sis of new glyceride ester bonds. 3. Free labelled chimyl alcohol has been isolated from the intestinal contents after feeding labelled chimyl dioleate indicating an exchange and release of fatty acids in both 1-, and 2-position in the mixed ether-glyceride. 4. In lymph lipids more than half the radioactivity was associated with palmitic acid, indicating a splitting of the ether bond in mucosa cells. 5. It is concluded that chimyl alcohol can be absorbed unchanged but that it is extensively metabolized already in the mucosa cells, whether it is fed as free chimyl alcohol or as an alkoxydiglyceride.

1. Bergström, S., Blomstrand, R., *Acta Physiol. Scand.,* 1956, v38, 166.

2. Blomstrand, R., Ahrens, E. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 802.

3. Holmes, H. N., Corbet, R. E., Geiger, W. B., Kornblum, N., Alexander, W., *J. Am. Chem. Soc.,* 1941, v63, 2607.

4. Borgström, B., *Acta Physiol. Scand.,* 1952, v25, 111.

5. Bergström, S., Blomstrand, R., Borgström, B., *Biochem. J.,* 1954, v58, 600.

6. Desnuelle, P., Constantin, M. J., *Biochim. et Biophys. Acta,* 1952, v9, 531.

7. Mattson, F. H., Benedict, J. H., Martin, J. B., Bech, L. W., *J. Nutrition,* 1952, v48, 335.

8. Borgström, B., *Arch. Biochem. and Biophys.,* 1954, v49, 268.

9. Reiser, R., Dieckert, J. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 649.

10. Borgström, B., *Biochim. et Biophys. Acta,* 1954, v13, 491.

11. Blomstrand, R., Tryding, N., Westöö, G., *Acta Physiol. Scand.,* 1956, v37, 91.

Received August 31, 1959. P.S.E.B.M., 1959, v102.

Inhibition of Gastric Secretion in the Rat by Normal Human Gastric Juice.* (25354)

RENE MENGUY AND WILLIAM O. SMITH (Introduced by James F. Hammarsten)
Depts. of Surgery and Medicine, Univ. of Oklahoma Medical Center and Vet. Admin. Hosp., Okla. City

Presence of a substance in normal human gastric juice which inhibits Heidenhain pouch secretion in dogs when administered intravenously has now been well established(1,2). This substance is not dialyzable, is resistant to boiling and is largely inactivated by drastic acidification(2). It appears to inhibit specifically hydrochloric acid and water secretion (2). Studies aimed at identification and isolation of the active substance have been hindered by certain disadvantages inherent to the Heidenhain pouch preparation. Of these, the foremost is gradual development of gastric atrophy after the animal received several injections of gastric juice(3). Our purpose was to determine the feasibility of using the pylorus ligated rat preparation(4) for determination of inhibitory activity of preparations of normal human gastric juice.

Materials and methods. Gastric juice was collected from hospital patients previously found free of any gastrointestinal pathology. Following an overnight fast, a 4 hour collection of insulin stimulated gastric secretion was carried out. Immediately after collection, the juice from several subjects was pooled, dialyzed 36 hours against distilled water and lyophilized. Approximately 1 mg of material/1 ml of gastric juice was obtained. Immediately prior to use, this material was resuspended in normal saline and brought to pH 7.0. Gastric secretion was measured by pylorus ligation technic(4) in 65 male Holtzman rats weighing 160 to 220 g. Prior to experiments, rats were fasted 48 hours in individual cages with wide mesh wire bottoms. Water was given *ad lib.* Rats were operated upon under light ether anesthesia, and pylorus was ligated with a 4-0 silk ligature. Rats were divided randomly into control group and 3 treatment groups. Control rats were given 1 ml of normal saline; treated rats received

* The authors acknowledge valuable technical assistance of Howard Guiles.

TABLE I. Effects of Human Serum Albumin and Normal Human Gastric Juice on 4 Hour Gastric Secretion in the Pylorus Ligated Rat.

	No. rats	Wt (g)†	Vol (ml)†		Free HCl (meq/l)†	
Controls, 1 ml saline*	17	181 ± 12	6.3 ± 1.5		110 ± 22	
Treated, 6 mg albumin*	15	179 ± 10	6.0 ± 1.7		111 ± 7	
" , 2 mg human gastric juice*	14	176 ± 12	3.2 ± 1.8	p .001	94 ± 25	p .1
" , 6 mg " " " *	19	175 ± 14	1.3 ± .6	p .001	43 ± 28	p .001

* Intrav. inj. † Mean ± stand. dev. p = probability of significance of t value.

either 6 mg of human serum albumin, 6 mg of normal human gastric juice or 2 mg of normal human gastric juice in 1 ml of normal saline. Doses were on basis of 200 g of rat weight and were given intravenously by single injection into the vena cava at time of pyloric ligation. Exactly 4 hours later the animals were killed by exsanguination, the stomachs removed and gastric content was measured individually for volume, free acidity and in some instances for peptic activity. The latter was measured by modification of the method of Anson and Mirsky(5).

Results. The results are summarized in the Table. In animals receiving normal saline the mean volume of 4 hour gastric secretion was 6.3 ml with a free HCl concentration of 110 meq/l. Essentially no changes in these values occurred in rats given human serum albumin. In rats given 2 mg of human gastric juice the mean volume of 4 hour gastric secretion was 3.2 ml with a free HCl concentration of 94 meq/l. These values were 1.3 ml and 43 meq/l respectively in rats receiving 6 mg of the material. In the few experiments in which it was measured, peptic activity of gastric content was not altered by administration of human gastric juice.

Rectal temperatures of rats were taken before and after injections. No fever was produced by the gastric juice preparation.

Discussion. Characteristically, the rat stomach secretes a large volume of highly acid interdigestive secretion which is quite consistent from one animal to another of similar weight. For this reason the rat is an excellent animal in which to study agencies inhibiting gastric secretion. Previous attempts to use the rat for assay of other biologic gastric inhibitors such as enterogastrone(6) have failed. However, it appears that "gastrone" which is the name given by Code to the inhibitory substance present in human gastric juice has a consistent inhibitory effect on the stomach of the rat. Although doses required to produce inhibition in rats are relatively larger than those resulting in the same degree of inhibition in dogs, the total doses are considerably smaller.

No change in pepsin output was observed, thus confirming an earlier observation on dogs (2); the inhibitory effect is specific for water and acid secretion. Failure of human serum albumin to alter gastric secretion suggests that the action of human gastric juice is not a nonspecific one due to a foreign protein.

The active principle in human gastric juice responsible for inhibition of gastric secretion in animals has not yet been identified. The recently described resin column chromatography of Richmond *et al.*(7) for separation of large molecular components of gastric juice may be useful in isolation of the inhibitory factor. Considering the small amounts of fractionated material available for assay and the many assays which may be necessary, the pylorus ligated rat preparation appears to be the most satisfactory test preparation for this purpose.

Summary. Human gastric juice from subjects free of gastrointestinal pathology was collected, pooled, dialyzed and lyophilized. The material thus obtained consistently inhibited gastric secretion of pylorus ligated rats when administered intravenously in amounts of 2 or 6 mg at time of pyloric ligation.

1. Blackburn, C. M., Code, C. F., *Am. J. Physiol.,* 1948, v155, 427.

2. Smith, W. O., Hoke, R., Landy, J., Caputto, R., Wolf, S., *Gastroenterology,* 1958, v34, 181.

3. Smith, W. O., Joel, W., Wolf, S., *Tr. A. Am. Physicians,* 1958, v71, 306.

4. Shay, H., Sun, D. C. H., Gruenstein, M., *Gastroenterology,* 1954, v26, 906.

5. Anson, M. L., Mirsky, A. R., *J. Gen. Physiol.,* 1932, v16, 59.

6. Morris, C. R. Grossman, M. I., Ivy, A. C., *Am. J. Physiol.*, 1947, v148, 382.
7. Richmond, V., Caputto, R., Wolf, S., *Gastroenterology*, 1955, v29, 1017.

Received August 31, 1959. P.S.E.B.M., 1959, v102.

Counting Efficiency of Co^{56}, Co^{58} and Co^{60} with 2 Counters and 3 Counting Technics. (25355)

LEON ELLENBOGEN (Introduced by B. L. Hutchings)
Lederle Laboratories Division, Am. Cyanamid Co., Pearl River, N. Y.

The technic for measuring Vit. $B_{12}Co^{60}$ of low specific activity contained in one liter of solution on a NaI well scintillation counter has been described(1). Hine and Miller(2) reported on efficiency of counting Vit. $B_{12}Co^{60}$ in a large plastic scintillator capable of holding 60-80 ml. Recently the Co^{58} and Co^{56} isotopes have been used in studies with radioactive Vit. B_{12}. This paper reports on the counting efficiency of Co^{56}, Co^{58}, Co^{60} as a function of sample volume in both a modified plastic scintillator and the conventional NaI well and the merit of the counting systems

Methods. Vit. $B_{12}Co^{58}$ and Vit. $B_{12}Co^{60}$ were purchased from Merck & Co. and Vit. $B_{12}Co^{56}$ was generously supplied by Dr. E. Lester Smith of Glaxo Labs., Greenford, England. 0.25 μc aliquots of Vit. $B_{12}Co^{56}$, Co^{58} and Co^{60} were counted in the NaI well using a standard vial up to a maximum capacity of 4 ml and in a beaker containing one liter adapted to fit on and around the NaI well. In addition the 3 isotopes were counted in the plastic well scintillation counter. The counting efficiency of the 3 isotopes as a function of sample volume was compared. The NaI well type scintillation counter for counting up to 4 ml samples as well as the beaker to fit on and around the counter capable of counting 1 liter of solution was previously described(1). The counter* consists of an RCA 5819 phototube, a thallium activated sodium iodide crystal, 1¾″ diameter and 1″ thick with a hole ¾″ in diameter and 1″ deep, and a cathode follower preamplifier. The plastic scintillator† was modified and enlarged on the advice of Dr. G. J. Hine from that previously described and diagram shown in Fig. 1. The plastic well was surrounded by 3 in. of lead shielding except at the tube opening. Test tubes to contain the samples in this well were 38 x 300 mm.

Results. Small Volumes (1-4 ml.) If one counts samples in small volumes (1-4 ml), the capacity of most commercially available NaI well crystals, it is apparent from Table I that the plastic well is as efficient as the NaI well for Co^{56} and more efficient than the NaI well for Co^{58} and Co^{60}. The NaI crystal used in these studies is slightly less efficient than the more recent commercially available crystals. Since average background rate of the plastic well is about 400 cpm as compared to 120 cpm for the NaI crystal, the ratio of R_S^2/R_b (R_S = total counting rate, R_b = background rate) is greater for the NaI well. It has been shown that the higher the ratio of R_S^2/R_b (merit ratio) the less the counting time necessary for a given accuracy(3).

Intermediate Volumes (4 ml-250 ml). The plastic scintillator is highly efficient and the efficiency is almost independent of sample size from 10-100 ml for the 3 isotopes. Larger NaI wells holding more than 4 ml are difficult and very expensive to build. Baskin *et al.*(4) described a NaI well crystal with a capacity of 20 ml and an efficiency for Co^{60} of 22.6%. Thus the plastic well appears to be the most efficient method for counting gamma radiation contained in sample volumes of 10 to 250 ml. Because of relative independence of volume size on efficiency and the larger size of plastic well, whole mice, rats up to about 75 g and

* Model S_1—purchased from H. O. Anger, Berkeley, Calif.

† Pilot Scintillator B, Pilot Chemicals. Specific gravity = 1.03 Atomic ratio $\frac{H}{C}$ = 1.10