

in the epithelium and myometrium, and the high incidence of stromal mitoses.

Classification of the uterotrophic responses noted in the present study is difficult. The digoxin-treated uteri showed definite vesiculation of the stromal nuclei and prominent stromal nucleoli, but these were the only characteristics of progestational stimulation. On the other hand, the edematous stroma without mitoses, the hypertrophic surface epithelial cells and the repaired muscle cells may be used to classify the effect of digoxin as an estrogen-like response. The appearance of the vaginal smears of the digoxin-treated rats, some showing proestrus and some late metestrus, strongly suggests estrogenic stimulation, however small this stimulation may be. The magnitude of the changes evoked in uteri which received both digoxin and estrone suggests that the uterotrophic influence of digoxin may have been added to or superimposed upon the estrone effect.

The ultimate fate of digitalis glycosides is still disputed. Because of the structural simi-

larity between cardiotonic glycosides and steroid hormones, one may speculate that a metabolite of digoxin, utilizing the cyclopentanoperhydrophenanthrene radical common to both groups of compounds, has estrogen-like qualities. Such a possibility as well as the roles of the pituitary, adrenals and liver are being investigated.

Summary. Digoxin administration to mature ovariectomized rats resulted in uterotrophic response evidenced by heightening of epithelium and enlargement of epithelial nuclei, edema of the stroma with vesiculation of stromal nuclei and prominence of stromal nucleoli, and recovery of the myometrium. The response of castrate uteri to estrone was intensified by the concurrent administration of digoxin.

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Production of Gastric Ulcers in Dogs on Protein Depletion Regime.* (23178)

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In higher mammals production of hemoglobin takes precedence over formation of most body proteins. Advantage was taken of that fact in 1939 when we were able to demonstrate that in the dog, hemoglobin production could be limited by protein intake(1). By "forced hemoglobin production" in dogs concomitantly maintained on a low protein intake circulating and reserve proteins were rapidly depleted. In recent studies in which a similar technic of protein reduction was used in studies of anaphylaxis(2) and homo-transplants† we have been impressed with the high incidence of gastric ulcer and particularly where a perforation was the immediate cause

of death. These lesions varied quite considerably as to severity and location. In some instances there were patchy and in others quite extensive denudement of the gastric mucosa. In a few, deep indurating ulcers involved not only the mucosa but the muscularis. Very frequently complete perforation of the stomach occurred with appearance of "punched out" lesions frequently seen at human autopsies. Some of these changes can be seen in Fig. 1, 2 and 3. Most frequently the lesion was seen on the greater curvature or above the pyloric sphincter and uncommonly in the duodenum.

Methods. The procedure can be described briefly as follows: The dogs were bled $\frac{1}{4}$ of estimated circulating blood volume (72 ml/kg) on each of 3 successive days. The same

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† In preparation.

TABLE I. Low Protein Diet Used with Forced Hemoglobin Production.

	g	Prot.	Fat (g)	CHO	Total cals.
Bananas	300	3.6	.6	69	300
Salmon	100	20.6	9.6	0	168
Cod liver oil	40		40		360
Karo syrup	120			120	480
Wesson salts	1				
	560	24	50	190	1308
		7.4% prot. cal.	34% fat cal.	58% CHO cal.	

Fed 50 g/kg = 117 cal./kg.

amount of blood was removed on the 5th day and this usually resulted in establishment of a temporary anemic state at about $\frac{1}{2}$ normal hematocrit value. Phlebotomy was then carried out at such intervals as to maintain the hematocrit at approximately this level. From this time iron as saccharated oxide ("Pro-ferrin" was administered at time of each bleeding in amounts of approximately 1 mg for each ml of red cells removed. Normally this would result in maintenance of iron balance and since under this procedure, the erythrocytes become hypochromic, one was giving essentially an excess of iron over and above that removed. From the time of establishment of the anemic state, the dogs were maintained on a diet low in proteins as well as other dietary essentials such as certain vitamin fractions. Its composition is shown in Table I.

Results. A group of 6 litter-mate dogs was being depleted of proteins by the method described above. Two of these animals died of perforating ulcers 6 and 7 months respectively after institution of the diet, bleeding and iron injections. This called our attention to the incidence of such changes and we realized that in the past we might have missed at autopsy a number of instances in which non-perforating ulcers had existed in the stomach or duodenum. Therefore, in the present communication we shall be concerned mainly with the next 15 animals which were placed on this regime and examined carefully at autopsy for gastric and duodenal lesions.

Two litter-mate animals, 55-7 and 55-8, were treated by the same procedure and died

in 3.5 months of perforating ulcers. The first had an ulcer of the lower greater curvature which had perforated and also other ulcers involving the gastric and duodenal mucosa. The second had perforating pyloric and duodenal ulcers. The next group of 4 litter-mate dogs, 55-53 through 55-56, on the same regime with no other treatment survived 2, 3, 3 and 3.5 months respectively. Three out of 4 of these animals showed lesions of the mouth and fore and hind legs. Two had no ulcerative changes in the gastro-intestinal tract. On the other hand, 2 of them had perforating pyloric ulcers and 1 of these in addition had a perforating duodenal ulcer. The other with the perforating gastric ulcer showed a hemorrhagic ulcer of the greater curvature. The next 3 animals were litter-mates 55-82 through 55-84. These animals were on the same regime as the other dogs except that 2 of them received daily supplements of vitamins as follows: Thiamine = 50 mg; riboflavin = 5 mg; nicotinamide = 50 mg; ascorbic acid = 12 mg and Brewers yeast = 1.5 tablets daily. The third animal received no vitamins. The latter animal survived 5 months and cause of death was unknown. At autopsy the gastric wall showed internal hemorrhages and the usual fatty liver was present. One of the vitamin treated animals was sacrificed at this time for comparative purposes and showed a normal stomach. The liver showed grossly very slight fatty infiltration. The second animal receiving vitamins died 8 months after being placed on this regime and showed internal hemorrhage of the gastric wall.

The next 7 dogs were litter-mate animals, 55-43 through 55-49, of which one died of extraneous causes before the regime began. Three months after beginning the low protein-forced hemoglobin production regime, 3 of the animals were given a single intravenous injection of radioactive colloidal gold of 30 mc. Following depletion of their circulating protein they were all used as candidates for skin homografts following which they were later studied for tuberculin sensitivity 4 weeks following subcutaneous administration of bacto-adjuvant. It is interesting to note that 5 out of 6 of the animals survived for 5

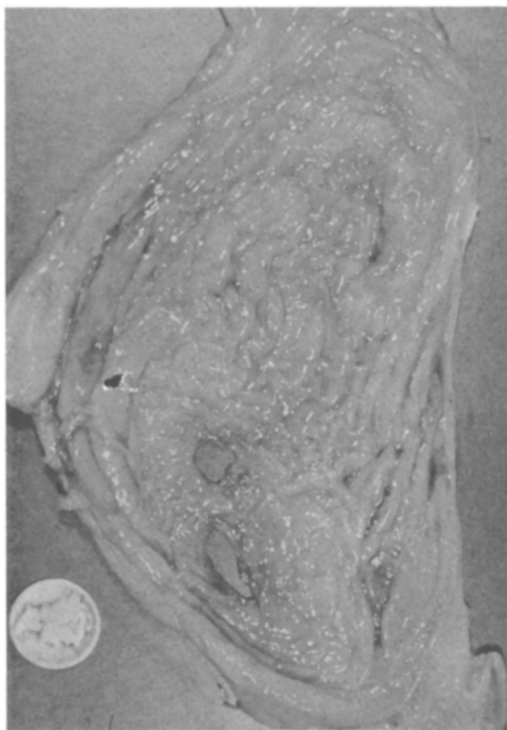


FIG. 1. Dog 55-48: Internal wall of stomach showing 2 indurating ulcers on curvature.

months all dying spontaneously within a period of 1 week. The last animal survived $6\frac{1}{2}$ months. Four of the 6 animals showed absence of mucosa in the greater curvature. The 2 others showed deep indurating ulcers of the pyloric mucosa and duodenum. Two of the animals had, in addition, perforating ulcers above the pyloric sphincter.

The animals on this regime lost a considerable amount of weight and, indeed, this was the most striking feature as far as clinical signs were concerned. Elsewhere(2) we have shown that the electrophoretic patterns of dogs on such a regime undergo a considerable change as far as distribution of the plasma protein constituents although the total plasma proteins are not markedly altered.

Aside from the high incidence of peptic ulcer in these animals several other points should be mentioned briefly. In every case there was a very considerable amount of deposition of subcutaneous fat varying from 1.5 to 3 cm in thickness. This was such as to preclude the successful use of skin homografts. This factor will be described in detail

elsewhere. This lipid deposition, it should be noted, took place in spite of the uniform degree of emaciation in these animals. It was very nearly liquid in appearance suggesting a high degree of unsaturation. In all animals there was at autopsy gross evidence of extensive fatty infiltration of the liver although in the 2 vitamin treated animals this seemed to be somewhat less than in the others. In most of the animals there also developed rather deep ulcerative lesions usually involving the mouth and quite characteristically also appearing at the ankle joints. We had noted these lesions in our earlier work involving the use of forced hemoglobin production in protein depletion(1) and found that such lesions very promptly disappeared within a few days following the administration of whole liver.

Of the 15 dogs consecutively used the time from institution of the low protein-forced hemoglobin regime to death averaged 4.5 months.

Discussion. Li and Freeman(3) reported 47% incidence of peptic ulcers in dogs maintained on diets which were free from protein except for that contained in yeast which made

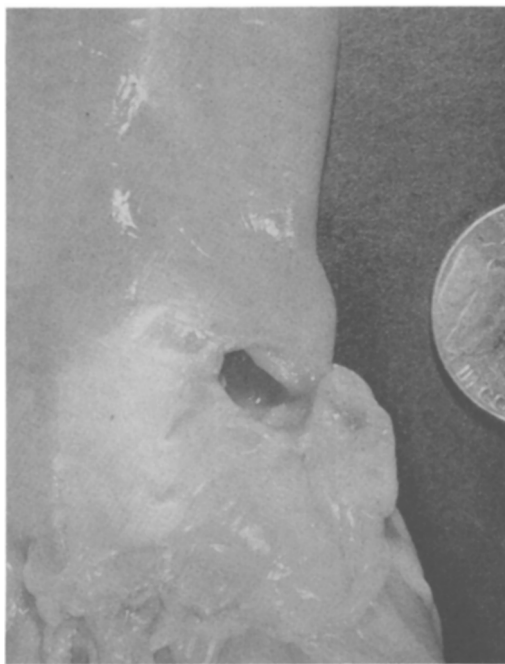


FIG. 2. Dog 55-7: Exterior wall of lower portion of stomach and duodenum showing perforating ulcer.



FIG. 3. Dog 55-48: External wall of stomach showing one indurating and one perforating ulcer near pyloric area.

up 5% of each diet. Twelve weeks on such a regime seemed to be the minimum of time required for development of any peptic ulcer. Of 32 protein deficient dogs studied 15 had solitary peptic ulcers, 13 of which animals had ulceration on the pyloric side of the sphincter. Fouts(4) fed dogs a low protein (casein) diet supplemented with thiamine, nicotinic acid, riboflavin, pyridoxine and pantothenic acid over extended periods of time. They lived on an average of 175 days developing anorexia, weight loss, anemia, skin ulcers, peptic ulcers and fatty cirrhotic livers. Weech and Paige(5) maintaining their dogs in the study of nutritional edema on a diet consisting of carrots, rice, lard, cod liver oil and sugar and salt mixture found a high incidence of peptic ulcers of the stomach and duodenum. Among 22 animals receiving the diet for an average of 90 days 36% exhibited true peptic ulcers at autopsy. Of the remaining 14, 23% showed erosions of gastric or duodenal epithelium without true ulcer formation. The incidence of ulcers could not be correlated with duration of the diet, degree of emacia-

tion as shown by weight loss or degree of anemia which was established. There seemed to be the possibility of a correlation between serum albumin levels with development of the ulcers. Other investigators have described occurrence of gastro-intestinal ulcers in rats and mice maintained on various protein deficient diets.

We do not feel that induction of skin grafts or sensitization to bacto-adjuvant with subsequent tuberculin test were likely to have affected the induction of the ulcerous lesions. The incidence of overall lesions is higher in that group than in the group of dogs as a whole but the number involved is too small to permit evaluation. The closeness of time of death in the siblings of this group however is very striking as mentioned above.

Simple iron-deficiency anemia maintained in dogs by phlebotomy while on normal or low-iron diets over many years does not result in gastric lesions in the experience of one of the authors (PFH).

On the basis of the findings we are not prepared to speculate on the relationship of lowered and altered distribution of circulating proteins or depletion of body protein stores on the induction of ulcers.

Summary. Dogs maintained in the anemic state by phlebotomy with intravenous injection of amounts of iron equivalent to that removed in hemoglobin and concomitantly fed a low protein diet, are quite rapidly depleted of their protein reserves and the circulating plasma proteins are markedly altered. Weight loss is invariably considerable. In these animals the incidence of peptic ulcer is extremely high with a large percentage developing perforating ulcers causing death.

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