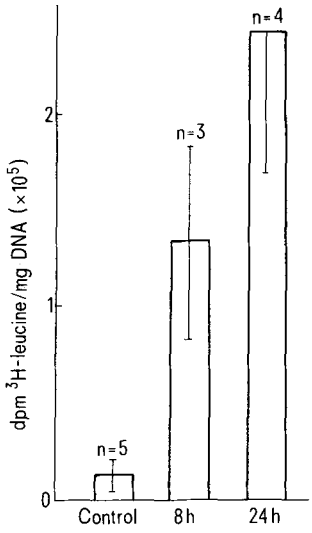
also exhibits this parameter of oestrogenic response which can be demonstrated in vitro only from 15 days postnatally in the rat[6] and which then increases to 25 days. However, even at 25 days, the rate of amino acid incorporation only increases 1.8 times in the rat. It is interesting to note that increased progesterone receptor concentrations can first be detected in the foetal guinea-pig uterus by 6 h after oestradiol treatment and are maximal by 24 h[3]. Nevertheless, although increased amino acid incorporation could be demonstrated, there was no concomitant net increase in total protein concentration, as shown in the table, and at the same time there was no increase in total DNA. Whether this increased ^{3}H-leucine incorporation reflects increased protein synthesis or a mere variation of the intracellular amino acid uptake or pool remains to be elucidated. However, the observation that oestradiol can stimulate amino acid incorporation into proteins before significantly increasing the total protein concentration has also been made in the studies of the postnatal development of uterine responsiveness to oestradiol in the rat[7]. Moreover, the effects of oestradiol on the incorporation of ^{3}H-thymidine into DNA and on the total uterine DNA content are also dissociated temporally in the rat. Thus, the foetal uterus of the guinea-pig also responds to oestradiol treatment by an increased incorporation of ^{3}H-leucine into total uterine proteins without any net increase in the total concentration of uterine protein, and this now represents another parameter of oestrogenic action which can be provoked in the foetus.



Effect of oestradiol treatment on the incorporation of ^{3}H-leucine into acid-insoluble proteins of the foetal guinea-pig uterus. Pregnant guinea-pigs were injected with 1 mg oestradiol/kg b.wt and after 8 h and 24 h (controls received vehicle alone) the female foetuses were injected in vivo and in situ with 60 µCi of ^{3}H-leucine. After 30 min, the animals were sacrificed and the foetal uteri were excised. A PCA precipitate of the uterine proteins was prepared and the radioactivity counted. The columns represent the means ± SEM of n determinations.

1 J.R. Pasqualini and B.L. Nguyen, Experientia *35*, 1116 (1979).
2 J.R. Pasqualini and B.L. Nguyen, Endocrinology *106*, 1160 (1980).
3 C. Sumida and J.R. Pasqualini, J. Receptor Res. *1*, 439 (1981).
4 K. Burton, Biochem. J. *62*, 315 (1956).
5 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
6 D. Sömjen, G. Sömjen, R.J.B. King, A.M. Kaye and H.R. Lindner, Biochem. J. *136*, 25 (1973).
7 A.M. Kaye, D. Sömjen, R.J.B. King, G. Sömjen, I. Icekson and H.R. Lindner, Adv. exp. Med. Biol. *44*, 383 (1974).

Changes in gastric parietal cells during prolonged intermittent corticosteroid treatment

M. Munichor, S. Finkelbrand and M. Silbermann[1]

Division of Morphological Sciences, Faculty of Medicine, Technion, Israel Institute of Technology, 12 Haaliya Street, Haifa 31 096 (Israel), 22 October 1980

Summary. Immature A/J mice were treated for up to 7 weeks with intermittent doses of triamcinolone hexacetonide and were thereafter allowed to recover for 7 weeks. Structural examinations and morphological measurements were performed on the parietal cells in the gastric mucosa. By the 3rd injection a significant decrease was noted in the number of the above cells - a feature that lasted throughout the experimental period. In contrast, the diameter of the parietal cells increased. However, following recovery, the latter returned to their normal size.

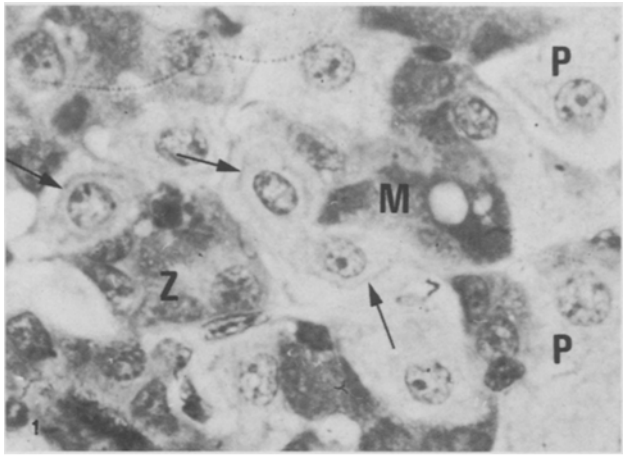
Gastrointestinal complications that have been associated with corticosteroid therapy include peptic ulcer[2]. Possible mechanisms whereby corticosteroids may cause gastric ulceration have been investigated. Treatment of immature rats with high doses of glucocorticoid hormones has been shown to retard significantly the proliferative activity of cells along the gastric mucosa[3]. Reduction in the quantity of gastric mucus and changes in its quality have been described[4] along with a reduction in the turnover of gastric epithelial cells[5,6]. Among the various strains of mice, the A/J strain exhibits an exceptionally high degree of sensitivity to glucocorticoids due to the high level of glucocorticoid receptors in their cells[7]. The purpose of the present study was to examine: a) the effects of prolonged intermittent corticoid administration on the gastric mucosa, and b) the potential of recovery of mucosal cells following longterm hypercorticoidism. To the best of our knowledge, this study is the first one to use the highly sensitive A/J strain for such investigations.

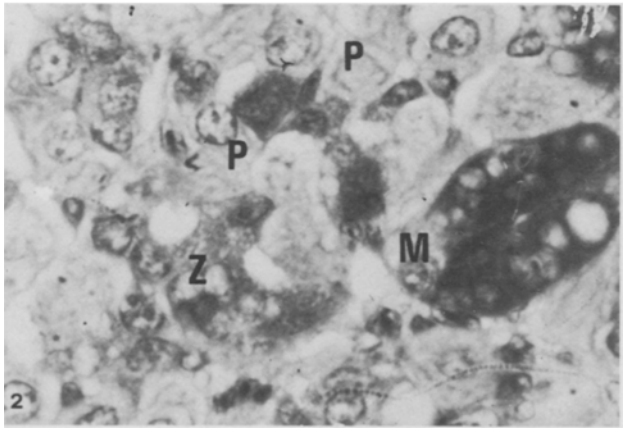
Material and methods. 7-week-old A/J mice, fed Purina laboratory chow and drinking water ad libitum, were given intermittent (every 4 days) i.m. injections of 4 mg/kg b.wt of triamcinolone hexacetonide (Aristospan, Cyanamid Co.). Nontreated animals served as controls. Test and control animals were sacrificed after 3, 7 and 14 consecutive injections, respectively. Additional groups of treated and control animals were allowed to recover for 7 weeks following the cessation of the hormonal treatment. On sacrifice, the stomachs were removed intact and were immediately fixed in a mixture of 4% formaldehyde and 2%

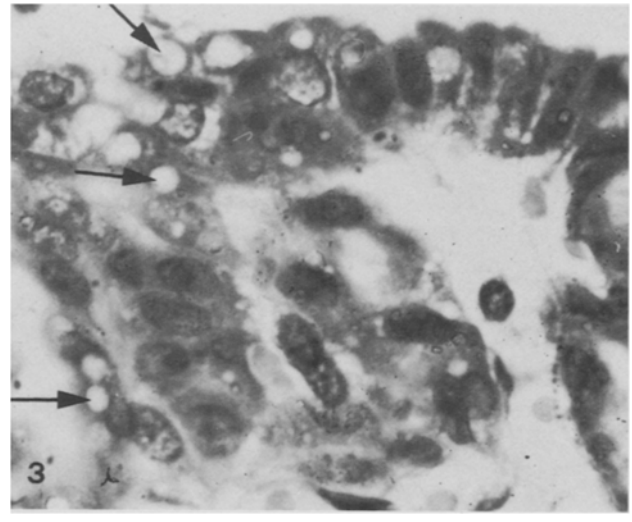
glutaraldehyde in phosphate buffer (pH 7.2), embedded in paraplast, sectioned at 6 µm and stained with haematoxylin and eosin or with periodic acid-Schiff (PAS). Measurements on the parietal cells were made using an ocular micrometer and included the total number and size of these cells per μm^2 of gastric mucosa. The measurements were carried out around the glandular neck area, a region which contains a large number of parietal cells. For each group (test and control) the mean values were calculated from at least 80 determinations (5 determinations per section, using 4 different sections of the same animal and at least 4 animals per group). Means and standard errors were calculated from all numerical data and the comparisons of the means were determined by Student's t-test.

Results. The number and size of parietal cells in normal mice did not change significantly with increased age. A significant decrease was already found in the number of parietal cells after the first 3 injections - a feature that lasted throughout the experimental period (table). The diameter of the above cells increased significantly after the first 7 injections; however, signs of recovery were noted following the cessation of the hormonal treatment, and by the end of the recovery period no significant differences were observed between test and control specimens (table). Morphologically, there were specimens that revealed obvious histopathological changes along the surface epithelium and in the gastric glands. In these cases both the surface lining cells, as well as the glandular ones, showed distinct intracellular vacuolization. In addition, an intensification of the PAS reaction was noted along the intraccellular canaliculi of the parietal cells (figs 1-3).

Discussion. The present study clearly indicates that the gastric mucosa is highly sensitive to the antianabolic effects of triamcinolone, a potent fluorinated synthetic analogue of cortisol, even when the hormone is administered intermittently. Mucous, zymogen and parietal cells were found to undergo marked structural alterations. The most distinctive feature, however, related to the changes in the number and size of the parietal cells. 3 doses of the hormone sufficed to bring about a significant decrease in the number of the parietal cells, a feature that was followed by an appreciable hypertrophy of these cells. The steroid-induced changes in







Figs. 1-3. Portions of gastric mucosa of Triamcinolone-treated mice. Fig. 1. A section reacted with periodic acid-Schiff indicating the intracellular canaliculi (arrows) within parietal cells (P). Note the intracellular vacuolization in a mucous cell (M). Z, zymogen cell. Animal received 8 injections. ×730. Fig. 2. The appearance of zymogen cell (Z), mucous cell (M) and parietal cells (P) in an animal that received 14 injections. H&E, ×730. Fig. 3. Appearance of the surface epithelium and its underlying tunica propria following 7 injections of the hormone. Note the widespread intracellular vacuolization (arrows). H&E, ×820.

The effect of triamcinolone upon the number and diameter of parietal cells in the stomach of A/J mice

Number of injections	Number of parietal cells per μm^2 mean ± SD	Significance	Diameter of parietal cell (µm) mean ± SD	Significance
0	1235 ± 34 (7)	-	14.40 ± 0.20 (7)	-
3	938 ± 75 (4)	$p < 0.002$	15.06 ± 0.23 (4)	NS
7	960 ± 21 (4)	$p < 0.001$	15.20 ± 0.07 (4)	$p < 0.01$
14	908 ± 33 (4)	$p < 0.005$	15.60 ± 0.09 (4)	$p < 0.05$
14 + 50 days of recovery	902 ± 15 (7)	$p < 0.001$	14.00 ± 0.35 (7)	NS

Number of animals in parentheses. NS, not significant.

the size of individual parietal cells were found to be transient in nature, as following the cessation of the hormonal treatment the size of these cells returned to normal. On the other hand, the overall number of the parietal cells did not respond respectively, as the significant decrease in the number of these cells was sustained till the end of the recovery period. The present study, however, tends to indicate that the parietal cells are not involved solely in the process of glucocorticoid-induced gastric disorder. It became apparent that almost all the cell types composing the mucosal lining of the stomach are intimately involved. Hence, it would be reasonable to assume that the pathology resulting from triamcinolone is the result of a concomitant adverse effect upon the heterogenous cellular population responsible for the structural and metabolic integrity of the gastric mucosa.

1 The authors wish to thank Mrs M. Brenman for her excellent technical assistance.
2 M.E. Rimza, Am. J. Dis. Child. *132*, 806 (1978).
3 J.N. Loeb, N. Engl. J. Med. *295*, 547 (1976).
4 A.B. Myles and J.R. Daly, in: Corticosteroid and ACTA Treatment, p. 183. The Williams & Wilkins Co., Baltimore 1974.
5 F. Glenn and W.R. Grafe, Ann. Surg. *165*, 1023 (1967).
6 T. Rasanen, Acta physiol. scand. *58*, 201 (1963).
7 D.S. Salomon and R.M. Pratt, J. Cell Physiol. *97*, 315 (1978).

Active and inactive renin in dog plasma before and after bilateral nephrectomy

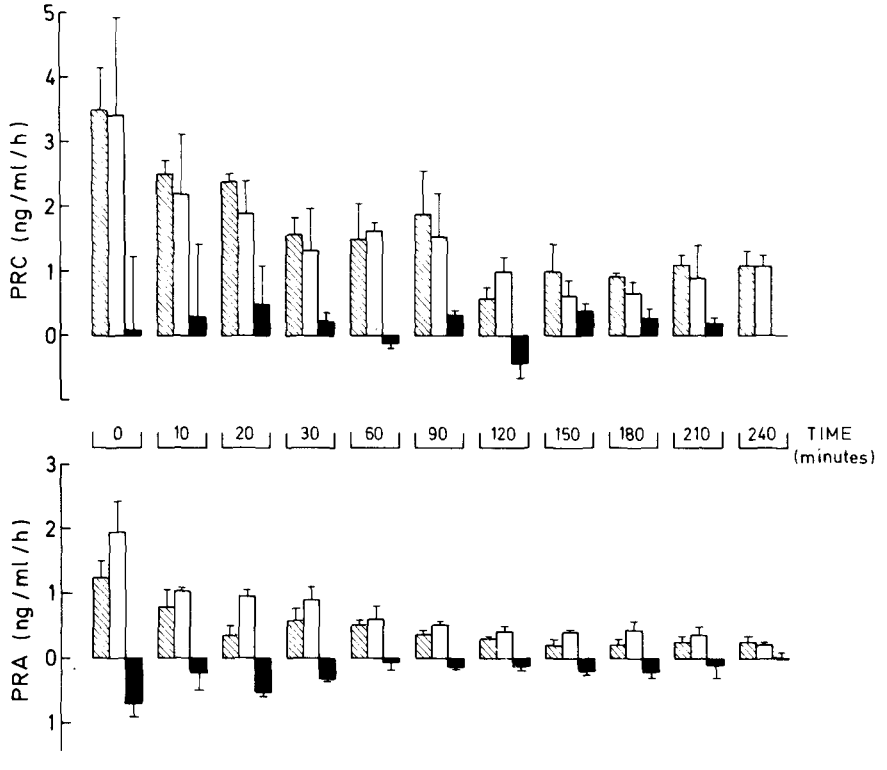
J. Staessen[1], P. Lijnen, R. Fagard and A. Amery[2]

Hypertension and Cardiovascular Rehabilitation Unit, Department of Pathophysiology, University of Leuven, B-3000 Leuven (Belgium), 13 October 1980

Summary. No significant amounts of inactive renin could be demonstrated by in vitro treatment (acidification or cryotreatment) of dog plasma obtained before and after bilateral nephrectomy. After bilateral nephrectomy, total and active renin were cleared from the plasma following similar disappearance curves, and dropped to half of their initial value within 30 min.

In a previous paper we reported on the disappearance of endogenous renin from the circulation of the dog after bilateral nephrectomy[3]. However, plasma renin was measured by a method involving acidification of the plasma to remove the endogenous renin substrate and an excess of exogenous renin substrate was then added for the generation of angiotensin I[4]. In the absence of a direct measurement, the plasma renin level is expressed as the amount of angiotensin I generated per ml of plasma/h. Meanwhile it has been recognized that the acidification of the plasma may activate an inactive form of renin[5]. The earlier described disappearance curve of renin after nephrectomy in dogs might represent a combination of the disappearance curves of active and inactive renin[3].

Inactive renin is not only activated by acidification but also by cold treatment of plasma[6]. In the present study we



Total (▨), active (□) and inactive (■) plasma renin concentration (PRC) and activity (PRA) before and after bilateral nephrectomy.