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Preparation and Assay of Inhibitor of Gastric Secretion and Motility from Normal Human Urine.

H. NECHELES, MARTIN E. HANKE* AND ERICH FANTL.

From the Department of Gastro-Intestinal Research, Michael Reese Hospital, and the Department of Biochemistry, University of Chicago.

Necheles and Lim[1] prepared biodialysates from the blood of dogs, which produced an increase of pancreatic and gastric secretion. In some experiments, however, inhibition of gastric secretion was observed. The latter finding was not followed up until reports of a number of hormones in the urine of man and animal prompted one of us (H.N.) to search for the presence of a gastric inhibitory principle in human urine.[2]

Lim, *et al.*,[3] described the extraction of a gastric inhibitory substance from intestines which they called enterogastrone. They found small amounts of this substance in other tissues and in a sample of Ivy's cholecystokinin. Walawski[4] described a substance with similar properties extracted from the large intestine. Lim's findings were confirmed by Ivy's laboratory, and they (and Sandweiss) confirmed our findings of an enterogastrone-like substance in human urine.[5]

Chemical Methods. Normal human urine is treated with 1% of $(NH_4)_2SO_4$, and the gelatinous precipitate (which forms in part even without $(NH_4)_2SO_4$), is washed twice with 1.5% $(NH_4)_2SO_4$, and then stirred vigorously with about 1 volume of water and 4 volumes of 95% alcohol. Thus there is obtained a 65% alcohol infusion, equal to about 1.5% of the volume of the original urine. The solid is collected by centrifugation, and the supernatant treated with one volume of 95% alcohol which causes the formation of a copious white precipitate equivalent to about 15 mg per liter of urine. Both solids are progressively fractionated by (1) extracting with water, (2) adding 2.1 volumes of 95% alcohol

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1 Necheles, H., and Lim, R. K. S., *Chin. J. Physiol.*, 1928, **2**, 415; Necheles, H., *Chin. J. Physiol.*, 1927, **1**, 69.

2 Necheles, H., communications to Dr. D. J. Sandweiss and Dr. A. C. Ivy, 1937; *Am. J. Digest. Dis.*, 1938, **5**, 471; *Internat. Physiol. Congr.*, Zurich, 1938.

3 Kosaka, T., and Lim, R. K. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 890; Lim, R. K. S., *Quart. J. Exp. Physiol.*, 1933, **23**, 263.

4 Walawski, J., *Med. Doswiadcz. i. Spot.*, **11**, 1930, 348.

5 Gray, J. S., Bradley, Wm. B., and Ivy, A. C., *Am. J. Physiol.*, 1937, **463**, 118; Gray, J. S., Wieczorowski, E., and Ivy, A. C., *Science*, 1939, **89**, 489.

and 0.2% NaCl to get a 65% alcohol precipitate. (3) adding an equal volume of alcohol to the supernatant fluid to get an 80% alcohol precipitate. These fractionations are repeated according to a definite plan, until at the ninth reprecipitation 2 water-soluble fractions are obtained, one completely precipitated by 65% alcohol and the other entirely soluble at 65%, but precipitated at 80% alcohol. Both give strong protein tests, and can be precipitated from aqueous solution by ammonium sulfate (45% of saturated), picric or tannic acid.

Biological Assay. The substances were assayed for toxicity, and for effects on motility and secretion. Crude preparations increased secretion of bile and contracted the gall bladder. Purer preparations did not affect the gallbladder, blood pressure, respiration and secretion of saliva, urine or bile. Gastric secretion was assayed on Pavlov dogs following a standard meat meal, and motility on gastrostomized dogs following injection of insulin. These procedures were chosen after many others had been tried and found unsatisfactory. With 0.5 mg injected intravenously, the volume of secretion was decreased from 0.6 to 0.3 cc per minute, and the acidity from 100 free and 130 total, to 60 and 105 clinical units, respectively. With 1 mg the volume was changed from 1.0 to 0.1 cc per minute, and the acidity from 130 free and 150 total, to 0 and 25 clinical units respectively. On motility 2 mg caused a slight inhibition for 1 hour, while 4 mg caused complete inhibition for more than 3 hours. Secretion is inhibited by a smaller dose than is motility.

The fact that a large number of preparations show the same relative effect on secretion and motility, indicates that the same chemical substance inhibits both functions. Our substance may be identical with Lim's enterogastrone; however, the inhibition of gastric motility and secretion with various peptones[6] leaves this question open. Refractoriness to our preparations as described by others[7] was not observed. Small doses of our substance do not inhibit the stomach, when given *per os.* Boiling for 2 minutes destroys about 50% of the activity.

Summary. Method of extraction and assay of a substance from normal human urine are described. The substance is non-toxic and depresses or abolishes gastric secretion and motility in doses of 1-4 mg. The same chemical substance seems to inhibit both functions.

[6] Brunemeier, E. H., and Carlson, A. J., *Am. J. Physiol.*, 1914-15, **36**, 191; Dadley, J., and Koskowski, W., *C. R. Soc. Biol.*, Paris, 1932, **109**, 1028; Thomas, J. E., and Crider, J. O., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 825.

[7] Gray, J. S., and Wieczorowski, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 324.