

# EFFECT OF ADRENALECTOMY AND ADMINISTRATION OF PREDNISOLONE ON GASTRIC ULCER FORMATION IN FORESTOMACHECTOMIZED SHAY ALBINO RATS

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**Summary :** The gastric ulcerogenic effect of the glucocorticoid, prednisolone, was studied in albino rats. Shay operation in forestomachectomized rats with intact adrenals induced acute gastric ulcers in the corpus, whereas adrenalectomy prevented the development of gastric ulcers in another group of forestomachectomized Shay rats. Only superficial erosion of gastric epithelium was noticed in 66.67% of these rats.

Administration of prednisolone to another group of similarly operated rats increased the number and severity of gastric ulceration, whether the adrenals were ablated or otherwise. From the observation of acid and mucous content of the stomach, it is postulated that the reduced mucous secretion may be an important predisposing factor in gastric ulcerogenic effect of steroids.

**Key words :** adrenals                      adrenocorticoids                      gastric ulcers                      rats

## INTRODUCTION

Spontaneous gastric ulceration does not occur in rats under conditions existing in the laboratories (5,16). However, several procedures, surgical and otherwise, have been employed to induce gastric ulcers in rats and other laboratory animals. These include the 'Shay' operation (pyloric ligation) originally performed by Shay *et al.* in rats (26), the Mann-Williamson operation of surgical duodenal drainage (13,15) gastric freezing (1), thermal injury (28), implantation of the lower end of the bile duct into the stomach (11), electrical stimulation of the hypothalamus (25) and vascular occlusion of the gastric artery (24). Of these, pyloric ligation (26) appears to be a reliable and simple technique to induce gastric ulceration particularly in mice and rats and has been extensively used in various experimental studies (4,7,14,19,20,26).

Clinical observations and experimental evidences indicate that adrenocorticoids are also involved in the formation and enhancement or aggravation of gastric ulcers (4,5,6,10,12,21,22,23). Several factors have been suggested in the etiology and

pathogenesis of steroid induced gastric ulcers such as decreased mucous secretion (17, 21, 22, 23), increased acid and pepsin secretion (9, 6, 21), as also the antiphlogistic property of steroids (21) and direct tissue action (2). An important characteristic of the steroid induced ulcer in rats is its location. It is always found in the glandular portion of the stomach, where the mucosa is similar to that of the human stomach and generally the steroid induced ulcer of the rat resembles rather closely human peptic ulcer (21). Hence, steroid induced gastric ulcers were studied in the rat so that the results can be comparable with those observed in humans either on the basis of increased adrenocortical secretions caused by stress or by the therapeutic use of steroids. But the rat stomach contains a thin transparent non-glandular portion called rumen or forestomach which is susceptible to ulcer formation but has no equivalent in man and related species (3). In view of this forestomachectomized rats were used.

The present investigation was conducted to support for prove otherwise the gastric ulcerogenic property of adrenocorticoid prednisolone and the likely mechanism of production and/or enhancement, namely increased acid and pepsin secretion and/or decreased mucous production. Forestomachectomized 'Shay' rats, with or without adrenals, were used.

## MATERIAL AND METHODS

Male albino rats, 90-100 days old of Holtzman's strain weighing 140-150 g were forestomachectomized as described earlier (18). Ten days after operation, these forestomachectomized rats were used for experimentation.

All the rats were starved for 48 hours with water *ad lib*, in individual cages with a care to avoid coprophagy. Under light ether anaesthesia a mid line incision was made and the pyloric duodenal junction was ligated as per the method of Shay *et al.* (26). Care was taken not to injure any gastric vessel or cause traction to the stomach. The Sham operated controls had a loose ligature over the pylorus.

Adrenalectomy was performed as per the method described by Firor and Grollman (8) at the initiation of starvation and these rats received 0.9% drinking saline

5.0 mg of prednisolone (Calbiochem, USA) suspended in 1 ml. of 0.9% saline/100 g body weight, was administered subcutaneously twice daily at a 12 hr interval after adrenalectomy, the last dose being administered immediately after pyloric ligation. Drinking water or saline was withdrawn immediately after pyloric ligation. Suitable controls were maintained. The animals were autopsied 14 hours after pyloric ligation. The stomach was dissected out and the volume, pH, free, total and sialic acids of the gastric juice were determined. The tissue (corpus) sialic acids content was also determined as per the method of Warren (30).

The stomach was opened along the greater curvature and examined for the ulceration under a magnifier (x10). The number of ulcers was recorded and their severity was graded according to a scale ranging from 0 to 4+, based on the depth of the largest ulcer noted and later checked by sectioning and staining the ulcerated region. The ulcer index was calculated as the sum of ulcers plus severity plus % incidence divided by 10 (21). The ulcerated region was fixed in Bouin's fixative, sectioned in paraffin at 10  $\mu$  thickness and stained in Harris' haematoxylin-eosin for histological studies. The results were analysed by applying student's 't' test.

## RESULTS

Sham operated controls did not exhibit any gastric ulceration.

*Forestomachectomized Shay rats* : 3-5 subacute ulcers were observed in the corpus with a severity of 1.5-2+ in 66.67% of rats and resulting in an ulcer index of 11.67 caused by accumulated gastric juice in stomach (Table I, Figs. 1, 2).

TABLE I : Effect of adrenalectomy and administration of prednisolone on gastric ulcer formation and gastric secretions in forestomachectomized 'Shay' albino rats.

	5 mg prednisolone/100 g bodyweight twice daily during starvation, total doses 4				
	Sham operated	Treatment Shay operated			
		+ Saline	+ Prednisolone	+ ADX	+ ADX + prednisolone
48 hr of starvation and 14 hr of pyloric ligation	(5)	(6)	(5)	(3)	(2)
<i>Glandular ulcers/rat :</i>					
(a) Ulcer number (M $\pm$ S.E.)	—	3.50 $\pm$ 0.71	5.0* $\pm$ 0.87	2.00* $\pm$ 0.58	9.00** $\pm$ 1.00
(b) Ulcer severity (M. $\pm$ S.E.)	—	1.50 $\pm$ 0.26	2.40* $\pm$ 0.41	0.67* $\pm$ 0.56	3.00* $\pm$ 0.00
(c) % incidence	—	66.67	80.00	66.67	100.00
(d) Ulcer index	—	11.67	15.40	9.34	22.00
<i>Gastric secretions :</i>					
<i>I Gastric juice :</i>					
(a) Volume (ml) (M $\pm$ S.E.)	—	2.97 $\pm$ 0.22	1.10*** $\pm$ 0.05	—	—

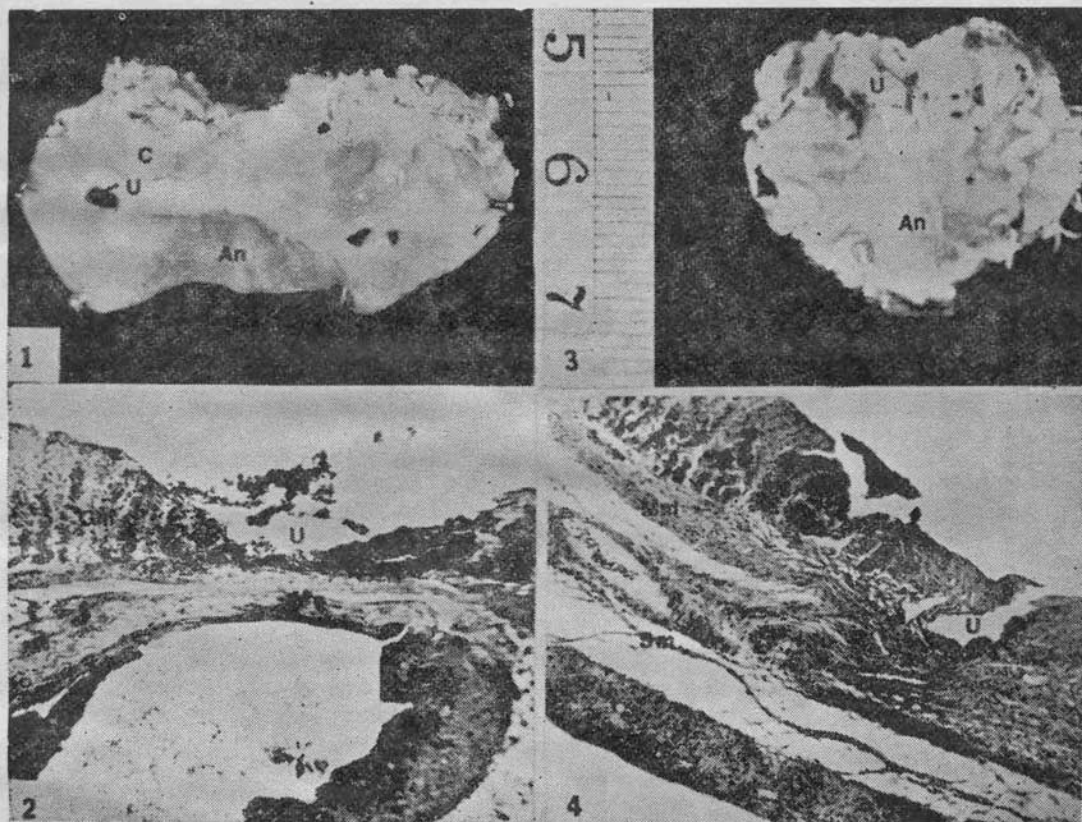
(b) pH	—	4.1	3.9	—	—
(c) Free acid (mEq)	—	6.0	7.0	—	—
(d) Total acid (mEq)	—	6.0	8.0	—	—
(e) Sialic acids ( $\mu$ moles NANA)/10 ml	—	0.65	—	—	—
II. Tissue (corpus) sialic acids ( $\mu$ moles NANA)/100 mg	1.18	0.52	0.23	0.23	0.15
% Mortality	20.00	±	±	57.14	66.67

Adrenalectomy (ADX) was performed at the beginning of starvation. Number in parentheses denotes the number of rats survived. Dead rats deleted.

The gastric juice was collected at autopsy 14 hrs after Shay operation, pooled groupwise and analysed. Ulcer index = Number of ulcers/rat + Severity + % incidence  $\times 10^{-1} \text{M} \pm \text{S.E.} = \text{Arithmetic mean} \pm \text{Standard error}$ .

\*Nonsignificant; \*\* $P < 0.02$  — Significant \*\*\* $P < 0.001$  — highly significant.

The respective control and treated group were compared for 't' test.

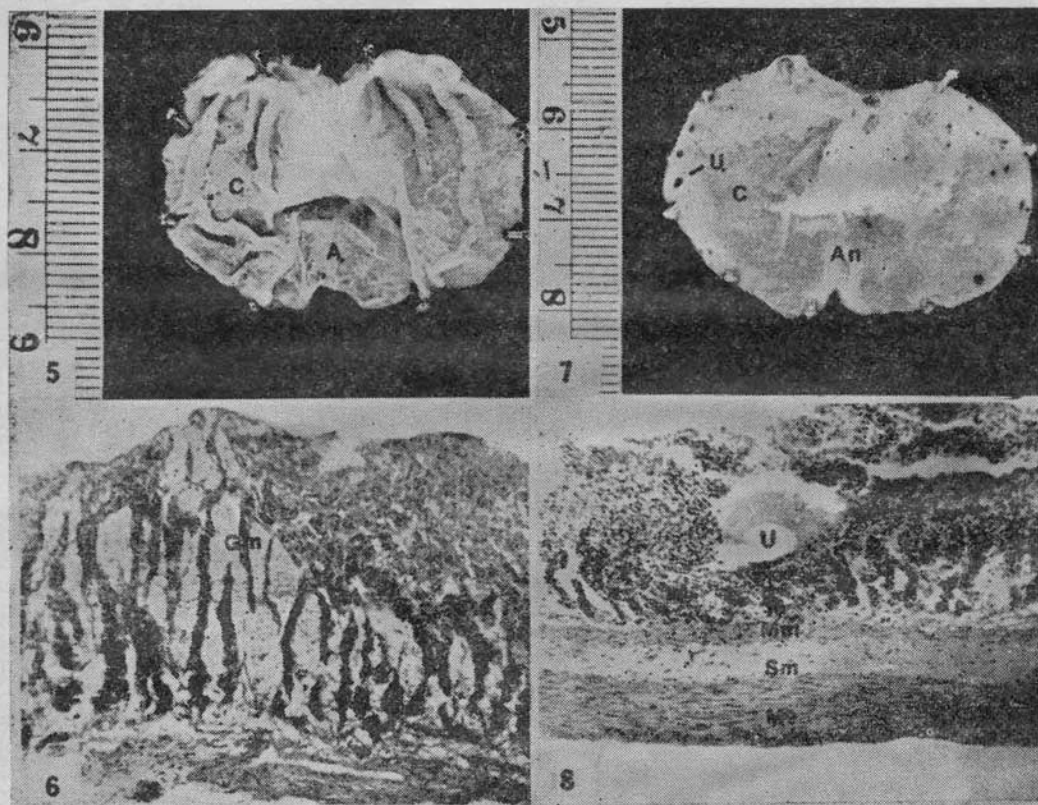


Figs. 1-8: Gross and microscopic examination of stomach of forestomachectomized Shay rat showing subacute ulceration (1,2), absence of well defined ulcers (U) in the corpus (C) with vacuolization of gastric mucosa (Gm) and deeply stained gastric glands seen after adrenalectomy (5,6), multiple haemorrhagic ulcers with erosion of the gastric mucosa up to the muscularis mucosa (Mm) after treatment with prednisolone (3,4), and small superficial haemorrhagic ulcers in the corpus after adrenalectomy and subsequent treatment with prednisolone (7,8).



*Forestomachectomized Shay Rats (adrenals ablated)* : Only superficial erosions of gastric mucosa were seen with a severity of 0.67+. There were 2-3 eroded spots, counted as ulcers, wherein the gastric mucosa showed considerable vacuolization of gastric glands (Table I, Figs. 5,6).

*Administration of prednisolone to forestomachectomized shay rats* : An increase incidence of ulcers was observed wherein 5-6 subacute ulcers with a severity of 2-2.5+ occurred in 80% of the rats. The ulcer index was 15.40. The gastric secretion was considerably reduced. Though the gastric juice was acidic, there was not much change in the free and total acids when compared to the controls. The sialic acid content in the gastric juice was practically nil, (showing absence of mucous) with considerable reduction in tissue sialic acid (Table I, Figs. 3,4).



*Administration of prednisolone to forestomachectomized Shay Rats (Adrenals ablated)* : There were small multiple haemorrhagic ulcers in the corpus with a severity of 2-3+, the gastric mucosa being eroded upto the muscularis mucosa or the submucosa. The gastric juice content of the stomach was practically nil, with a concomitant reduction in tissue sialic acid and absence of it in the gastric juice (Table I, Figs. 7,8).

## DISCUSSION

Adrenalectomy in the Shay rat prevents the formation of forestomach ulcers (14) as well as lowers the volume of gastric secretion (14,31), with a significant decrease in secretion of acid. Sheriff (27) observed superficial erosion of the mucosa of the corpus in adrenalectomized Shay rats. In the present study also, only superficial ulceration of the mucosa occurred in forestomachectomized Shay rats in which adrenals were ablated. Besides, the sialic acid content of the corpus was considerably reduced. All mucous secretion contains a sialoprotein, a glycoprotein containing sialic acid as a characteristic sugar component. Hence sialic acid is indicative of mucous secretion (32).

Administration of corticoids to Shay rats enhanced ulceration and increased the number and severity of gastric ulcers. The volume of gastric juice secreted was also considerably reduced with a concomitant decrease in the mucous secretion, as indicated by the low sialic acid content of the corpus. Similar results were obtained after administration of prednisolone to forestomachectomized Shay rats after adrenal ablation.

The above observations support the view of other workers that the main causative factor for the induction of gastric ulcers by steroids seems to be an absence or reduction of the mucous barrier (17, 21, 22, 23) with the result that the acidic gastric secretion, though reduced, may be sufficient to cause acute gastric ulcers. This is contrary to the concept of Spiro and Milles (29), who hypothesized an increased gastric secretory potential by steroids to explain the ulcerogenic property of steroids.

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## REFERENCES

1. Atik, M., L.A. Balart, F. Isla and G. McHardy. Ulceration from gastric freezing. *J.A.M.A.*, **191** : 386, 1965.
2. Beck, I.T., H.W. Fletcher, R.D. McKenna and H. Griff. Effect of small and massive doses of prednisolone on gastric secretory activity. *Gastroenterology*, **38** : 740, 1960.
3. Berg, B.N. Pathological changes in the nutritional gastritis in rats. *Am. J. Pathol.*, **18** : 49, 1942.
4. Bonta, I.L. A study of the effect of some glucocorticoids and ACTH on artificially induced gastric ulcers of the rat. *Arch. Int. Pharmacodyn.*, **132** : 147, 1961.
5. Bonta, I.L. Effect of corticoids and ACTH on the induction of gastric ulcers in laboratory animals. *Methods in Hormone Research*. **IV** : 235, 1965.

6. Chaikof, L., W.H. Janke, P.C. Pesoros and B.E. Brush. Effect of prednisolone and corticotrophin on gastric secretion. *Arch. Surg.*, **83** : 32, 1961.
7. Cohen, R.L. and K.M. Tvede. The screening of antiulcer drugs by quantal procedure. *Proc. Soc. Exp. Biol. Med.*, **78** : 708, 1951.
8. Firor, W.M. and A. Grollman. Studies on adrenals : Adrenalectomy in mammals with special reference to the white rat. (*mus Norvegicus*). *Am. J. Physiol.*, **103** : 683, 1933.
9. Gray, S.J., J.A. Brown, R.W. Reifstein and H.M. Spiro. Effect of corticotrophin (ACTH) and cortisone on gastric secretion. *J.A.M.A.*, **147** : 1529, 1951.
10. Ingle, D.J., M.C. Grossman and J.E. Nezamis. Effect of administering large doses of cortisone acetate to normal rats. *Am. J. Physiol.*, **166** : 171, 1951.
11. Kirk, R.M. Experimental gastric ulcers in rat. *Brit. J. Surg.*, **57** : 521, 1970.
12. Kowalewski, K. Effect of certain androgenic steroids and cortisone on gastric ulcerogenesis in fasting rats. *Proc. Soc. Exp. Biol. Med.*, **101** : 147, 1959.
13. Kuroyanagi, Y. and H. Necheles. Mann-Williamson rat. *Proc. Soc. Exp. Biol. Med.*, **108** : 771, 1961.
14. Madden, R.J. and H.H. Ramsburg. Adrenalectomy in Shay rat. *Gastroenterology*, **18** : 128, 1951.
15. Mann, C.F. and C.S. Williamson. The experimental production of peptic ulcer. *Ann. Surg.*, **77** : 404, 1923.
16. McCarrison, R. Some surgical aspects of faulty nutrition. *Lancet*, **1** : 1151, 1931.
17. Menguy, R. and Y.F. Master. Effect of cortisone on mucoprotein secretion by gastric antrum of dogs : Pathogenesis of steroid ulcers. *Surgery*, **54** : 19, 1963.
18. Nazeer Ahmed, R., E.M. Sheriff and M. Appaswamy Rao. Gastric ulcerogenic effect of prednisolone administered orally in forestomachectomized albino rats. *Karnatak Univ. J. Sc.*, **XIX** : 49, 1974.
19. Ogawa, T. and H. Necheles. Gastric secretion in the mouse. *Am. J. Physiol.*, **194** : 303, 1958.
20. Paul, F., A.N. Wick and E.M. Mackay. An assay method for antiulcer substances. *Gastroenterology*, **8** : 774, 1947.
21. Robert, A. and J.E. Nezamis. Ulcerogenic property of steroids. *Proc. Soc. Exp. Biol. Med.*, **99** : 443, 1958.
22. Robert, A. and J.E. Nezamis. Effect of prednisolone on gastric mucous content and on ulcer formation. *Proc. Soc. Exp. Biol. Med.*, **114** : 545, 1963.
23. Robert A. and J.E. Nezamis. Histopathology of steroid induced gastric ulcer. *Arch. Pathol.*, **77** : 407, 1964.
24. Seirafi, R. and L.C. Reid. The vascular component in the causal genesis of peptic ulcer. *Surgery*, **51** : 233, 1962.
25. Sen, R.N. and B.K. Anand. Effect of electric stimulation of the hypothalamus on the gastric secretory activity and ulceration. *Ind. J. Med. Res.*, **45** : 507, 1957.
26. Shay, H., S.A. Komarov, S.S. Fel, D. Meranze, M. Gruenstein and H. Sipler. A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology*, **5** : 43, 1945.
27. Sheriff, E.M. Studies on the histopathology of experimentally induced gastric ulcers in toads, gerbills and albino rats. Ph.D. Thesis, Karnatak Univ. Dharwad, India, 1973.
28. Skoryna, S.C., D.R. Webster and D.S. Kahn. A new method of production of experimental gastric ulcer. The effect of hormonal factors on healing. *Gastroenterology*, **34** : 1, 1958.
29. Spiro, H.M. and S.S. Milles. Clinical and Physiologic implications of the steroid induced peptic ulcer. *New Engl. J. Med.*, **263** : 286, 1960.
30. Warren, L.L. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.*, **234** : 1971, 1959.
31. Welbourn, R.B. and C.F. Code. The effect of cortisone and adrenalectomy on secretion of gastric acid and occurrence of gastric ulceration in the pyloric ligated rat. *Gastroenterology*, **23** : 356, 1953.
32. Werner, J. Studies of glycoproteins from mucous epithelial secretions. *Acta. Soc. Med. Upsal.*, **58** : 1, 1953.