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Glucocorticoids inhibit the proliferation of mucosal cells and enhance the expression of a gene for pepsinogen and other markers of differentiation in the stomach mucosa of the adult rat

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Summary: Adrenalectomy caused a significant decrease in the mucosal level of pepsinogen in the adult rat stomach. This decrease was mainly due to a reduction in the level of the main component of rat pepsinogen isozymogens, namely, Pg1 and in the level of expression of Pg1 mRNA, with a maximal effect being evident 48 hours after operation. Immunohistochemical analysis revealed that the level of expression of Pg1 in the pepsinogen-producing cells was decreased throughout the stomach mucosa and this decrease was especially marked in the immature chief cells located in the glandular neck of the fundic mucosa. Adrenalectomy also increased the proliferation of mucosal cells and suppressed the expression of cathepsin E and class III mucin, markers of differentiated stomach mucosa. All these changes were reversed by hydrocortisone replacement and the extent of the recovery of the level of Pg1 mRNA was dependent on the dose of hydrocortisone, indicating the transcriptional control of the Pg1 gene by hydrocortisone. The observed results suggest that the continuous presence of glucocorticoids is necessary for active transcription of Pg1 gene in fully differentiated stomach mucosa and that glucocorticoids are important regulators of both the function and the morphology of stomach mucosal cells.

Introduction: The physiological significance of glucocorticoids in the regulation of pepsinogen gene expression is not fully understood. It has been repeatedly reported that glucocorticoids affect the function of pepsinogen-producing cells in the stomach mucosa that have not yet fully differentiated. For example, administration of glucocorticoids precociously increases the mucosal level of pepsinogen and the expression of its mRNA in the infant animal stomach (1-4). However, the effects of glucocorticoids on pepsinogen-producing cells in fully

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differentiated stomach mucosa are unclear. In adult animals, administration of hydrocortisone has been reported either to increase or to have no effect on secretion of pepsinogen and there is also a controversy as to the effect of adrenalectomy on the secretion of pepsinogen (5,6). In addition, the effects of glucocorticoids on expression of pepsinogen gene in fully differentiated stomach mucosa have not been examined. Therefore, in this study, we investigated the effects of adrenalectomy and hydrocortisone replacement on expression of pepsinogen gene together with the effects of these interventions on the proliferation and differentiation of the mucosal cells in the adult rat stomach.

Materials and Methods

Animals, surgery and treatment with hydrocortisone: Young adult male rats of the Wistar strain (8 weeks old; Charles River Japan Co.) were used in all experiments. The animals were anesthetized with ethyl ether and were either adrenalectomized or sham-operated by the dorsal approach. The adrenalectomized rats received subcutaneous injections of either physiological saline or various doses of hydrocortisone 21-sodium succinate (12.5-100 mg/kg body weight; Upjohn Co.) at 8-hour intervals after the operation for the analysis of the effects of hydrocortisone replacement. These rats were sacrificed daily to examine sequential changes in the stomach mucosal cells. Animals were killed after anesthesia with ethyl ether and their stomachs were quickly removed and cut open along the greater curvature. The resected stomach was divided into two parts; one was fixed with acetone for histochemical studies and the other was stored in liquid nitrogen until it was used for biochemical analysis.

Isolation and analysis of RNA: RNA was extracted from the stomachs by the guanidium isothiocyanate method and purified by CsCl density gradient ultracentrifugation, as described by Chirgwin et al. (7). A sample of 10 μg of RNA was denatured and subjected to electrophoresis on an agarose-formalin gel by the method of Goldberg (8). Then, the RNA was transferred to a nylon membrane and subjected to hybridization analysis, as described elsewhere (9). The PvuII-XbaI fragment of pRPC1, which contains a nearly full-length cDNA that encodes the major isozymogen of rat pepsinogens, Pg1 (10), was labeled with [α-^{32}P] dCTP by nick-translation and used as the probe for hybridization.

Assay of pepsinogen: Rat stomach mucosa was minced, homogenized and centrifuged at 105,000 xg for one hour as described elsewhere (1,2). The supernatant was used for assays of the potential peptic activity of pepsinogen by the method of Anson (11) with slight modifications. Protein concentrations were measured by the method of Bradford (12). For identification of pepsinogen isozymogens in the extract of stomach mucosa, polyacrylamide gel electrophoresis in 50 mM Tris-acetate buffer (pH 8.2) was performed as described previously (1,2).

Histological studies: For histological examinations, stomachs were fixed in pure acetone and embedded in paraffin. The avidin-biotin-peroxidase complex (ABC) method with antibodies raised against the Pg1 isozymogen (13) and against cathepsin E (14) was used to detect pepsinogen-producing cells and surface mucous cells, respectively. Paradoxical concanavalin A (Con-A) staining was used to detect mucosal cells that contained class III mucin (15). For studies of the proliferation of mucosal cell, three rats per day were injected intraperitoneally with 5-bromo-2'deoxyuridine (BrdU; 100 mg/kg body weight; Sigma) one hour before sacrifice. Nuclei in the cells that had incorporated BrdU were detected immunohistochemically.

Results: To investigate the effects of adrenal glucocorticoids on the function and morphology of pepsinogen-producing cells in the stomach mucosa of the adult rat, rats were

adrenalectomized to reduce levels of endogenous glucocorticoids. The body weight of the adrenalectomized rats was significantly lower than that of the sham-operated animals one week after the operation [control rats, 314.1±11.2 g; adrenalectomized rats, 261.7±15.6 g (mean ±SD; n= 10; $p<0.05$)]. To evaluate the effectiveness of adrenalectomy, serum corticosterone levels were measured by radioimmunoassay one week after operation. In the operated animals, the serum level was significantly lower than in the sham-operated animals, suggesting that the operation was successful [control rats, 400.3± 129.8 ng/ml; adrenalectomized rats, 60.2± 12.3 ng/ml (mean ±SD; n= 10; $p<0.05$)]. In adrenalectomized rats, there was a significant decrease in the mucosal level of pepsinogen one week after the operation [control rats, 0.82±0.12 u/mg protein; adrenalectomized rats, 0.56±0.08 u/mg protein (mean ± SD; n=10; $p<0.05$)]. Electrophoretic analysis of homogenates of mucosa from the rat stomach revealed four isozymogens of pepsinogen, as described previously (1). Adrenalectomy led to a reduction in the main isozymogen, Pg1, of rat pepsinogen while expression of other isozymogens remained at a relatively constant level (Fig. 1-A). In good agreement with the reduction in the mucosal level of Pg1, Northern blot analysis of total RNA from adrenalectomized rat stomachs showed that the level of Pg1 mRNA was also reduced (Fig. 1-B). The level of the mRNA decreased relatively slowly and maximal reduction was observed 48 hours after operation. Adrenalectomy did not have any

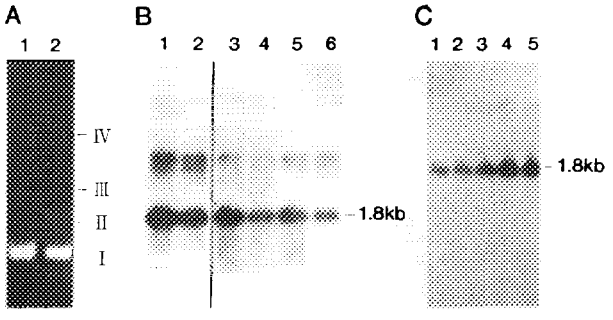

Figure 1. **A**, Effects of adrenalectomy on the mucosal level of pepsinogen isozymeogens in the rat stomach. The mucosal extracts from control (lane 1) and adrenalectomized (lane 2) rat stomachs one week after operation was analyzed by poiyacrylamide gel electrophoesis as described previously (1,2). The proteolytic bands I-IV correspond to the four isozymogens of rat pepsinogen, Pg1 to Pg4, respectively.
B, Effects of adrenalectomy on the expression of Pg1 gene in the rat stomach. Rats were sacrificed one week after operation and total RNA from control (lanes 1-3) and adrenalectomized (lanes 4-6) rat stomachs was isolated and analyzed by Northern blot hybridization with cDNA for rat Pg1 as probe.
C, Effects of hydrocortisone on the expression of Pg1 gene in the adrenalectomized rat stomach. Adrenalectomized rats were given various doses of hydrocortisone subcutaneously at 8-hour intervals (lanes 1-5; 0, 12.5, 25, 50 and 100 mg/kg body weight) one week after operation. Rats were sacrificed three days later and total RNA from the rat stomach was isolated and analyzed by Northern blot hybridization with cDNA for rat Pg1 as probe.

significant effect on numbers of pepsinogen-producing cells, as determined one week after operation. However, immunohistochemical analysis using antibodies against Pg1 revealed that the level of expression of Pg1 in the pepsinogen-producing cells was reduced throughout the stomach mucosa by adrenalectomy. This reduction was especially marked in the immature chief cells located in the glandular neck of the fundic mucosa (Fig. 2). The proliferation and

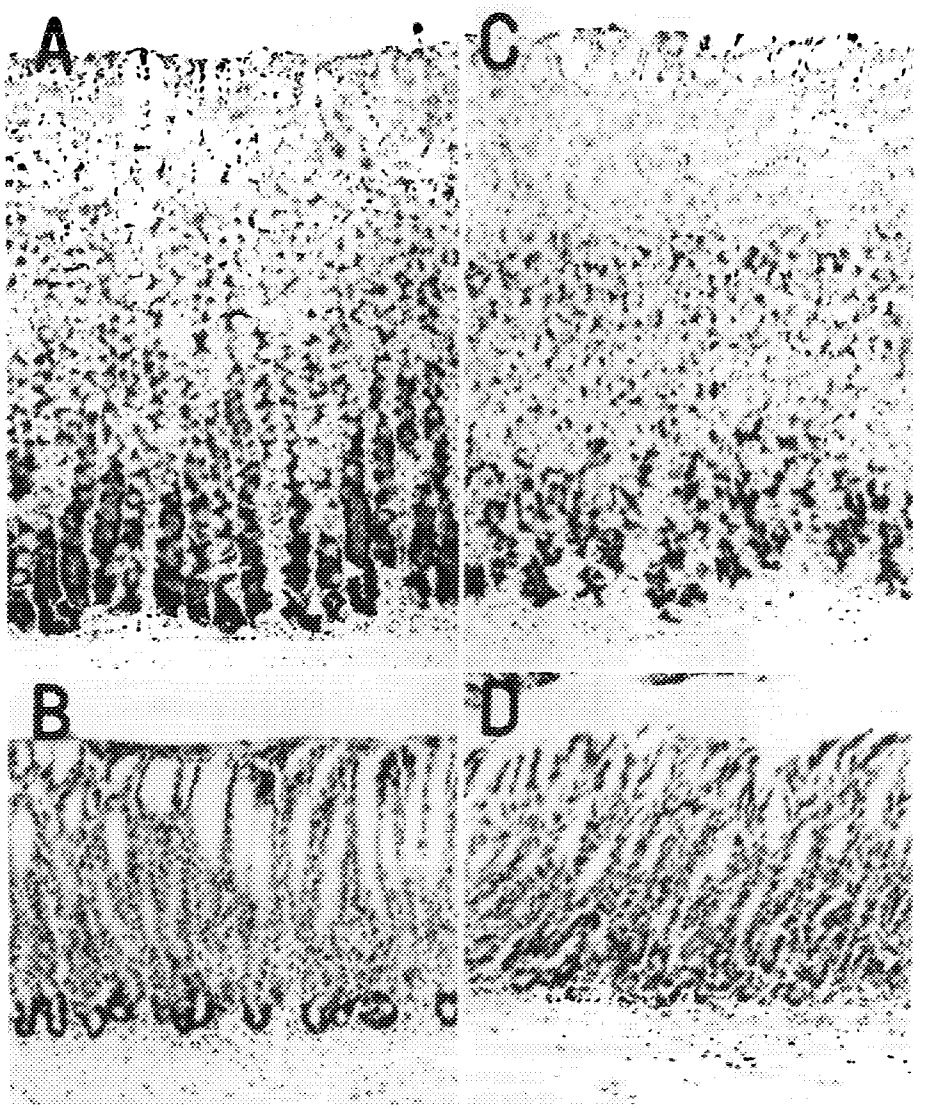

Figure 2. Effects of adrenalectomy on the expression of Pg1 in the rat stomach mucosa. Stomach mucosae from control (A and B) and adrenalectomized (C and D) rats one week after operation were immunohistochemically stained with antibodies against rat Pg1 by the ABC method. A and C; fundic gland mucosa. B and D; pyloric gland mucosa (x200).

differentiation of stomach mucosal cells were greatly influenced by adrenalectomy. The numbers of cells labeled with BrdU were elevated in adrenalectomized animals (Fig. 3). Histochemical analysis by paradoxical Con-A staining revealed the expression of class III mucin in the immature chief cells and in cells of the pyloric gland. Adrenalectomy also reduced the expression of class III mucin in these cells (Fig. 4). In addition, the level of expression of

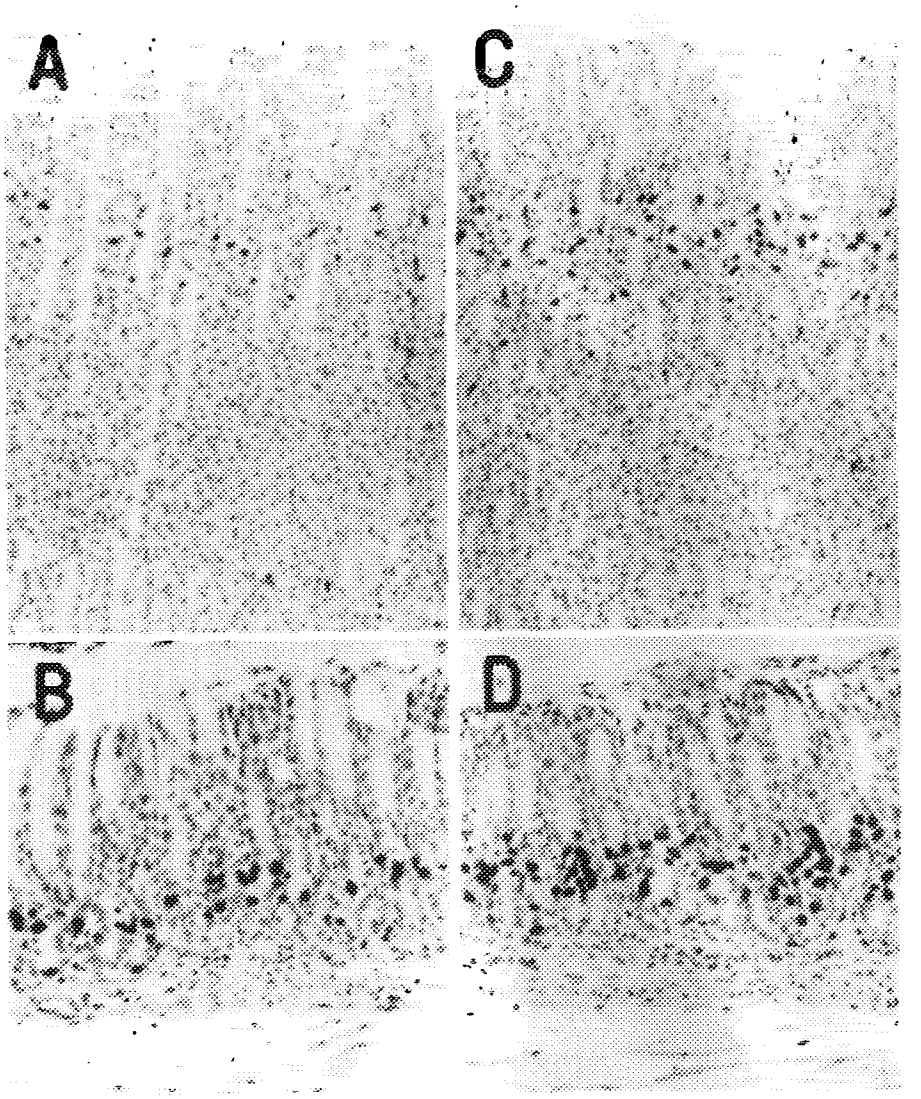

Figure 3. Effects of adrenalectomy on the proliferation of mucosal cells in the rat stomach. Control (A and B) and adrenalectomized (C and D) rats were injected with BrdU (100mg/kg body weight) one week after operation and nuclei in the cells that had incorporated BrdU were detected immunohistochemically. A and C ; fundic gland mucosa. B and D; pyloric gland mucosa (x200).

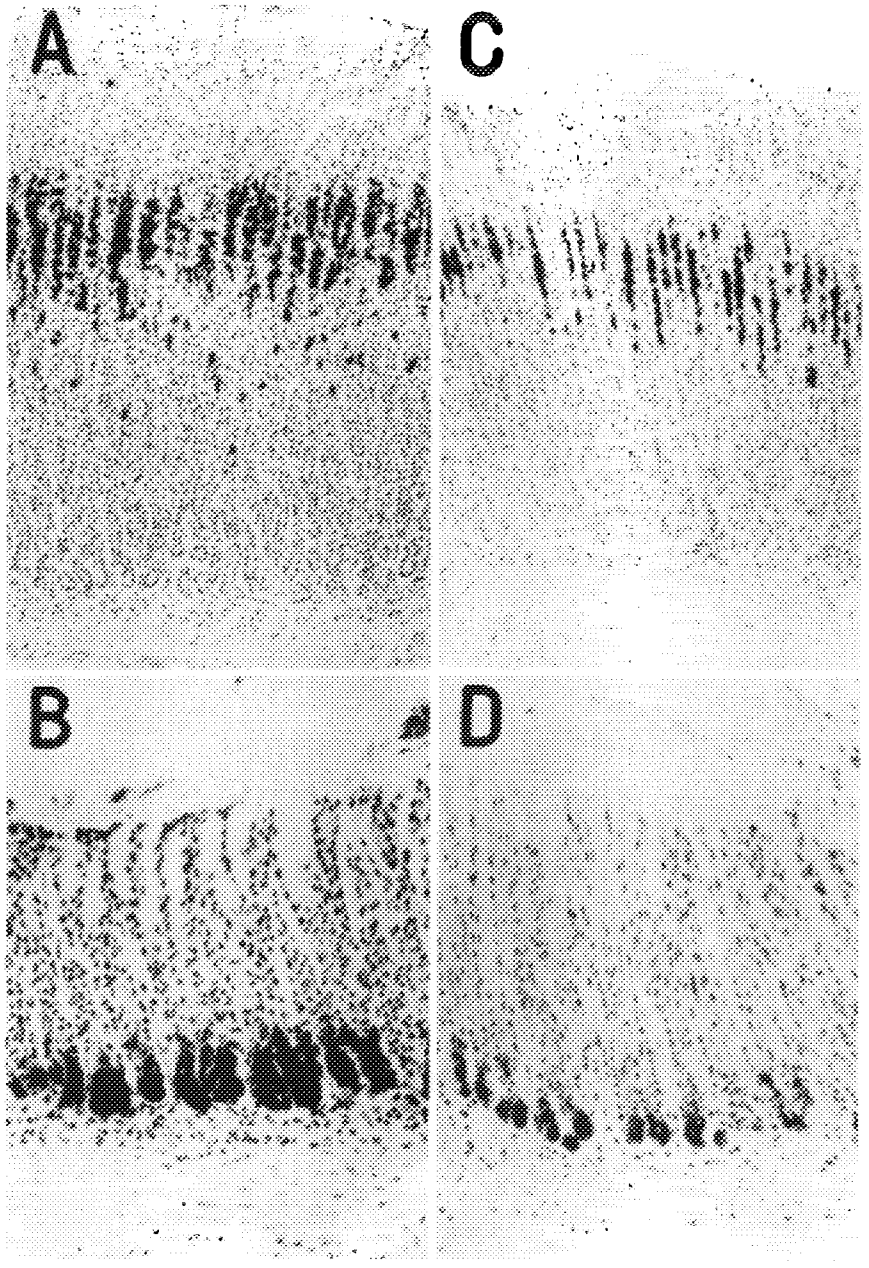

Figure 4. Effects of adrenalectomy on the expression of class III mucin in the rat stomach mucosa. Stomach mucosae from control (A and B) and adrenalectomized (C and D) rats one week after operation were stained with paradoxical Con-A staining to detect class III mucin. A and C; fundic gland mucosa. B and D; pyloric gland mucosa (x200).

cathepsin E in the surface mucous cells throughout the stomach mucosa was reduced (Fig. 5). These adrenalectomy-induced changes were apparent 48 hours after operation and the basic character of the changes remained unaltered even 28 days after operation. When hydrocortisone

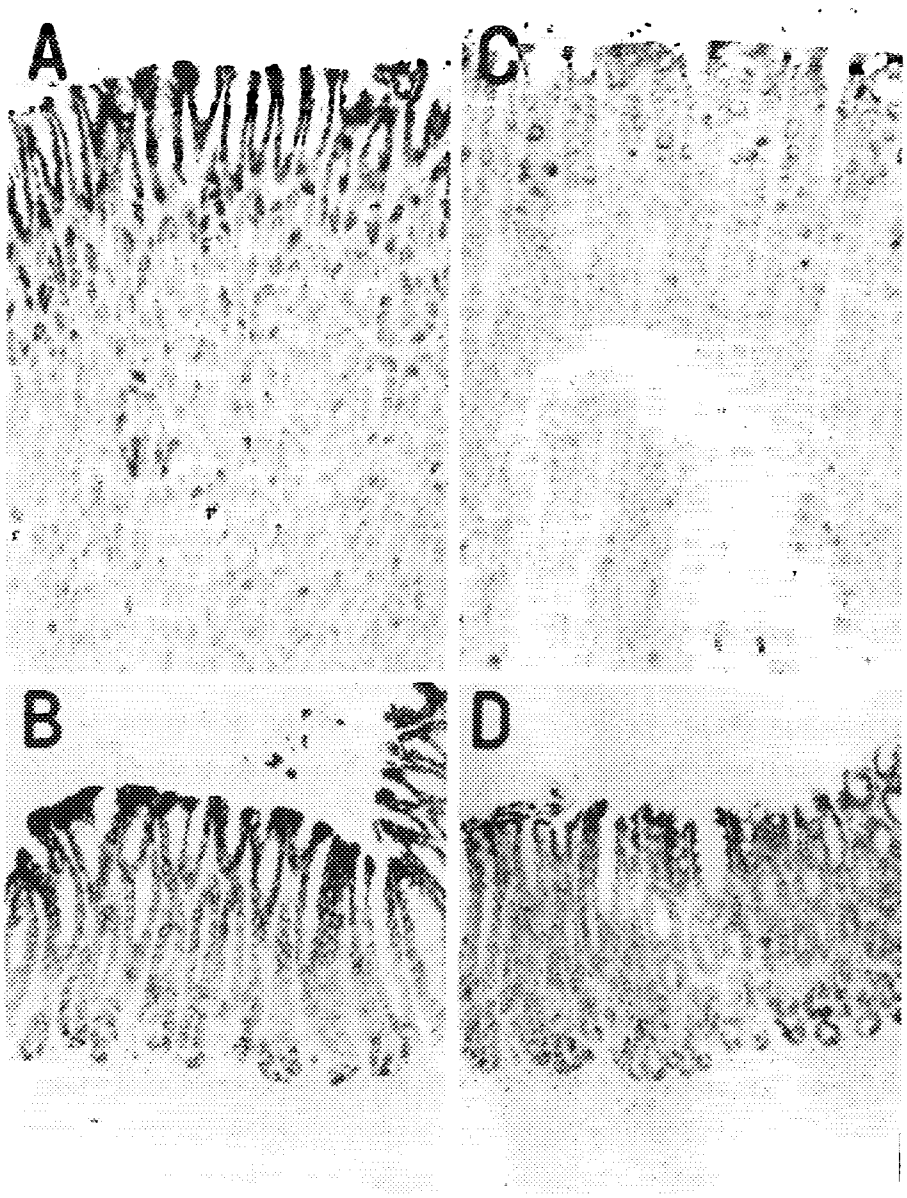

Figure 5. Effects of adrenalectomy on the expression of cathepsin E in the rat stomach mucosa. Stomach mucosae from control (A and B) and adrenalectomized (C and D) rats one week after operation were immunohistochemically stained with antibodies against rat cathepsin E by the ABC method. A and C; fundic gland mucosa. B and D; pyloric gland mucosa (x200).

was injected into adrenalectomized rats, the adrenalectomy-induced changes in the proliferation and differentiation of stomach mucosal cells were reversed within 48 hours. The extent of the recovery of expression of the Pgl gene was dependent on the dose of hydrocortisone, with a maximal effect being evident at a dose of 50 mg/kg body weight (Fig. 1-C).

Discussion: Previous studies involving adrenalectomy or hydrocortisone treatment demonstrated that glucocorticoids do not have any appreciable effect on the level of pepsinogen in the stomachs of adult animals (1,2). By contrast, in the present study, we carefully evaluated the effectiveness of adrenalectomy by monitoring the serum corticosterone level and found that adrenalectomy caused a significant decrease in the mucosal level of pepsinogen. The decrease was mainly due to a decrease in the main isozymogen of pepsinogen, namely, Pg1 and the level of expression of the corresponding gene was also decreased. Immunohistochemical analysis revealed that expression of Pg1 in the pepsinogen-producing cells throughout the stomach mucosa was affected by adrenalectomy, but a particularly strong response was observed in the immature chief cells located in the glandular neck of the fundic mucosa. Glucocorticoids are known to affect the expression of various tissue proteins by modulating the rate of gene transcription (16). In the present study, treatment of the adrenalectomized rats with hydrocortisone led to a recovery of both of the mucosal level of pepsinogen and of the expression of the Pg1 gene. Increases in the level of Pg1 mRNA were dependent on the dose of hydrocortisone administered, indicating that expression of the Pg1 gene is controlled at the transcriptional level by hydrocortisone. We demonstrated previously that exogenous hydrocortisone can increase the level of expression of the Pg1 gene in the developing rat stomach to the level seen in adults (4). The results of the present study extend this finding to the normal adult rat stomach and indicate that the continuous presence of glucocorticoids is necessary for the maintenance of active transcription of Pg1 gene in the fully differentiated stomach. The mechanism whereby glucocorticoids exert their effects on expression of the Pg1 gene remains to be elucidated. To date, the consensus sequence of glucocorticoid-responsive elements has not been found in the 5'-flanking region of the rat Pg1 gene (16,17). In addition, adrenalectomy and hydrocortisone replacement do not affect expression of the Pg1 gene instantaneously: The maximal effect of adrenalectomy was not observed until 48 hours after the operation. Thus, hydrocortisone may not interact directly with the Pg1 gene. It seems likely that the effect of hydrocortisone on expression of the Pg1 gene requires the induction of some other, as yet unidentified, regulatory protein factors. This type of indirect interaction has been reported in the case of glucocorticoid regulation of a gene for α_1-acid glycoprotein (18) and a gene for tryptophan 2,3-dioxygenase(19).

The effect of adrenalectomy was not directed exclusively at the expression of pepsinogen. In the surface mucous cells, the level of expression of cathepsin E, which is a marker of differentiation (20,21), was also decreased. In addition, the level of expression of class III mucin in the immature chief cells in the glandular neck of the fundic mucosa and in the cells of the pyloric gland was reduced. At the same time, adrenalectomy increased the number of

proliferating cells in the stomach mucosa, as revealed by immunohistochemical detection of BrdU-labeled cells. These results contrast with those of a previous study of the infant rat stomach in which it was demonstrated that corticosterone treatment does not change the overall pattern of cell proliferation (3). The changes observed in the present study were reversed by hydrocortisone replacement. Thus, it is apparent that, in the adult rat stomach, glucocorticoids have global effects on the proliferation and differentiation of mucosal cells. It is not known whether the factor that regulates the effects of glucocorticoids on the expression of the Pg1 gene is also responsible for the proliferation and differentiation of the stomach mucosa, but it appears probable that glucocorticoids modulate the expression of the Pg1 gene, at least in part, by regulating the differentiation of pepsinogen-producing cells. Together with the results of previous studies, the present results clearly indicate that glucocorticoids are important regulators of the function and morphology of the stomach mucosa beyond the developmental stage and into adulthood.

References

1. Furihata,C., Kawachi,T. and Sugimura,T. (1972) Biochem.Biophys.Res.Commun47; 705-711
2. Kumegawa,M., Takuma,T., Hosoda,S., Kunii,S. and Kanda,Y. (1978) Biochim.Biophys.Acta. 543; 243-250
3. Tseng,C.C. and Johnson, L.R. (1986) Am. J. Physiol. 250; G633-G638
4. Ichinose,M., Miki,K., Tatematsu,M., Furihata,C., Matsushima,M., Ichihara,Y., Tanji,M., Konishi,Y., Ohara,M., Inoue,H., Kurokawa,K., Takahashi,T., Kageyama,T. and Takahashi, K. (1990) Biochem.Biophys.Res.Commun. 172; 1086-1093
5. Cooke,A.R. (1967) Gastroenterol. 52; 272-281
6. Samloff,I.M. (1971) Gastroenterol. 60; 586-604
7. Ulrich,A., Shine,J., Chirgwin,J., Pictet,R., Tischer,E., Rutter,W.J. and Goodman,H.M. (1977) Science 196; 1313-1319
8. Goldberg,D.A. (1980) Proc.Natl.Acad.Sci.USA, 77; 5794-5798
9. Ichinose,M., Miki,K., Furihata,C., Tatematsu,M., Ichihara,Y., Ishihara,T., Katsura,I., Sogawa,K., Fujii-Kuriyama,Y., Tanji,M., Oka,H., Matsushima,T. and Takahashi,K. (1988) Cancer Res. 48; 1603-1609
10. Ichihara,Y., Sogawa,K., Morogashi,K., Fujii-Kuriyama,Y. and Takahashi,K. (1986) Eur. J. Biochem. 161; 7-12
11. Anson,M.L. (1939) J. Gen. Physiol. 22; 79
12. Bradford,M. (1976) Anal.Biochem. 72; 248-254
13. Furihata,C., Saito,D., Fujiki,H., Kanai,Y., Matsushima,T. and Sugimura,T. (1980) Eur.J. Biochem. 105; 43-50
14. Yonezawa,S., Takahashi,T., Ichinose,M., Miki,K., Tanaka,J. and Gasa,S. (1990) Biochem. Biophys.Res.Commun. 166; 1032-1038
15. Katsuyama,T. and Spicer S.S. (1978) J.Histochem.Cytochem. 26; 233-250
16. Yamamoto,K.R. (1985) Annu.Rev.Genet. 19; 209-245
17. Cordingly,M.G., Riegel,A.T. and Hager,G.L.(1987) Cell 48; 261-270
18. Klein,E.S., Reinke,R., Feigelson,P. and Ringold,G.M. (1987) J.Biol.Chem. 262; 520-523
19. Nakamura,T., Niimi,S., Nawa,K., Noda,C., Ichihara,A., Takagi,Y., Anai,M. and Kurtz,D.T. (1987) J.Biol.Chem. 262; 727-733
20. Tsukada,S., Ichinose,M., Miki,K., Tatematsu,M., Yonezawa,S., Matsushima,M., Kakei,N., Fukamachi,H., Yasugi,S., Kurokawa,K., Kageyama,T. and Takahashi,K. (1993) Biochem. Biophys.Res.Commun. 187; 1401-1408
21. Kageyama,T., Ichinose,M., Tsukada,S., Miki K., Kurokawa,K., Koiwai,O., Tanji,M., Yakabe, E., Athauda,S.B.P. and Takahashi,K. (1992) J.Biol.Chem. 267; 16450-16459