Cycloheximide Inhibition of Gastric Secretion in the Rat* (33660)

S. D. J. YEH AND M. E. SHILS
Division of Medical Research, Sloan-Kettering Institute, New York, New York 10021

In studying the influence of various inhibitors of protein synthesis on intestinal absorption, it was noted that the treated rats often had a delayed gastric emptying of nutrients, especially of lipids (1). In addition, cycloheximide decreased intrinsic factor production (2). Further studies of the effect of this inhibitor on gastric secretion of acid and pepsin are reported here.

Method. Adult rats of both sexes were fasted overnight and the pylorus was ligated under light ether anesthesia. Cycloheximide (0.5 mg/kg in 0.9% saline) was given i.m. immediately after surgery. At the end of the experiment, gastric contents were carefully removed and then the stomach lumen washed with cold saline. The volume and pH of the initial contents were measured; the contents and washings were pooled and titrated to pH 7.0 with dilute NaOH and results expressed as milliequivalents of total acidity. Pepsin levels in fresh gastric juice were estimated by the method of Klotz (3) using both radioactivity and optical density measurements. The protein content of gastric juice was calculated from the optical density at 260 and 280 mμ or by the weight of dialyzed and lyophilized pooled samples. For stimulating gastric secretion, 2 ml of 6% alcohol were introduced intragastrically shortly after pylorus ligation. Crystalline insulin and gastrin pentapeptide[1] were given i.m. according to the dosage schedule in the appropriate table. The 2-deoxyglucose was given rapidly via the penile vein. Plasma glucose was determined by a glucose oxidase method (4).

Results. Cycloheximide resulted in significant decreases in the total volume and acidity in the gastric juice collected over a period of 4 or 6 hr following pylorus ligation and drug injection (Table I). Pepsin activity and protein content in the gastric juice of treated rats were likewise decreased (Table II). The ability of the gastric secretory mechanism to respond to stimulation was tested with a variety of agents. The response is shown for control and treated animals to alcohol, gastrin pentapeptide, insulin, and 2-deoxyglucose (Fig. 1). The control rats responded to all of these agents excepting alcohol with appreciable and significant increases in volume; acid production increased significantly with each, being most marked

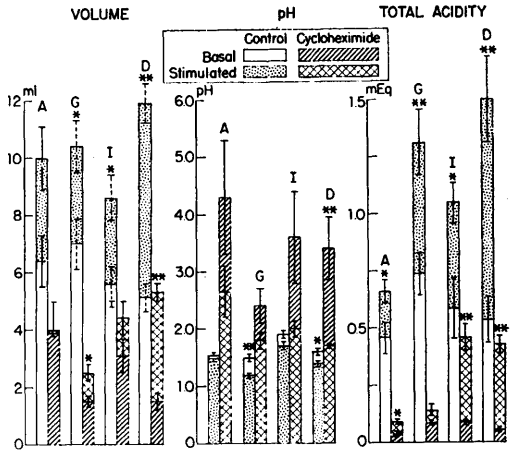

FIG. 1. Effect of cycloheximide on gastric secretion and response to various stimulants. Cycloheximide (0.5 mg/kg of body wt.) or saline to controls was given i.m. immediately after pylorus ligation. 200 g female rats were used in all studies excepting that with 2-deoxyglucose when 350 g males were utilized. ‡ = mean ± SE; * = $p<0.05$; ** = $p<0.01$. Sampling procedures are given in text. (A) Two ml of 6% alcohol or water were given intragastrically immediately after cycloheximide or saline injection and the animals were sacrificed 5 hr later; (G) gastrin pentapeptide (30 μg/kg) or saline was given i.m. hourly 4 times with the first dose immediately after cycloheximide or saline; rats were sacrificed 1 hr after last dose; (I) crystalline insulin (2 U/kg) or saline was given i.m. 2 hr after cycloheximide or saline and the rats were sacrificed 2 hr later; and (D) 2-deoxyglucose (150 mg/kg) or saline was given i.v. 1 hr after cycloheximide or saline and the rats were sacrificed 3 hr later.

* Supported in part by PHS Grant CA-08748 from the National Cancer Institute.

[1] AY-6608 Courtesy of Ayerst Research Laboratory, New York City.

TABLE I. Effect of Cycloheximide on Gastric Secretion.

Cycloheximide (mg/kg)	Duration (hr)	Gastric secretion ($\bar{x} \pm$ SE)		
		Volume (ml)	pH	Total acidity (meq)
0 (5)[a]	4	4.32 ± 0.57	1.84 ± 0.10	0.34 ± 0.05
0.5 (5)	4	1.16 ± 0.34[b]	6.57 ± 0.51[b]	0.02 ± 0.02[b]
0 (6)	6	6.70 ± 0.83	1.57 ± 0.08	0.70 ± 0.09
0.5 (6)	6	2.27 ± 0.30[b]	5.00 ± 0.71[b]	0.04 ± 0.03[b]

[a] Number of rats given in parentheses; males of 250–300 g were used.

[b] $p < 0.01$ in comparing control with treated rats at identical times.

with 2-deoxyglucose and insulin and least with alcohol in the doses used. The cycloheximide-treated animals also responded to these stimuli with increased acid secretion which was statistically significant at the 1% level in all instances except with gastrin pentapeptide; gastric volume did not increase significantly with alcohol or insulin. However, the treated animals, starting at a much lower baseline secretion than their controls had much smaller absolute increases in acid secretion with stimulus than did their counterparts. The absolute increases above the baseline in mean total acidity of treated as compared to controls in response to stimulation was 25% for alcohol, 10% for pentapeptide, 79% for insulin, and 38% for deoxyglucose. In no instance did the gastric acid secretion of the stimulated cycloheximide-treated rats achieve the level of the *unstimulated* controls. No sex difference was noted in the inhibitory action of cycloheximide on gastric secretion nor in the response to alcohol, gastrin pentapeptide, or insulin; deoxyglucose was tested only with males.

Cycloheximide-treated rats without insulin had significantly lower fasting plasma glucose levels than their controls; the hypoglycemic response to crystalline insulin was appreciably less than that of the controls (Table III). The expected blood glucose rise associated with deoxyglucose was approximately the same in control and cycloheximide-treated rats; again the cycloheximide-treated group without the vagal stimulus had a lower baseline level than the control group (Table III).

Discussion. Our studies have demonstrated that a single dose of cycloheximide which reduces by 50% the incorporation of ^{14}C labelled leucine into the proteins of stomach also decreases the secretion of intrinsic factor (2), gastric juice, pepsin, and hydrochloric acid. The greatest decrease noted was that of acid production (10 times or more). Our microscopic examination of hematoxylin–eosin stained sections of stomach failed to demonstrate any obvious gross structural differences between the control and cycloheximide-treated rats.

TABLE II. Effect of Cycloheximide on Gastric Pepsin Level.

Cycloheximide (mg/kg)	Gastric vol (ml)	Protein content (mg)	Pepsin activity (OD units)[b]	Radioactivity (%)[c]
0 (5)[a]	9.56 ± 1.53[d]	805 ± 129	284 ± 25	24.0 ± 3.4
0.5 (5)	1.76 ± 0.35[e]	295 ± 38[e]	83 ± 5[e]	4.7 ± 0.2[e]

[a] Number of rats given in parentheses; females of 200 g; these were sacrificed 6 hr after cycloheximide injection and all data are expressed for 6-hr period.

[b] One OD unit = change of OD of 0.001 (at 280 mμ) × 10^4.

[c] Percentage of initial ^{131}I labeled albumin present in supernatant after 1/15 of 6-hr gastric juice was incubated in 1 ml of 6% bovine albumin and 0.02 μCi of RISA followed by precipitation with trichloroacetic acid.

[d] $\bar{x} \pm$ SE.

[e] $p < 0.01$.

TABLE III. Plasma Glucose in Cycloheximide-Treated and Control Rats Given Insulin or Deoxyglucose.

Cycloheximide (mg/kg)	Plasma glucose (mg/100 ml)[a]			
	Insulin[b]		Deoxyglucose[c]	
	(−)	(+)	(−)	(+)
0 (4)[d]	117 ± 7.5	10 ± 3.7	82 ± 5.7	106 ± 3.3
0.5 (4)	95 ± 3.8[e]	30 ± 3.3[f]	57 ± 3.3[f]	85 ± 3.1[f]

[a] Blood was obtained by aortic puncture under light ether anesthesia.
[b] Female rats.
[c] Male rats.
[d] Number of rats given in parentheses.
[e] $p < .05$.
[f] $p < .01$.

The reduced secretion of pepsin or intrinsic factor may be presumed to be secondary to the interference of cycloheximide with the discharge of activated amino acid from aminoacyl sRNA to ribosomes (5). While it is tempting to invoke the same explanation as the mechanism of impaired acid secretion, the evidence for this is insecure. To our knowledge there is no good information for or against the view that transport of hydrogen ion across the membrane of parietal cells into the lumen of the stomach requires a carrier protein the synthesis of which may be inhibited by cycloheximide. It is possible that synthesis of key enzymes in the mucosa may be inhibited by this compound. These would include carbonic anhydrase (6), histidine decarboxylase (7), and enzymes required for oxidative metabolism to provide energy needed to transport hydrogen ion (8). This possibility would require a marked decrease in enzyme concentration to occur within 4 hr by the inhibition of new enzyme formation. Since the half-lives of the pertinent enzymes are unknown, one can only speculate on this aspect. If their half-lives are no longer than the adaptive enzyme tryptophan pyrolase with a half-life about 2 hr (9) this mechanism would be possible. We have made certain observations (unpublished) which argue against significant changes in oxidative metabolism; cycloheximide-treated rats were able to oxidize glucose-^{14}C at the same rate as controls in 4 hr and there were no differences in ATP concentrations in the blood and liver of control and treated rats. If the activity of a key enzyme in gastric acid secretion had fallen to a critical level as the result of cycloheximide, then one would have to postulate that the rise in gastric secretions occurring with vagal and other stimuli in the treated rats is secondary to changes in substrate availability, product removal, or some other process not directly related to enzyme concentration. *In vitro* kinetic studies of gastric mucosa may be informative in shedding light on these problems.

Gastrin released from the antrum plays a key role in gastric acid secretion (10). Cycloheximide may affect gastric acid secretion by inhibiting synthesis of this heptadecapeptide, by impairing its release from the antral cells or by preventing its action in parietal cells. Administration of the gastrin pentapeptide [active in many species of animals including rats (11)] did not cause much change in the treated rats. Therefore, decrease in gastrin production or inhibition of its release from antral cells are not the key factors responsible for the cycloheximide-induced reduction of acid secretion. Other polypeptide hormones appear to activate protein synthesis in the target organs in achieving their response (12) and it is possible that gastrin also depends upon this type of mechanism. In a preparation of bullfrog gastric mucosa tested *in vitro* (13), acid secretory response to gastrin or pentapeptide over a period of 6 hr was not modified by the addition of puromycin to the medium whereas leucine incorporation

into mucosal protein was inhibited by 97%. The cause for this discrepancy between these *in vitro* findings with puromycin and our *in vivo* observations with cycloheximide is not apparent at this time.

The effect of cycloheximide in delaying gastric emptying (2) and in decreasing the response to vagal stimuli may be related to an inhibition at cholinergic nerve endings or on other neural activity. However, the failure of gastrin pentapeptide to increase acidity suggests that cycloheximide inhibits a basic mucosal mechanism.

To our knowledge, cycloheximide in the dosage used has no pharmacologic action other than inhibition of protein synthesis. However, physiologic disturbances secondary to this effect or in association with other unknown biochemical actions cannot be absolutely ruled out. However, with cycloheximide at 0.5 mg/kg rats have unaltered rectal temperature and are normal in appearance and activity. Histology of the liver and jejunum was normal in our animals after 6 hr.

Summary. Cycloheximide, at a dose (0.5 mg/kg, i.m.) which is known to inhibit incorporation of amino acids in the proteins of the stomach was found to decrease gastric secretory volume and acid and pepsin production in rats with pylorus ligation. When various stimuli to gastric secretion (alcohol, insulin, gastrin pentapeptide, and 2-deoxyglucose) were given, there were smaller absolute responses in the cycloheximide-treated rats than in controls.

1. Yeh, S. D. J. and Shils, M. E., Intern. Congr. Biochem., 7th, Tokyo **5**, 923, (1967).

2. Yeh, S. D. J. and Shils, M. E., Federation Proc. **27**, 359 (1968).

3. Klotz, A. P. and Duvall, M. R., J. Lab. Clin. Med. **50**, 753 (1957).

4. Saifer, A. and Gerstenfeld, S., J. Lab. Clin. Med. **51**, 448 (1958).

5. Siegel, M. R. and Sisler, H. D., Biochim. Biophys. Acta. **87**, 70 (1964).

6. Emas, S., Gastroenterology **43**, 557 (1962).

7. Levine, R. J., Federation Proc. **24**, 1331 (1965).

8. Durbin, R. P. and Kasbekar, D. K., Federation Proc. **24**, 1377 (1965).

9. Feigleson, P., Rashman, T., and Margolis, F., Arch. Biochem. Biophys. **85**, 478 (1959).

10. Grossman, M. E., Am. J. Digest. Diseases **11**, 90 (1966).

11. Gregory, R. A., Proc. Roy. Soc. (London) Ser. B. **170**, 81 (1968).

12. Wool, I. G., *in* "Mechanism of Hormone Action" (P. Kerlson, ed.) p. 98. Academic Press, New York (1965).

13. Davidson, W. D., Lemmi, C. A. D., and Thompson, J. C., Endocrinology **82**, 416 (1968).

Received July 11, 1968. P.S.E.B.M., 1969, Vol. 130.