3 cell types and total WBC returned toward the control values although they were slightly higher.

Discussion. The results indicate that the leucocytosis which develops in animals fed a magnesium deficient diet is coincident with development of hyperemia of the ears. During this phase of the magnesium deficient syndrome Tufts and Greenberg(2) reported that plasma magnesium concentration is at its lowest, a slight rise occurring with disappearance of the hyperemia. Belanger *et al.* (3) has recently reported that dermal mast cells are degranulated during the hyperemic phase of magnesium deficiency followed by regranulation with the disappearance of hyperemia.

It seems likely that these observations are interrelated. Belanger *et al.*(3) suggested that sudden deprivation of magnesium results in histamine liberation from mast cells which may be the cause of the hyperemia. The low magnesium levels, or histamine release, or both, may be the cause of the leucocytosis which occurs during this phase, especially the eosinophilia.

In the present report, the blood counts for all rats for day 8 were grouped regardless of the hyperemic state of the ears. If only those rats with 3+ to 4+ ear scores are considered, the leucocyte counts, especially that of the eosinophiles, average considerably higher. In this case, the eosinophiles were 1480/mm^3, mononuclear leucocytes 29,400/mm^3, and neutrophiles 16,400/mm^3. The eosinophile counts were now 11.6 times their respective control value, while mononuclears were 2.1 times the control value and neutrophiles 3.9 times their control value. Obviously, the eosinophiles were elevated relatively more than the other leucocytes.

It is known that the leucocyte count in rats increases with age from the immature to adult stage. However, the control leucocyte counts reported here increased slightly more than expected. A change in diet from pellets to the synthetic diet may have contributed to this unexpected rise. It should be emphasized that there was no macroscopic evidence of infection in the control group at autopsy.

Summary. Rats fed a magnesium deficient diet develop a leucocytosis during the hyperemic phase of the syndrome. Mononuclear leucocytes and neutrophiles contribute to the leucocytosis but eosinophiles are elevated relatively more, being increased over 1,000%. Possible relationships to the development of the leucocytosis are discussed.

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Received July 11, 1958. P.S.E.B.M., 1958, v99.

Ulcerogenic Property of Steroids. (24378)

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A serious side-effect of ACTH and corticoid therapy in man is development or reactivation of gastroduodenal ulcers(1-9). To understand the pathogenesis of their production and to find means of preventing their appearance, it became desirable to find a method to produce these ulcers in experimental animals. For various reasons, the existing methods of producing ulcers experimentally do not lend themselves to quantitative evaluation of the effect of steroids. The ulcers obtained by some technics, such as with the Shay rat, are inhibited by concurrent treatment with corticoids(10). Injection of a small amount of formaldehyde into the gastric wall of a rat leads to formation of a necrotizing ulcer which is aggravated by cortisone, but it is difficult to quantitate the degree of the lesion(11). In

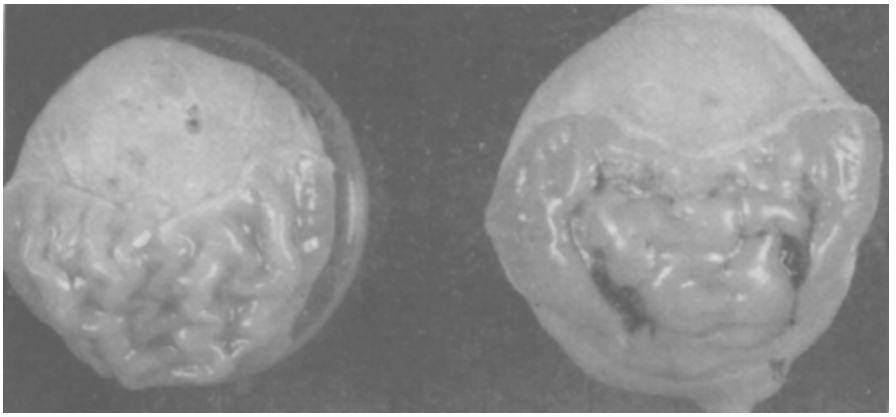

FIG. 1. Steroid induced ulcer. Open stomachs of animals fasted for 4 days. Left: no treatment. Note ulcers in forestomach and intact mucosa in pyloric portion. Right: Δ^1-COL, 4 mg, daily, S.Q. No forestomach ulcers but multiple and deep ulcers in the pyloric portion.

this study, multiple gastric ulcers were produced in rats by intensive treatment with corticoids.

Materials and methods. Female rats of the Upjohn strain (originally from Sprague-Dawley) of a body weight ranging from 150 to 165 g were divided in groups of 10 animals, placed in individual cages, and fasted for 4 days starting at 8 a.m. Either cortisol (COL) or Δ^1-cortisol (Δ^1-COL) were injected at various doses (Fig. 2) in a volume of 0.2 ml of CMC (carboxymethylcellulose, polysorbate 80, propylparaben and water) subcutaneously once daily. Control animals received only the vehicle. In some experiments, antibiotics (penicillin G crystalline, 5,200 μ plus dihydrostreptomycin sulfate, 4 mg, mixed into the same syringe in a volume of 0.2 ml, subcutaneously twice a day) were also administered in order to prevent development of infection. Addition of antibiotics was found not to interfere with formation of ulcers. After 4 days, the animals were killed with chloroform, the stomachs dissected, opened along the lesser curvature, and the mucosa observed under a magnifier.

Results. Following this treatment, gastric ulcers regularly developed (Fig. 1). They were usually multiple, varying in size from 0.5 to 3 mm and located only in the pyloric (glandular) portion of the stomach (both antrum and fundus). The percentage of animals showing ulcers was determined, severity of the lesions expressed according to an arbitrary scale of 0 to 3+ and number of ulcers per animal was recorded. Ulcer index was calculated by adding incidence divided by 10, severity (in pluses) and number of ulcers per rat. Table I gives the results of a typical experiment and shows how the ulcer index is determined. Maximum ulcer index possible is about 20, which represents 100% incidence, 3+ severity, and 7 ulcers per rat.

The relative ulcerogenic properties of COL and Δ^1-COL were compared (Fig. 2). Results indicate that (a) Δ^1-COL is 2 to 3 times as ulcerogenic as COL; (b) ulcerogenic property of a steroid is directly related to the dose; (c) the assay is reliable and reproducible, as proved by the curve shown in Fig. 2 which includes experiments performed during a year's time. When-

TABLE I. Steroid Induced Ulcers.

		Controls	COL, 10 mg
Initial body wt		165	163
Final " "		124	112
Body wt difference		−41	−51
Forestomach	Edema (%)	60	40
	Ulcers		
	%	60	0
	No./rat	3.5	
Pyloric ulcers	Incidence (%)	0	90
	Severity (+)		1.1
	No./rat		5.3
	Ulcer index	0	15.4

COL was administered daily for 4 days to fasted animals.

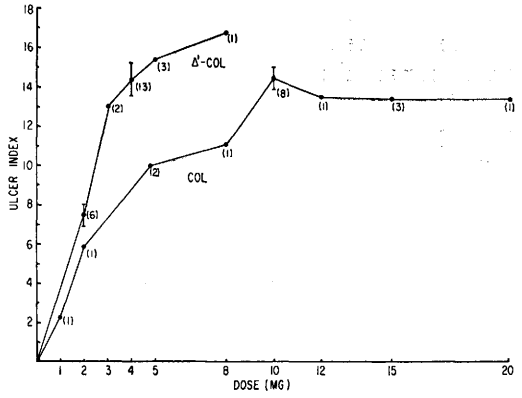

FIG. 2. Relative ulcerogenicity of COL and Δ^1-COL. Number in parentheses refers to number of experiments performed for each dose, each experiment corresponding to 10 animals. Standard deviation, shown for values obtained from 6 or more experiments, is low.

ever standard error could be calculated between experiments on a same dose, it was low. (d) In the case of COL, a plateau is reached with a dose of 10 mg. This may be due to the fact that on injecting more than 10 mg, the proportion of material absorbed is not appreciably increased. This view is supported by the fact that at autopsy large amounts of unabsorbed compound are still found in the subcutaneous tissue when 10 mg or more are injected.

Histological study of these stomachs will be reported in detail elsewhere. Briefly, in the steroid-treated animals, there is a general thinning of the gastric mucosa; at first tiny and later extensive hemorrhages are seen within the glands and eventually parts of tissue become necrotic. Necrosis involves only the mucosal surface when the dose is low and extends to the submucosa with a high dose. In a full-blown ulcer, the mucosa has completely disappeared leaving a crater whose bottom consists of necrotic debris and polymorphonuclear infiltration overlying a moderately edematous submucosa.

In the forestomach the reverse situation exists. In at least half of the control animals fasted for 4 days and not treated with steroids, the forestomach wall is edematous and contains numerous ulcers. These are similar to those of the Shay rat except that they are less severe and never lead to perforation. When steroids of the cortisol type are administered, these ulcers never appear and most often edema is either absent or only slight in the forestomach. As in the case of the Shay rat, cortisol exerts a paradoxical effect in that it promotes formation of ulcers in the pyloric portion of the stomach and prevents their appearance in the forestomach.

Discussion. These experiments indicate that it is possible to produce gastric ulcers in the rat with steroids. The test can be used quantitatively since it is expressed in terms of ulcer index ranging from 0 to 20. The response is dose-related and is obtained within a short period of time, that is, 4 days. Steroid-induced ulcers will also appear without fasting but they develop more slowly and are frequently complicated by uncontrollable generalized infection. In experiments not reported here, ulcers were produced in nonfasted rats after 10 to 15 days of treatment with large doses of steroids.

An important characteristic of the steroid-induced ulcer is its location. It is always found in the *glandular* (pyloric) portion of the stomach where the mucosa is similar to that of the human stomach. Generally, the steroid-induced ulcer of the rat resembles rather closely human peptic ulcer. In both cases, there is hemorrhage, necrosis of a segment of the mucosa, and formation of a crater whose bottom is composed of necrotic debris and leukocytes. Differences between this and the human ulcer are: (a) perforation has never occurred. This could be due to the fact that assay time is too short for perforations to occur. Actually, if the test is extended over 4 days, many animals die. It is possible that by maintaining these animals with parenteral glucose and amino acids, it would be possible to increase the severity of the disease to the point of perforation. (b) The ulcers are usually multiple, in general 2 to 4 in number, although as many as 8 in some cases of heavily treated animals. This difference may be due to the very acute and severe conditions of the experiments, namely, complete fasting and heavy dosage with potent glucocorticoids. It could be that man placed in similar conditions would also develop multiple ulcers. (c) Duodenal ulcers have not been encountered. This may be a species difference.

Ingle *et al.*(12) made the incidental observation that some rats, nonfasted and receiving high doses of cortisone acetate, developed ulcers in 3 weeks. Several of the treated animals also exhibited signs of general infection due to cortisone overdosage. It has been our experience that the stomach is a site of predilection for development of infection in such conditions. There is a possibility that some of the lesions represented local infection of the gastric wall, although we have also confirmed development of ulcers without infection in nonfasted rats. Baker(13) also reported formation of gastric ulcers in a few rats receiving 6 mg of ACTH daily.

The mechanism by which the steroid ulcers develop is obscure. Among the hypotheses that can be formulated, 3 seem to be of particular interest: (a) Corticoids may increase secretion of gastric juice. This has been shown repeatedly in man and several animal species(14,15). To our knowledge it has never been demonstrated in rats and preliminary experiments from our laboratories indicate that such an increase in either acid or pepsin does not take place after corticoid treatment in rats. The only change observed was an increase in volume of gastric juice in animals treated with an ulcerogenic dose of corticoids. (b) The ulcers may appear because of the antiphlogistic property of the steroids. Normally, an irritant stimulates an inflammatory defense reaction in the tissues where it is applied. In the case of an animal fasted during 4 days, gastric juice, unbuffered by food, constitutes a strong irritant. If high doses of antiphlogistic corticoids are administered, as in the present experiment, the inflammatory reaction is blocked and the irritant (gastric juice) may damage the gastric mucosa. In other words, ulcer formation would result from a misplaced antiphlogistic effect. (c) It has been suggested that corticoids both in man and animals(16,17) decrease the secretion of mucus by the surface epithelium. Adrenalectomy in the rat produces the opposite effect(18). It is conceivable that a decrease in mucus, which has been considered as a protective barrier, favors the attack and digestion of the mucosa by gastric juice.

The fact that cortisol prevents appearance of ulcers in the forestomach while it promotes their formation in the pyloric portion may be explained as follows: in the *forestomach* of a fasting animal or of a rat with a ligated pylorus, the lesion is an inflammatory reaction initially caused by concentrated gastric juice. This lesion is characterized first by a tremendous edema of the forestomach wall (which is increased about 10-fold in thickness). The edematous tissue contains inflammatory cells (polymorphonuclears and round cells). Only after this has progressed for some time do the ulcers develop. They probably appear because, the vessels being compressed by the pronounced edema, a state of malnutrition of the surface epithelium, which is already avascular, develops. Such a malnourished tissue is not as resistant to gastric juice as normally and therefore undergoes necrosis. Cortisol prevents this initial inflammatory reaction, there is no edema, no ischemia and the mucosa retains its integrity.

In the *pyloric portion*, on the other hand, the mucosa is thick, richly vascularized and not or only slightly edematous. Therefore, there is no ischemia. Cortisol then produces ulcers through a different mechanism, according to one or more of the hypotheses mentioned above.

Summary. An assay for the ulcerogenic property of steroids is described. The test compound is administered subcutaneously once a day for 4 days during which the animals are fasted in individual cages. At autopsy, the incidence of animals showing ulcers is noted, severity is estimated, and number of ulcers per rat is counted. These 3 values are combined and expressed in terms of ulcer index ranging from 0 to 20. It was shown that response is directly related to dose and that Δ^1-cortisol is 2 to 3 times as ulcerogenic as cortisol. Hypotheses to explain this effect of steroids are mentioned, namely, increased secretion of gastric juice, antiphlogistic property of steroids favoring necrosis of the mucosa, and decrease of mucus formation in steroid-treated animals.

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Received July 24, 1958. P.S.E.B.M., 1958, v99.

Mode of Action of Insulin, Carbutamide, and Tolbutamide.* (24379)

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Carbutamide and Tolbutamide may lower the concentration of blood glucose in several ways, one of which is release of insulin by the pancreas. Although based on strong experimental evidence(1-16), this mode of action cannot be accepted as the sole explanation for hypoglycemia unless it can be shown that other metabolic effects of insulin are also shared by the drugs. Results of experiments designed to study this point have not been uniform. One group of investigators believes that tolbutamide, like insulin, stimulates peripheral glucose utilization(7), while other groups could find no evidence for this stimulation(8,9). The action of sulfonylureas on muscle glycogen is also uncertain, as it has been reported that its synthesis *in vivo* is stimulated(10), inhibited(11) or not affected (12,13) by tolbutamide. Contrary to insulin, carbutamide and tolbutamide stimulate synthesis of liver glycogen in fasted animals(11-15), but tolbutamide does promote glycogen deposition into peripheral fat as does insulin (15). Blood pyruvate increases during insulin hypoglycemia(16,17), but it was found increased(18), unchanged(17,19) or decreased(16,20,21) during sulfonylurea hypoglycemia. Like insulin, tolbutamide depresses ketosis(15), but, unlike insulin, it does not affect the disappearance of D-xylose and L-arabinose from the blood(21). The reasons for these contradictory results are not clear: perhaps the sulfonylureas act on the liver as well as on the pancreas(13,22), or perhaps insulin, secreted directly into the portal system under sulfonylurea stimulation, acts differently from insulin injected into the periphery. Purpose of this work was to investigate this possibility.

Methods. Forty-seven mongrel dogs of both sexes were fasted for 16-18 hours and anesthetized with a mixture of sodium pentobarbital (35 mg/kg) and sodium barbital (100 mg/kg), injected intravenously. This anesthetic mixture provided the rapid induction and the long lasting, uniformly deep anesthesia with complete muscle relaxation, which is essential to prevent excessive fluctuations in blood pyruvate and lactate, unrelated

* Aided by Grant from Nat. Inst. of Arthritis and Metabolic Diseases, U.S.P.H.S.

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