Effect of a Histamine Releaser on Steroid Induced Ulcers. (27311)

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Two histamine releasers, compound 48/80 and polymyxin B, have been reported to produce acute gastric hemorrhagic necrosis of the mucosa only a few hours after a single intraperitoneal injection in the rat(1,2,3). When the animals are killed up to 7 days later, deep well-defined ulcers are found in the glandular portion of the stomach(2) and they eventually heal. On the other hand, gastric ulcers of different histological structure can be produced in the rat by extensive treatment with glucocorticoids(4). The defects are localized in the glandular portion, appear after about 3 days of treatment, and are multiple. They bear a close resemblance to human peptic ulcers, especially to those that develop during steroid therapy. Paradoxically, it was found that a short treatment with glucocorticoids (cortisol(5), prednisolone(6)) prevents development of polymyxin B ulcers. Another steroid, desoxycorticosterone, which has been shown previously to exert antiphlogistic activity when given at high doses(7), likewise inhibits these acute gastric ulcers(6).

It was then deemed of interest to investigate whether reciprocal protection would take place, namely, would treatment with progressively increasing doses of polymyxin B, known to release most of body histamine (8), influence the ulterior development of steroid induced ulcers? If the latter were prevented by such a treatment, it would suggest that mediation by products liberated from mast cells by polymyxin B (histamine, possibly serotonin and other amines) is involved in the ulcerogenic property of steroids.

Materials and methods. Sixty female Sprague-Dawley rats of an average body weight of 128 g were divided into 6 equal groups and treated as indicated in Table I. Groups II, V and VI were given the following increasing doses of polymyxin B sulfate, twice daily: days 1 and 2, 200 μg; days 3 and 4, 400 μg; day 5, 600 μg; day 6, 800 μg; days 7 through 15, 1000 μg. Each dose was injected subcutaneously in a volume of 0.2 cc of saline. The other groups received as many injections of saline. After the first day, an anaphylactoid reaction began to develop (3), characterized by swelling and redness of the paws, the snout, and redness of the ears. The animals also had a tendency to scratch their noses. This reaction was maximal after 3-4 days of treatment after which it gradually faded so that after 11 days the anaphylactoid reaction was no longer visible. On the twelfth day, all the animals were fasted in individual cages. Groups III through VI were treated with various doses of prednisolone (Table I), in one subcutaneous daily injection of 0.2 cc, following the usual procedure to study the ulcerogenicity of steroids(4). Vehicle (a solution of carboxymethylcellulose, polysorbate 80 and benzyl alcohol in isotonic saline) was given to Groups I and II. After 4 days of fasting and steroid injection, during which polymyxin B continued to be given to the same groups as before fasting, the animals were killed with chloroform. One hour before killing, however, 25 mg of ferric chloride in 2 cc of water was administered orally. Iron was found to be retained electively by the ulcers and the latter can thus be counted accurately by development of the Prussian blue reaction(9,10). At autopsy, the stomachs were opened along the lesser curvature, washed under running water and spread over the round end of a glass tube. They were immersed in this position in a solution of 2% potassium ferrocyanide and 1% hydrochloric acid for 10 minutes. After that time, they were removed from the glass surface and washed again with running water. The excess blue present on the surface of the stomach was gently rubbed off with fingers. The stomachs were then examined with a binocular 2 $\times$ magnifier for presence of ulcerations. The number of ulcers was used as endpoint since it was found earlier to be directly related to the dose of steroid(9,10).

Results. By using the ferrocyanide technic ulcers are stained deep blue (Prussian blue)

TABLE I. Effect of Polymyxin B Pre-treatment on Development of Steroid Ulcers. Severity of forestomach ulcers expressed according to a relative scale from 0 to +++.

	I	II	III	IV	V	VI
	Vehicle	Polymyxin B	Prednisolone, 1 mg	Prednisolone, 3 mg	Polymyxin B Prednisolone, 1 mg	Polymyxin B Prednisolone, 3 mg
Body wt (g)	128	128	128	127	127	128
Day 1–2		200 μg × 2				
3–4		400 μg × 2			Same doses of polymyxin B as Gr. II	
5		600 μg × 2				
6		800 μg × 2				
7–12		1000 μg × 2				
Body wt	160	162	159	160	162	162
Day 12–15, fasting + prednisolone		1000 μg × 2				
Final body wt	114	109	103	101	100	98
Body wt diff.	−46	−53	−56	−59	−62	−64
Forestomach						
Edema, %	30	10	40	33	10	10
Ulcers, %	50	60	80	44	20	40
Severity (+)	.9	1.1	1.0	.3	.2	.3
Glandular ulcers						
Incidence, %	0	0	100	100	100	100
No./stomach			8.3	22.0	8.0	20.7
Blood in intestine, %	40	40	30	70	30	90

whereas the background is colorless or only faintly stained. Table I indicates that there was no significant influence of polymyxin B pretreatment on the development of steroid (glandular) induced ulcers, both with regard to incidence of animals showing ulcers and their number per stomach. Similarly, polymyxin B did not affect the incidence of animals having visible blood in the intestine consequent to bleeding of the steroid ulcers. Forestomach fasting ulcers were identical with and without polymyxin B (Groups I and II). As previously noted(4), forestomach ulcers, due to prolonged fasting, are less severe in steroid treated animals. Since forestomach ulcers are probably due to mucosal irritation by gastric juice, as in the case of Shay ulcers, inhibition by glucocorticoids appears to result from their anti-inflammatory property.

Discussion. There have been several reports showing that blood and tissue polymorphonuclear eosinophils contain appreciable amounts of histamine and it was suggested that these cells would serve as carriers for histamine(11,12). If this were the case, one would expect that glucocorticoids, by their destructive effect on eosinophils, would acutely release histamine into the tissues where these cells are abundant. The stomach contains an especially large number of eosinophils in the connective tissue of the mucosa and the submucosa and upon corticoid administration they can no longer be found(13). It became logical to assume that sudden liberation of histamine, an ulcerogenic substance, from these cells could account for development of steroid ulcers.

The results of the present experiment seem to rule out such a hypothesis. Indeed, even when more than 90% of skin histamine has been depleted by progressive treatment with polymyxin B(8), the ulcerogenicity of prednisolone is unimpaired. One can further conclude from this study that other products of mast cell granules probably play no important role in production of steroid ulcers, at least in the rat. This kind of lesion must be mediated through other mechanisms. Polymyxin B, however, liberates very little serotonin from the tissues and moreover it does not appear to come from the mast cells(8). It would therefore be of interest to perform a

similar study using a serotonin releaser, like reserpine. Such an experiment could throw some light on the pathogenesis of steroid induced ulcers since 5-hydroxy-tryptophan, a serotonin precursor(14) and serotonin(15) were found to be ulcerogenic in rats.

It is interesting to note also that the animals of all groups gained weight equally during the pretreatment period with polymyxin B (Table I). When, however, these animals were fasted, whether in addition they received prednisolone or not, they lost more weight than their respective controls (not receiving polymyxin B). It would seem that previous depletion of histamine renders the animal less resistant to fasting in terms of body weight loss.

Summary. Administration of polymyxin B releases chiefly histamine from tissues. When rats are depleted of histamine by increasing doses of polymyxin B, the ulcerogenic property of prednisolone is unimpaired. This seems to rule out mediation of histamine in the pathogenesis of steroid induced ulcers, at least in the rat.

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Placental Transport of Model Amino Acids.* (27312)

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Amino acids are known to be concentrated in their transfer across the placenta(1). In exploring such transport processes, one needs to exclude irrelevant metabolic attack of the solute in question. For this purpose amino acids not susceptible to modification by the animal organism have proved useful. The present study explores the utility of such model amino acids for investigation of placental transport.

Experimental. Pregnant guinea pigs were used when estimated to be within one week of parturition. The weights of the fetuses on subsequent delivery were sufficiently uniform (65 to 75 g) to support this estimate. The gestation period is 68 days. Each animal was injected intraperitoneally with 10 μc of the 4 carboxyl-C^{14}-labeled amino acids. For α-aminoisobutyric acid this dose represented 0.22 mg, for l-aminocyclopentanecarboxylic acid, 1.3 mg, and for β-monofluoro and β,β,β-trifluoro-α-aminoisobutyric acid, about 3 mg. These amino acids have all been shown to yield extremely small amounts of radioactive CO_2 after injection into the mouse or rat; in the case of the first no other radioactive substance besides the injected amino acid has been detected in the urine(2,3). Therefore we assume that distribution of radioactivity measured distribution of the amino acid in the guinea pig.

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