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Effect of Prednisolone on Gastric Mucus Content and on Ulcer Formation. (28728)

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During the thirteen years that corticosteroids and ACTH have been used clinically, development or reactivation of peptic ulcer has been one of the serious side effects. In carefully controlled studies, the incidence after prolonged treatment was found to range from 10 to 30%(1). Steroid ulcers were also produced experimentally in rats(2), dogs (3,4), and guinea pigs(5). Several theories have been proposed to explain the pathogenesis of this drug-induced lesion, each placing the emphasis on one of the various effects of corticosteroids: stimulation of acid and pepsin secretion(3,6,7,8), increase in number of parietal cells(9,10,11), local release of histamine from gastric mast cells(11), diminution of tissue resistance related to the anti-inflammatory action of corticosteroids(12), change in gastric vascularity (hemorrhagic thrombosis)(13) and diminution in mucus secretion (1,2,14,15).

Recent observations suggest that the rate of mucus formation may play a major role in the natural defense of the stomach against ulcerogenic agents. It was reported that:

1. Ulcers, produced in rats by prolonged fasting, appear coincidental with a diminution of the amount of mucus in both gastric juice and tissue(16).

2. Prednisolone produces gastric ulcers in the rat and at the same time reduces the mucus content of the stomach(1,14,15).

3. The antrum of the rat stomach, unlike the corpus, does not ulcerate after either fasting(16) or steroid treatment(1) and this portion of the stomach contains twice as much mucus as the corpus(16).

4. In the dog, cortisone reduces the volume of juice (mostly mucus) secreted by a denervated antral pouch(15).

5. On the edges of steroid ulcers, immediately adjacent to the crater, there is always a

group of gastric glands proliferating (high mitotic rate) and actively secreting mucus (PAS positive material) (1). This finding was interpreted as a local defense reaction through which this newly formed mucus, covering the defect, would help to arrest further extension of the ulcer and favor healing.

This report concerns our investigation of the effect of a corticosteroid, prednisolone, on mucus formation. The mucus content of gastric juice and tissue was determined in fasted rats receiving prednisolone for various periods from 1 to 6 days. A correlation was attempted between the changes in mucus observed and formation of steroid ulcers.

Materials and methods. Female Sprague-Dawley rats weighing 195-210 g were used. Two separate studies were performed, one on gastric juice, the other on gastric tissue.

1. *Gastric juice.* Groups of animals were fasted in individual cages for one through 6 days after which the pylorus was ligated under ether anesthesia. The night before surgery, the rats were placed in a stainless steel cylindrical tube, as previously described(16), and were returned to the tube as soon as they had recovered from anesthesia. This procedure prevented them from eating hair or feces and licking the surgical incision. They were killed by chloroform 5 hours after pylorus ligation and the gastric contents were collected in a graduate, measured and homogenized. Acidity was titrated with NaOH .005 N using a glass electrode (free acid at pH 3.5; total acid at pH 8.5) and is expressed in mEq/l (concentration) and mEq/5 hours (output). Hexosamine, used as an estimation of mucus, was determined using a modification(16) of Boas's method(17). Results are expressed in mg/ml (concentration) and mg/5 hours (output).

Five mg of prednisolone in 0.2 ml was administered subcutaneously once a day to some of the rats whereas the controls received the vehicle only. The last injection was given immediately before pylorus ligation.

2. *Gastric tissue.* Other rats were treated exactly as described above except that they were not operated, and a group of fed untreated rats was added. At autopsy, the stom ach was dissected out, opened along th greater curvature and washed with lukewarm water. The forestomach was sectioned and discarded. The antrum was separated from the corpus—both areas are sharply demarcated—and both portions were dried overnight (16-18 hours) in an oven at 75-78° to constant weight. Hexosamine was determined in the dry tissue, using a technique previously described(16) and the results expressed in mg/100 mg of dry weight of corpus or antrum (*i.e.*, mg%).

Ulcer formation. Gastric ulcers produced by prednisolone were graded in terms of "ulcer index" according to a method previously described(2). Briefly, the "ulcer index" for each group is the sum of per cent incidence divided by 10, average severity (from a scale of 0 to 3+) and average number of ulcers per stomach.

Results. General condition of the animals. As expected, prednisolone-treated rats lost more weight during fasting than did controls (Table I). A few animals died after 5 and 6 days of fasting. The mortality rate, although low and not influenced by prednisolone, was slightly more elevated in rats that had undergone surgery.

TABLE I. Body Weight Loss and Mortality During Prolonged Fasting.

	Fed	Days of fasting				
		1	2	3	4	5
Exp 1—With pylorus ligation						
Untreated						
No. of animals*		15	15	15	15	20
Body wt loss (g)		27	36	39	47	56
Mortality (%)		0	0	0	0	10
Prednisolone-treated						
No. of animals*		15	15	15	15	20
Body wt loss (g)		34	48	55	62	71
Mortality (%)		0	0	0	0	0
Exp 2—Without pylorus ligation						
Untreated						
No. of animals*	38	23	30	30	50	56
Body wt loss (g)		22	31	40	47	57
Mortality (%)		0	0	0	0	13
Prednisolone-treated						
No. of animals*		7	15	15	35	8
Body wt loss (g)		32	42	50	61	71
Mortality (%)		0	0	0	0	0

* Initial.

Gastric juice. Volume (Fig. 1). The volne collected during the 5-hour period deeased gradually as fasting progressed, aching on the sixth day 58% of the value obtained after only 24 hours of fasting. Prednisolone, after one day of fasting, inhibited the volume (by 29% when compared to the volume of untreated rats fasted for the same

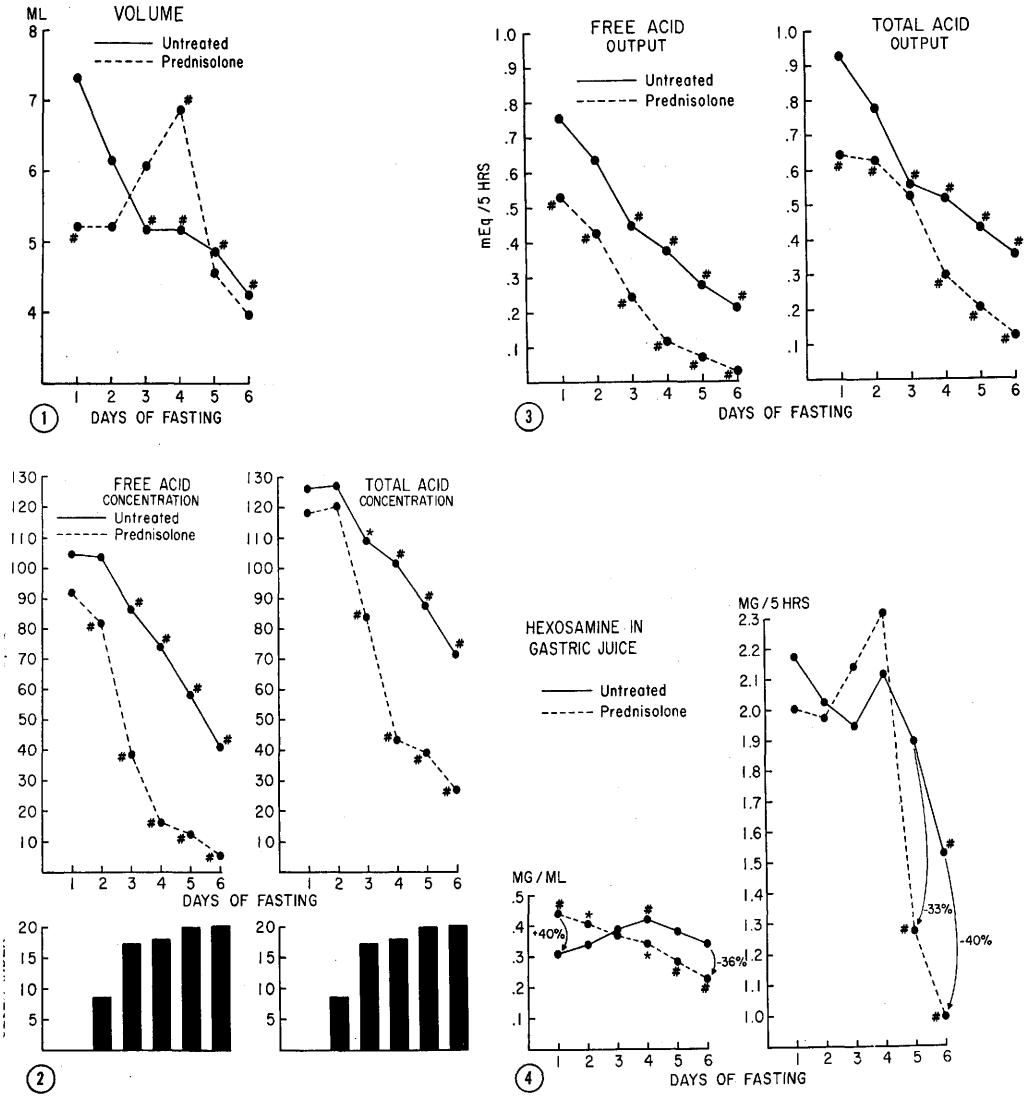

FIG. 1. Effect of fasting and of prednisolone on volume of gastric juice. "Untreated" curve: #: "P"<.01 when compared to value of day 1. "Prednisolone" curve: #: "P"<.01 when compared to value of untreated animals, fasted for identical time interval.

FIG. 2. Effect of fasting and of prednisolone on acid concentration of gastric juice. "Untreated" curve: #: "P"<.01 when compared to value of day 1. *: "P"<.05 when compared to value of day 1. "Prednisolone" curve: #: "P"<.01 when compared to value of untreated animals, fasted for identical time interval. "Ulcer Index": for prednisolone-treated animals only. See under Materials and Methods.

FIG. 3. Effect of fasting and of prednisolone on acid output of gastric juice. "P" value: same as for Fig. 1.

FIG. 4. Effect of fasting and of prednisolone on hexosamine concentration (left) and output (right) of gastric juice. "P" value: same code as for Fig. 2. Curved arrows indicate % difference, between 2 points produced by prednisolone,

length of time), but later, on Day 4, there was a stimulation (of 32%). Thereafter, the 2 curves coincided.

Acidity (Fig. 2 and 3). Free and total acid (concentration as well as output) progressively decreased during fasting and the differences from the values of Day 1 were statistically significant from the third day. Prednisolone accelerated the fall in acidity until, after 5 and 6 days, free acid had almost disappeared. The differences due to prednisolone are statistically highly significant.

In untreated rats, fasting ulcers appeared in the corpus after 5 and 6 days with the following incidence:

with pylorus ligation: 5 days, 33%; 6 days, 52%;
without pylorus ligation: 5 days, 7%; 6 days, 28%.

These ulcers are easily distinguishable from steroid ulcers: they are black due to embedded blood clots and usually do not have a crater. They have also been termed "intramucosal hemorrhages"(1). Their incidence was higher in pylorus-ligated rats presumably because of the retention of gastric juice.

Hexosamine (Fig. 4). Fasting alone tended to increase the concentration of hexosamine (significant after 4 days) and did not influence its output except after 6 days. In prednisolone-treated animals, the concentration was increased by 40 and 20% on the first and second day of fasting respectively; from the fourth day, it was lower than that of the controls. The output was not altered appreciably by prednisolone during the first 4 days of fasting but on the fifth and sixth days, the steroid produced a sharp fall (of 33 and 40% respectively).

Gastric tissue (Fig. 5). The concentration of hexosamine in the antrum was found to be twice that of the corpus at all times. During fasting, the hexosamine content, as compared to the values of fed rats, increased in both portions of the stomach; the rise was progressive in the corpus, and in the antrum a peak was reached after 2 days. All differences from values of fed animals are highly significant.

Prednisolone exerted the opposite effect: it completely prevented the rise of hexosamine in corpus and antrum normally elicited by fasting. The values, compared to those of rats fasted for the same period, showed a highly significant difference. When compared to the baseline of fed animals, there was a significant decrease in both portions only on the third day.

Ulcerations. The "ulcer index" for each group of prednisolone-treated animals is shown in Fig. 2 and 5. Ulcers were limited to the corpus and appeared on the second day; they were maximally developed on the third day. A full histological description of these ulcers is given elsewhere(1).

Discussion. Fasting up to 6 days induced in rats a progressive reduction in volume and acidity of gastric juice, but did not affect its mucus content expressed in terms of hexosamine. Not until the sixth day of fasting was the output, but not the concentration, of mucus decreased. It is significant that only after 5 and 6 days did fasting ulcers appear in control animals. As previously reported (16), a correlation exists between formation of these ulcers and diminution in rate of mucus secretion.

The decrease in gastric juice acidity seen during fasting was accentuated by prednisolone administration; actually, acidity was the lowest when steroid ulcers were maximally developed (from the third day). The marked acid decrease after prolonged fasting, with or without prednisolone, may be attributable in part to the buffering effect of blood present in gastric juices of animals with steroid ulcers. This explanation, however, does not account for the low acidity of gastric juice collected during the first 2 days of fasting and steroid treatment because this was relatively free of blood. It appears probable, therefore, that in the rat, steroid ulcers are not related to hyperacidity. A similar conclusion has been reached by others(18,19, 20) who failed to observe an increase in gastric acidity after treatment with corticosteroids.

Prednisolone produced a biphasic change in gastric juice mucus: there was first an

increase in hexosamine concentration followed by a marked diminution, of about 35%, of both concentration and output after 5 and 6 days. The rise in tissue mucus (corpus and antrum) normally elicited by fasting was completely prevented by prednisolone throughout fasting. The relatively high content of gastric juice hexosamine in prednisolone-treated animals during the first 4 days of fasting (Fig. 4) may reflect the ulcerogenic property of the steroid. Under such treatment, the mucosa is progressively eroded and the first cells to desquamate into the lumen are those of the surface epithelium, composed exclusively of mucus cells. It appears likely, therefore, that much of the mucus extracted from the juice at this time came from mucus cells debris and did not represent actual secretion. The sediment in gastric juice of prednisolone-treated rats was more abundant than that of the control animals, which supports this interpretation. After 5 and 6 days of fasting, however, most of the surface epithelium and part of the mucus neck cell layer had desquamated and passed into the small intestine; consequently, hexosamine content of the juice was very low and corresponded more truly to the actual rate of secretion. According to this interpretation, a high output of mucus in the juice after prednisolone does not imply a stronger mucus barrier, but paradoxically a weaker one since it is due to detachment of part of the mucosa. The mucus content of the tissue is revealing in this respect. The rise in mucus, in both corpus and antrum, normally elicited by fasting was completely prevented by prednisolone throughout fasting; there was a difference of about 25% in the values of prednisolone-treated animals as compared to those of the controls (Fig. 5). From these data, one can conclude that: a) a breakdown in the mucus barrier of the tissue precedes the appearance of steroid ulcers and thus appears to play an etiologic role, and b) the rate of mucus secretion in the juice after treatment with prednisolone is difficult to determine accurately because of admixture of whole mucus cells that desquamate as part of the ulcer process.

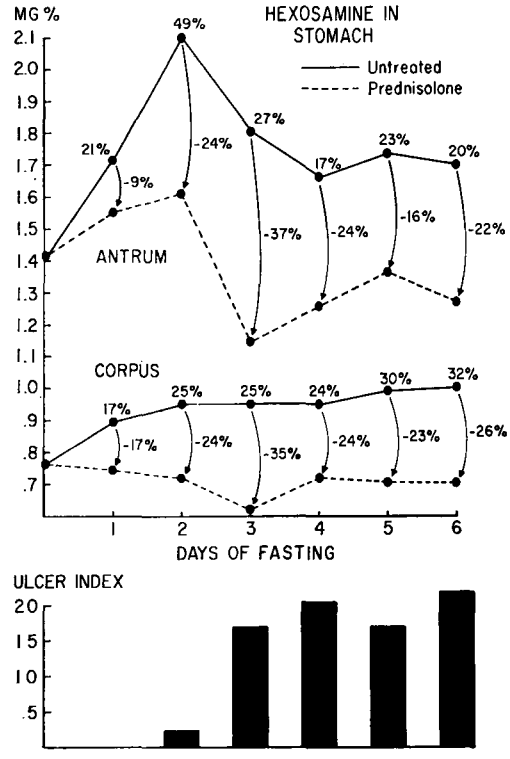

FIG. 5. Effect of fasting and of prednisolone on hexosamine concentration of corpus and antrum. "Untreated" curve: numerals above curve indicate % difference from value obtained in fed animals. All changes statistically significant ("P" <.01). "Prednisolone" curve: curved arrows indicate % difference between 2 points. All changes (except −9%) statistically significant ("P" <.01). "Ulcer Index": for prednisolone-treated animals only. See: Materials and Methods.

The diminution by prednisolone of gastric tissue mucus, as measured biochemically here, is in agreement with histological data reported by Baker and Bridgman(21) who found that PAS-stained material was depleted in mucus cells of the gastric mucosa in rats treated with cortisone. In man, corticosteroids were found to decrease the viscosity of gastric juice(22), a change interpreted as reflecting a diminution in mucus secretion. Cortisone was found to alter mucus secreted from an antral pouch in the dog (15). Although it decreased the volume regularly, changes in chemical composition of mucus (hexosamine, hexoses, fucose) were inconstant. Concentration of sialic acid in gastric juice was diminished by cortisone in 3 out of 4 dogs.

Our findings support a hypothesis(1,2, 14,15), based on a decrease in gastric mucus, to explain the appearance of steroid-induced ulcers. Corticosteroids would render the mucosa more sensitive to the digestive and irritative properties of gastric juice by weakening the mucus barrier. In this respect, it is significant that the diminution in mucus in the stomach itself (Fig. 5) took place before ulcers developed, which favors the concept of an etiologic role of mucus in ulcerogenesis. The low acidity of gastric juice after prednisolone overshadows the widely accepted theory that ulcers are necessarily related to hyperacidity.

Summary. Prednisolone, administered subcutaneously to rats, produced gastric ulcers and reduced the mucus of gastric juice and tissue. Determination of hexosamine was used as a measure of mucus. Ulcers were limited to the corpus and never appeared in the antrum. The antrum was found to contain twice as much mucus as the corpus. Gastric juice acidity was markedly reduced. These data favor a hypothesis ascribing a protecting role to mucus in the natural defense of the gastric mucosa against ulcerogenic agents. Steroid ulcers may be explained by a diminution of gastric mucus.

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In vivo and *in vitro* Effect of Glucagon on DL-Leucine-1-C^{14} Incorporation Into Protein of Rat Pancreas.* (28729)

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It has been found(1,2) that administration of glucagon to rats and rabbits results in a depletion of zymogen granules of acinar cells of the pancreas. Neither adr nalectomy, hypophysectomy, atropinizatio nor fasting had any effect on this action

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