at the dose of 30 mg (15.7% excretion of radioactivity) (Table IV).

We did not test the IF activity *in vivo* of rat stomach, since the method used for this preparation was the one described by Nieweg(19) which is known to preserve its IF activity well.

The results of GPIMH assay and Schilling tests performed *on 5 native human gastric juices from normals and patients with peptic ulcers and atrophic gastritis,* as well as on *2 lyophilized juices from patients with pernicious anemia,* are listed in Table IV. All 5 native gastric juices from normal controls and patients with duodenal ulcer markedly enhanced the uptake of labeled B_{12} by the lyophilized GPIMH (Table IV). This enhancement ranged from 170 to 426% above control value. It was highest in 2 of the

juices at the 0.05 ml dose, and in 2 others at the 0.1 ml dose, when assayed with 2,500 $\mu\mu$g of labeled B_{12}. All 5 native gastric juices were also tested for IF activity on 3 patients with pernicious anemia in remission, by means of Schilling test, and were found potent, as shown by urinary excretion ranging from 6.2 to 18.4% at the dose of 30 ml (Table IV).

One native gastric juice of a patient with atrophic gastritis, which was stored for over 4 months in the refrigerator, did not show appreciable enhancement of B_{12} uptake on the GPIMH (Table IV). It was also devoid of IF activity on Schilling test (1.6% excretion with 70 ml dose of the gastric juice). Two dialyzed and lyophilized gastric juices of 2 patients with proven pernicious anemia also showed very little activity on the GPIMH system, and only enhanced the uptake of B_{12} from 29 to 58% at the 0.05-0.1 mg doses. Both of these pernicious anemia cases showed negative Schilling test which was corrected by administration of potent IF preparation. One of these pernicious anemia gastric juices was also directly tested for its IF activity on another patient with pernicious anemia in remission, by means of Schilling test. This resulted in urinary excretion of 1.85% of radioactivity at a 30 mg dose of the lyophilized gastric juice, confirming its inactivity as a source of IF.

Fig. 3 shows that immersion of the *IF*-containing materials from human and rat sources in *boiling* water for 10 minutes resulted in reduction of the $Co^{57}B_{12}$ uptake on the GPIMH system to the levels of the saline controls or close to it. Fig. 4 shows the effect of the *alkalinization of IF*-containing materials upon the GPIMH uptake system. Twenty ml aliquots of human gastric mucosa extracts were incubated in 0.1 M glycine buffers of various pH's (measured in Beckman Zeromatic pH meter) at 37°C for 30 minutes. IF activity was compared with that of the same materials when incubated for the same length of time with 0.1 M phosphate buffer at pH 7.4 and with the same batch of GPIMH. IF activity was hardly affected by alkalinization to pH 10.0-10.5, somewhat reduced at pH 11.0-11.7, and completely destroyed at pH about and above 12.0 (Fig. 4).

Discussion. The GPIMH system appears to be well suited for assay of IF activity of gastric materials derived from human gastric juices, extracts from mucosa of fundus and corpus of the stomach of man, and extracts from the whole rat stomach and rat gastric mucosa. By prior injection of atropine, which prevents the discharge of products of glandular secretion from the glands of the rats(20), we attempted to enrich the rat mucosal extracts in IF. These extracts were more active than those of the whole rat stomach prepared by the method of Nieweg *et al.*(19). This difference could have been due, however, to a different technic of preparing the extracts and to a lesser proportion of the glandular mucosa in the whole extracts.

The assay on GPIMH gains more significance if labeled B_{12} of high specific activity is used and if the dose of IF to labeled B_{12} is carefully determined for each of the materials tested. Therefore, it is necessary to perform the assay at various dose levels. This situation is similar to that observed in assays of IF activity in man(22,23), where an excess of IF may decrease the intestinal uptake of B_{12}(24). The same is true for *in vitro* IF assays on rat liver slices and homogenates (1-4), rat(11) and guinea pig(14,15) everted intestinal sacs, and rat intestinal mucosa homogenates(13).

The uptake of Vit. B_{12} by GPIMH follows the principle of regression of % efficiency on increase of the dose, which has been established for the absorption of B_{12} in the human intestine(25). As shown by data in Table III with increase of the B_{12} dose from 500 to 5000 $\mu\mu$g, there is a gradual decrease of % uptake by the saline control from 12-23% to 3.3-5.8%. Similarly to conditions observed in human intestine, this is paralleled by gradual increase of the amount of Vit. B_{12} taken up by GPIMH on increase of the dose.

The pools of dialyzed and lyophilized GPIMH give more uniformity in assays than freshly made homogenates, where the results depend on the individual features of the intestinal mucosa. This corroborates the conclusions of Herbert and Castle(13) and Sul-

livan *et al.*(17) as to the applicability of lyophilized intestinal mucosa homogenates for IF assay.

The well known thermolability of the IF was corroborated by the reduction of uptake of B_{12} by the GPIMH system to levels obtained with saline controls when IF material was heated prior to assay. The abolishment of the IF activity when extracts were exposed to the pHs above 12.0 has not to our knowledge been recorded in other IF assays. Its mechanism is not clear. It may be due to the inactivation of IF itself at this pH, to denaturation of the protein which may occur at this pH, or to the cleavage of the polysaccharide groups from the peptide moiety which is known to occur in mucopolysaccharides or mucoproteins at high alkaline pHs. Alkalinization of the human gastric mucosa extracts to pH of 10.0 did not affect IF activity in the GPIMH system, as it has previously been ineffective in regard to IF activity of human gastric juice on *in vivo* assays(26,27).

The lack of significant IF activity of lyophilized gastric juice from pernicious anemia patients in GPIMH agrees with what is known about the loss or reduction of IF in the stomachs of patients with pernicious anemia. Our preliminary (unpublished) data obtained on lyophilized and native gastric juices of other pernicious anemia patients and those with histamine fast anacidity without pernicious anemia, however, show some overlapping. This may suggest prudence in using the GPIMH assay for differentiation of these 2 conditions, and indicate that only the positive result of the test is significant as an assay of IF activity, while the negative result may have various interpretations.

Summary and conclusions. The results of the assay of IF activity on fresh or dialyzed and lyophilized guinea pig intestinal mucosa homogenates (GPIMH) with the use of $Co^{57}B_{12}$ are reported. This method has been found suitable for assay of IF activity of native and lyophilized human gastric juices, extracts from human gastric mucosa, rat whole stomach and rat gastric mucosa. The prerequisite for significant enhancement of B_{12} uptake by the IF-containing materials is an optimal ratio of concentrations of IF to B_{12} and use of labeled vitamin of high specific activity. Heating of IF sources in boiling water, as well as alkalinization to the pH of 12 and above, reduces B_{12} uptake to levels obtainable with saline. While native human gastric juices from normal controls or duodenal ulcer patients showed a marked enhancement of B_{12} uptake by GPIMH, lyophilized gastric juices of 2 pernicious anemia patients showed very low IF activity. A gross correlation was found between results of the GPIMH assays and IF activity of gastric juices when tested by Schilling test on pernicious anemia patients in remission.

1. Miller, O. N., Hunter, F. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 39.

2. Herbert, V., *ibid.*, 1958, v97, 668.

3. Minard, F. N., Wagner, C. L., *ibid.*, 1958, v98, 684.

4. Herbert, V., Castro, Z., Wasserman, L. R., *ibid.*, 1960, v104, 160.

5. Johnson, P. C., Driscoll, T. B., *ibid.*, 1958, v98, 731.

6. Herbert, V., *The Megaloblastic Anemias,* Grune & Stratton, N. Y., 1959, p162.

7. Gräsbeck, R., *Advances in Clin. Chem.,* Acad. Press, New York, 1960, v3, 299.

8. Barlow, G. H., Frederick, K. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 400.

9. Glass, G. B. J., Castro, Z., Uchino, H., Schwartz, G., Ishimori, A., *Symposium on Vitamin B_{12} and Intrinsic Factor,* H. C. Heinrich, Ed., F. Enke, 1962, 520.

10. Strauss, E. W., Wilson, T. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 224.

11. Herbert, V., *J. Clin. Invest.,* 1959, v38, 102.

12. Coates, M. E., Holdsworth, E. S., *Vit. B_{12} Symposium,* VIIth Internat. Congr. of Hematology, Rome, Hematologica Latina, 1959, v2, 199.

13. Herbert V., Castle, W. B., *J. Clin. Invest.,* 1961, v40, 1958.

14. Wilson, T. H., Strauss, E. W., Hotchkiss, A., *Am. J. Physiol.,* 1959, v197, 926.

15. Wolff, R., Nabet, P., *Symposium on Vitamin B_{12} and Intrinsic Factor,* H. C. Heinrich, Ed., F. Enke, 1962, 514

16. Cooper, B. A., Paranchych, W., Lowenstein, L., *J. Clin. Invest.,* 1962, v41, 370.

17. Sullivan, L. W., Herbert, V., Castle, W. B., *Clin. Research,* 1962, v10, 208.

18. Castro, Z., Glass, G. B. J., *ibid.*, 1962, v10, 197.

19. Nieweg, H. O., Arends, A., Mandema, E.,

Castle, W. B., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 328.

20. Hirschowitz, B. I., *Am. J. Phys.*, 1960, v198, 108.

21. Borgstrom, B., Dahlquist, A., *Acta Chem. Scand.*, 1958, v12, 1997.

22. Glass, G. B. J., Boyd, L. J., Rubinstein, M. A., Svigals, C. S., *Science*, 1952, v115, 101.

23. Best, W. R., White, K. C., Robbins, W. A., Landmann, W. A., Steelman, S. L., *Blood*, 1956, v11, 338.

24. Glass, G. B. J., Boyd, L. J., Stephanson, L., Jones, E. L., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 1.

25. Glass, G. B. J., Boyd, L. J., Stephanson, L., *ibid.*, 1954, v86, 522.

26, Flood, C., West, R., *ibid.*, 1936, v34, 542.

27. Gräsbeck, R., *Acta Med. Scand.*, 1956, Suppl. 314, 87pp.

Received July 3, 1962. P.S.E.B.M., 1963, v112.

Effect of Sympathetic Blocking Agent on Plasma FFA and on the Response Evoked by Catechol Amines. (28150)

AARNE KONTTINEN AND MATTI RAJASALMI (Introduced by M. D. Bogdonoff)
Wihuri Research Institute, Helsinki, Finland

The regulatory role of the autonomic nervous system on plasma free fatty acid (FFA) has been shown in several investigations(1,2). These fatty acids with a rapid metabolic rate(3,4) are known to represent a potential pool for energy requirements(5-7) and are mobilized from fat depots, as reviewed by Bogdonoff *et al.*(8). Administration of epinephrine or norepinephrine has resulted in a prompt rise in plasma FFA concentration(9-12). The increase observed in connection with different anxiety states has also been stated to occur under the influence of these amines.

The rise in plasma FFA concentration evoked by epinephrine was counteracted by the ganglionic blocking agent hexamethonium (11), but the ganglionic blocking agent trimethaphan was without effect in this respect (13). Of the adrenergic blocking agents, which are believed to make adrenergic nerves insensitive to catechol amines, dibenzyline and dibenamine did not depress the rise in plasma FFA evoked by administered amines but ergotamine did so occasionally(13). The present experiment was carried out to observe the effect of a new type of blocking agent, the sympathetic blocking agent guanethidine*, [2-(octahydro-1'-azocinyl)-ethyl]-guanidine-sulfate, on plasma FFA concentration. This compound has a post-ganglionic blocking effect, which is believed to be mediated by the depletion of epinephrine and norepinephrine(14-15).

Materials and methods. The present experiment was carried out with 16 rabbits averaging 2.2 kg in weight (range 1950-2350 g), fed hay and standard dry-pressed food supplemented with vitamins and minerals. Food and water were allowed *ad lib.* The animals were accustomed to this diet from birth, and to the external conditions for one month before beginning the trial. The study was in 2 parts with an interval of one month. In both instances blood was taken from the ear vein at the same hour in the morning.

The first blood sample was taken just before administration of epinephrine (8 animals) or norepinephrine (8 animals). These amines were injected into the ear vein in isotonic sodium chloride solution. The dose was 2 μg/kg. After 10 minutes the second blood sample was collected from the other ear vein. The other part of the experiment was done in the same manner under the influence of the sympathetic blocking agent, which was injected into the ear vein 24 hours earlier in a dose equivalent to 15 mg guanethidine per kg. Thus each animal served as its own control. FFA was determined from the plasma(16) in duplicate, giving a reproducibility of $\pm$ 10 μEq/l. In addition blood sugar was measured by Somogyi's method.

Results. Mean values for plasma FFA and

* Ismelin®, kindly supplied by Ciba A. G., Basel.