proximal precursors, stimulation of reductase activities by antimetabolites could be as damaging to cellular metabolism as inhibition.

The results of the study presented here reinforce the conclusions reached by Larsson and Reichard(1), Moore and Hurlbert(2), and others(3,8) that inhibitors and stimulators of ribonucleotide reductases control the supply of proximal precursors for DNA synthesis and, ultimately, cellular replication.

Summary. The purine ribonucleotide reductases from a mouse tumor were studied *in vitro*. Adenylate and guanylate reductions were subject to end-product inhibition. β-D-arabinosylcytosine produced paradoxical inhibitory effects on deoxyadenylate biosynthesis. The adenylate reductase was stimulated by dGTP and guanylate reductase was stimulated by β-D-deoxythioguanylate.

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Influence of Phenylbutazone on Gastric Secretion of Mucus.* (32288)

RENE MENGUY AND LAURENT DESBAILLETS

Department of Surgery, University of Chicago, Division of Biological Sciences, Chicago, Ill.

Gastrointestinal symptoms often complicate the therapeutic use of phenylbutazone. It has been estimated that at least 2% of patients receiving the drug develop a peptic ulcer or experience recurrence of a previously occurring ulcer(1). The onset of gastrointestinal bleeding with or without formation of a peptic ulcer occurs more often(2). Administration of phenylbutazone to animals causes ulcers in guinea pigs(3), dogs(4), and rats(5). Other effects are gastritis and mucosal hemorrhage. The pathophysiology of gastric mucosal injury by phenylbutazone remains unknown. In dogs(6), rats(5) and guinea pigs(7), phenylbutazone lowers gastric acid, thus excluding the possibility that its effect on the stomach is mediated by hyperacidity. One cannot invoke a local, necrotizing effect of the drug on the gastric mucosa, because gastric changes are as intense after parenteral as after oral administration of the drug(8).

It has been postulated that the layer of mucus secreted by mucus-producing cells of the mucosa, and in particular by the prepyloric glands, protects the underlying mucosa by preventing acid-peptic secretions from gaining free access to it. Previous studies from this laboratory have shown that compounds such as cortisone(9) and aspirin(10), which have antirheumatic properties similar to those of phenylbutazone and also cause gastric mucosal injury, diminish the secretion of mucus by the canine gastric mucosa. The purpose of the studies forming the basis for this report was to determine if phenylbutazone interferes with secretion of gastric mucus in a way that may explain the gastric mucosal injury that it occasionally causes.

Materials and methods. Vagally denervated gastric-antral pouches were constructed in 4 dogs weighing from 16 to 30 kg. Care was taken to exclude acid secreting mucosa from the pouches. Secretions were drained *via* a

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small, plastic cannula, and were collected in a condom. The volume of each collection was measured, after which it was dialyzed at 4°C in seamless, regenerated, cellulose tubing in a rotating dialyzer against multiple changes of distilled water for 24 hours. After dialysis, each individual collection was freeze-dried. By weighing each dry sample, the concentration of non-dialyzable mucosubstanes per ml of native mucus was measured. The concentration of nitrogen, hexoses, hexosamines, L-fucose and sialic acid in aliquots of each individual sample was measuerd. Nitrogen concentration was measured by the micro-Dumas method using an automated instrument.† Protein concentration was calculated from the measured nitrogen concentration (minus the nitrogen in the amino-sugars) by using 6.25 as correction factor. Neutral and amino-sugars were liberated by hydrolizing 10 mg aliquots of lyophilized mucus in 3 ml of 2N HCl at 100°C for 2 hours. The hydrolysates were dried *in vacuo*. Hexosamines were measured by the method of Dische and Borenfreund (11), using a 25μg standard. Hexoses and L-fucose were measured by the method of Dische and Shettles(12), using a standard consisting of galactose, mannose and L-fucose in the amounts of 25, 25 and 10 μg/ml respectively. To determine the concentration of sialic acid, 10 mg of lyophilized mucus were hydrolyzed for one hour at 80°C in 0.1 N sulfuric acid. The released sialic acid was measured by the thiobarbituric acid method of Warren(13). Results of the analyses were expressed as mμ moles/mg of lyophilized mucus. By adding values of hexoses, hexosamines, L-fucose and sialic acid, an estimation of total carbohydrate concentration was obtained from which the carbohydrate to protein ratio was calculated for each sample. Every day at 8:00 a.m. and 6:00 p.m. the dogs received a small meat ball. Daily collections of mucus were made from 11:00 a.m. to 2:00 p.m. during a 15 to 20-day control period followed by a 20-day test period, during which 3 of the dogs received 100 mg/kg of phenylbutazone daily. The tablets were placed in the bolus of meat with half of the total daily dose given in the morning, and the remainder in the evening. In one dog, the daily dose of phenylbutazone was administered only once every third day at 8:00 a.m. In this dog the mucus secreted on the test days was compared with the material secreted on the alternate control days. In all 4 dogs, collections of mucus were continued for 20 days after administration of the drug had been discontinued to study the recovery from its effects. Significance of changes in these values were evaluated by Student's t-Test.

† Coleman Nitrogen Analyzer.

Results. Rates of mucus secretion and results of biochemical analysis of the mucus secreted before, during and after administration of phenylbutazone are summarized in Tables I, II and III respectively. In all 4 dogs, administration of phenylbutazone caused a decrease in the rate of mucus secretion which was significant in 3 of the animals. The concentration of non-dialyzable mucosubstances per ml of native mucus increased significantly in all 4 dogs during the administration of phenylbutazone.

The data indicate that phenylbutazone decreased the concentration of carbohydrates in non-dialyzable mucosubstance. The carbohydrates most influenced by phenylbutazone were sialic acid, L-fucose and hexosamines. Changes in the concentration of hexoses were less pronounced. In almost all instances, these values returned to or above control levels during the recovery period of the experiment (Table III). The carbohydrate to protein ratio fell during the administration of phenylbutazone and returned to normal levels after the drug was discontinued.

Discussion. The data indicate that phenylbutazone impairs the secretion of mucus by the gastric antrum. It diminishes the quantity of mucus secreted, and decreases the amount of carbohydrate incorporated into mucosubstance. These changes result from a systemic effect of the drug, since it was administered to the dogs in a way that precluded contact with the mucus-secreting cells that were being studied. Although it could be argued that phenylbutazone might enter gastric juices from the blood stream, this possibility is excluded because saliva and gastric juice contain barely detectable amounts of phenylbutazone in contrast to high blood levels(14).

A priori, the doses of phenylbutazone used in this experiment may appear large when compared with the therapeutic dose in man. However, phenylbutazone has a biologic half-life of only 6 hours in the dog(14). In man it is 72 hours(15). Therefore, higher doses must be given to dogs to maintain a plasma concentration similar to that following therapeutic doses of the drug in man.

The changes in gastric mucous secretion occurring under the influence of phenylbutazone resemble those that we have observed in dogs

TABLE I. Canine Gastric Mucus Secretion Before Administration of Phenylbutazone.

Dog No.	Rate of secretion,* ml ± S.D.	Conc of muco-substance,† mg/ml ± S.D.	Conc of sialic acid,‡ mμM ± S.D.	Conc of L-fucose,‡ mμM ± S.D.	Conc of hexosamines,‡ mμM ± S.D.	Conc of hexoses,‡ mμM ± S.D.	Carbohydrate to protein ratio§
1‖	12.7 ± 3.6	27.8 ± 5.7	13.9 ± 1.2	28.5 ± 6.9	101.4 ±14.0	181.8 ± 14.2	.086 ±.006
2	12.3 ± 3.1	30.2 ± 5.7	19.0 ± 2.6	47.8 ±12.8	126.5 ±34.8	241.0 ± 46.4	.110 ±.014
3	11.2 ± 1.5	23.3 ± 3.2	17.4 ± 1.1	36.9 ± 7.3	114.1 ± 8.3	227.1 ± 25.0	.101 ±.008
4	11.6 ± 1.6	53.5 ± 9.4	17.3 ± 1.7	26.7 ± 3.6	80.5 ± 8.2	246.2 ± 37.3	.091 ±.010

* Average 3 hr output of mucus.

† Average concentration of non-dialyzable mucosubstance in mucus expressed as mg/ml of native mucus.

‡ Average concentration of carbohydrate expressed as mμM/mg of lyophilized mucosubstance.

§ Average ratio of the concentration of total carbohydrate measured (μg/mg) to that of protein (μg/mg) in lyophilized mucosubstance.

‖ This dog received phenylbutazone every third day during the test period.

Legends are identical in Tables II and III.

TABLE II. Canine Gastric Mucus Secretion During the Administration of Phenylbutazone.

Dog No.	Rate of secretion,* ml ± S.D.	Conc of muco-substance,† mg/ml ± S.D.	Conc of sialic acid,‡ mμM ± S.D.	Conc of L-fucose,‡ mμM ± S.D.	Conc of hexosamines,‡ mμM ± S.D.	Conc of hexoses,‡ mμM ± S.D.	Carbohydrate to protein ratio§
1‖	11.8 ± 1.7	40.0 ± 7.9	10.4 ± 3.2	19.4 ± 3.6	71.6 ± 7.0	164.2 ± 21.6	.064 ±.009
	Δ=−7% (p<.15)	Δ=+30% (p<.05)	Δ=−25% (p<.0005)	Δ=−32% (p<.0005)	Δ=−30% (p<.0005)	Δ=−10% (p<.005)	(p<.0005)
2	8.9 ± .5	38.2 ± 6.2	17.9 ± 2.4	35.7 ± 9.3	102 ±16.8	230.1 ± 42.4	.096 ±.014
	Δ=−28% (p<.0005)	Δ=+21% (p<.0025)	Δ=−6% (p<.025)	Δ=−22% (p<.0005)	Δ=−21% (p<.0005)	Δ=−2% (p<.45)	(p<.005)
3	10.0 ± 1.2	26.4 ± 3.0	14.7 ± 1.6	29.8 ± 4.1	95.6 ± 8.1	217.3 ± 19.4	.091 ±.006
	Δ=−11% (p<.01)	Δ=+12% (p<.005)	Δ=−16% (p<.0005)	Δ=−20% (p<.0005)	Δ=−26% (p<.0005)	Δ=−4% (p<.10)	(p<.0005)
4	8.0 ± 1.4	59.4 ± 6.9	15.3 ± 1.5	20.0 ± 4.6	70.7 ±11.6	165.8 ± 32.3	.068 ±.010
	Δ=−31% (p<.0005)	Δ=+10% (p<.025)	Δ=−12% (p<.0005)	Δ=−23% (p<.0005)	Δ=−12% (p<.0025)	Δ=−34% (p<.0005)	(p<.0005)

Δ = change from control value. p = significance of difference between means of control and test periods.

TABLE III. Canine Gastric Mucus Secretion After Administration of Phenylbutazone.

Dog No.	Rate of secretion,* ml ± S.D.	Conc of muco-substance,† mg/ml ± S.D.	Conc of sialic acid,‡ mμM ± S.D.	Conc of L-fucose,‡ mμM ± S.D.	Conc of hexosamines,‡ mμM ± S.D.	Conc of hexoses,‡ mμM ± S.D.	Carbohydrate to protein ratio§
1‖	13.0 ± 2.9	32.4 ± 5.1	15.7 ± 1.4	29.3 ± 7.4	102.1 ±14.2	196.2 ± 22.1	.085 ±.010
2	13.0 ± 1.2	38.4 ± 4.0	19.6 ± .8	40.3 ± 4.5	110.6 ± 6.4	250.5 ± 26.0	.105 ±.010
3	12.4 ± 2.8	23.3 ± 4.1	18.4 ± 2.7	38.3 ±10.3	120.5 ±24.5	239.2 ± 40.5	.106 ±.019
4	12.0 ± 1.2	59.9 ± 6.6	17.7 ± 1.2	17.8 ± 2.6	70.4 ± 5.4	285.3 ± 28.6	.099 ±.007

receiving cortisone(9) or aspirin(10). Both of these compounds decreased the rate of mucous secretion, increased the concentration of non-dialyzable mucosubstances per unit volume of native mucus, and decreased the concentration of sialic acid, hexosamines and L-fucose in antral mucus. Since we have recently observed identical changes in dogs receiving ACTH and indomethacin, it appears that this influence on the formation of mucus is a property common to antirheumatic drugs.

Obviously, this study does not prove that mucus does, indeed, have the protective role that has been attributed to it. However, if the thin layer of mucus adherent to the underlying epithelium does, indeed, play a role in protecting the mucosa from the acid-peptic secretions present in gastric juice, one would expect an increase in the likelihood of ulceration if the secretion of mucus were deficient or if it were altered. Both of these changes appear to occur when phenylbutazone is administered to animals. The rate of mucus secretion and the amount of carbohydrate in mucus both decreased. Since the presence of oligosaccharide side chains renders these carbohydrate-protein complexes resistant to proteolysis(16), a fall in their number should make the mucus more susceptible to digestion by proteinases in the proximal gastrointestinal tract.

Summary. This study was undertaken in an attempt to clarify the mechanism by which the therapeutic use of phenylbutazone sometimes causes gastric mucosal injury in the form of hemorrhage, erosions or frank ulcerations. Mucoid secretions from gastric-antral pouches of dogs were studied before, during and after the oral administration of phenylbutazone in doses of 100 mg/kg of body weight. This drug decreased the rate of mucus secretions and the carbohydrate to protein ratio in non-dialyzable mucosubstance. The data suggest that gastric mucosal injury by phenylbutazone may be due to altered mucosal defense mechanisms.

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