

A Case of a Pony with Cushing's Disease

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ABSTRACT. The pars intermedia of the pituitary gland, and plasma ACTH and cortisol levels in the pony, which was first diagnosed in Japan as indicating equine Cushing's disease, were examined by immunohistochemistry and radioimmunoassay, respectively. The pars intermedia was greatly enlarged and most of its cells were immunoreactive for antisera to both adrenocorticotropic hormone (ACTH) and β -endorphin (β -End). The plasma ACTH level was elevated when clinical symptoms appeared. The present results reveal that equine Cushing's disease in this pony was induced by the hypersecretion of ACTH and β -End from the enlarged pars intermedia of the pituitary gland.—**KEY WORDS:** ACTH, Cushing's disease, pony.

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Equine Cushing's disease (call it C's d throughout) is defined as pituitary-dependent hyperadrenocorticism [4]. There are many reports on equine C's d at overseas lands, which can present signs of hirsutism, polydipsia, polyuria, diabetes insipidus, many secondary infections and muscle wasting, and may be associated with adenoma in the pars intermedia [1, 3, 6, 8, 11]. In Japan, however, little information is available on equine C's d. On race horses without any of these clinical signs, we have observed pituitary adenoma, which is characterized by an enlarged pars intermedia, showing the marked increase in number and size of adrenocorticotropic hormone (ACTH) immunoreactive cells [9]. In the present study we examined the pituitary and adrenal glands, and the plasma ACTH and cortisol levels, in a pony which was first diagnosed with equine C's d in Japan, using immunohistochemistry and radioimmunoassay (RIA).

A pony aged 25 years was diagnosed with C's d from the clinical signs of hirsutism, remarkable dehydration of over all the hypoderm, and many secondary infections. Blood from the pony was sampled from February 1992 to the end of 1995. Plasma ACTH and cortisol levels of the pony were determined by RIA. Six normal ponies were used as control for RIA. The plasma ACTH level of the pony was elevated abnormally after February 1993 and reached a maximum at February 1994, when clinical signs appeared, and then tended to decrease (Fig. 1a). There was little difference in plasma cortisol level between the pony with C's d and normal ponies. But it reached its highest level in 1995, shortly before the pony died (Fig. 1b).

Shortly after its death, the pituitary and adrenal glands of the pony were taken for examination. The pituitary gland was bisected along the longitudinal midline and the adrenal gland was sliced to facilitate penetration. One half of the pituitary gland and slices of the adrenal gland were fixed in 10% neutral buffered formalin, dehydrated in a graded series of alcohol solutions, and embedded in paraffin. The tissue was cut into sections 10 μ m thick and stained with hematoxylin and eosin. For immunocytochemical studies, sections were processed according to the peroxidase-

antiperoxidase method. Immunolabelling was performed using antiserum to porcine ACTH (1:1,000) or to human β -endorphin (β -End) (1:1,600) produced in the rabbit. The other half of the pituitary gland was dissected into 1 mm³ blocks of the pars intermedia after fixing in 10% neutral buffered formalin. The blocks were post-fixed in 1% osmium tetroxide for 1.5 hr, dehydrated in a graded series of alcohol solutions, and embedded in Epon-Araldite. The tissues were cut into two serial sections 1 μ m thick. After removing the Epon-Araldite with Etoxide, they were then immunostained with each antibody referred to above. Four normal race horses aged 4, 10, 14, and 18 years were used as control.

The pars intermedia of the pituitary gland of the pony with C's d was greatly enlarged compared with that of a normal horse (Fig. 2). In normal horses, ACTH (Fig. 3a) and β -End (Fig. 4a) immunoreactive cells were observed in the pars distalis and pars intermedia. Both types of cells were more numerous in the pars intermedia than in the pars distalis. β -End immunoreactive cells in the pars distalis in the pony with C's d were less observed compared with those of normal horses (Fig. 4a and b). ACTH immunoreactive cells in the pony with C's d were distributed as in a normal horse (Fig. 3a and b). The ACTH (Fig. 3a and b) and β -End (Fig. 4a and b) immunoreactive cells hypertrophied and reacted more strongly in the affected pony than in normal horses. In the pars intermedia, the cells which are immunoreactive with antisera to both ACTH and β -End were observed in the normal horses and in the pony with C's d, and were more numerous and larger in the pony than in normal horses (Figs. 5 and 6). Compared with the adrenal gland of normal horses, that of the pony with C's d was enlarged markedly (Fig. 7a and b). Zona fasciculatae showed palisades in normal equines (Fig. 8a) but grew abnormally to disturb the arrangement in the pony with C's d (Fig. 8b).

Heinrichs *et al.* [3] reported that cells in the pars intermedia in normal horses secrete peptide hormones (ACTH, β -End, α -MSH etc.) derived from proopiomelanocortin. In this study, most cells in the pars

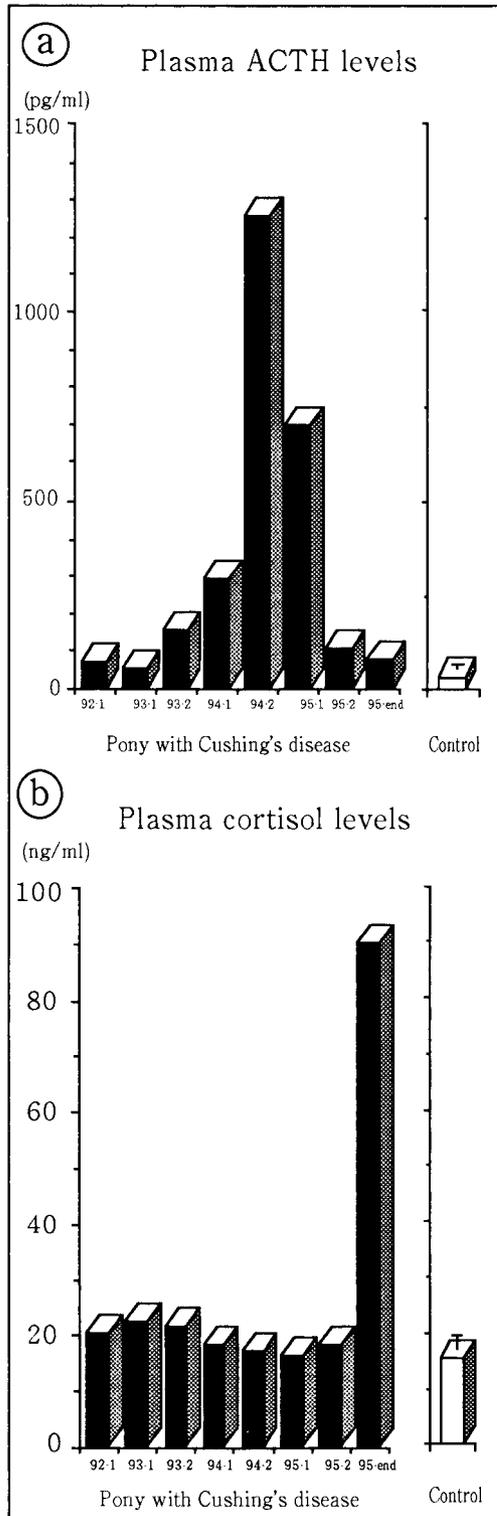


Fig. 1. Plasma ACTH level was elevated after February 1993 and reached maximum at February 1994. Plasma cortisol level was not elevated until the end of 1995. The axis of abscissa means the time of sampling (for example: 92-2=February 1992). Control value is the mean \pm SEM of 6 normal ponies. a; changes in plasma ACTH levels. b; changes in plasma cortisol levels.

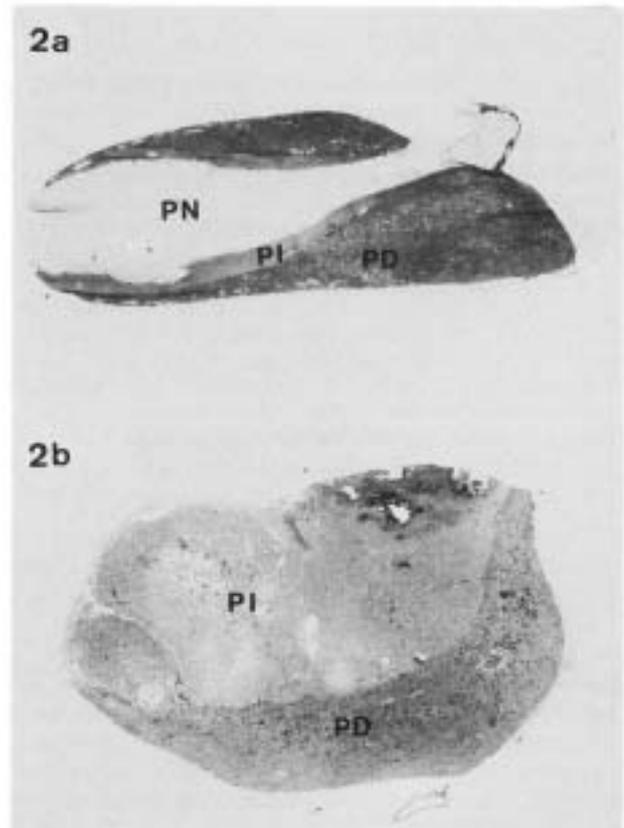


Fig. 2. a; Pituitary gland in sagittal plane of normal horse. Apart from the caudal part, the pars nervosa (PN) was surrounded by the pars intermedia (PI). In turn, the pars intermedia was surrounded by the pars distalis (PD), except for its posterior pole. H&E. \times 2.6. b; Pituitary gland of a pony with Cushing's disease. The pars intermedia (PI) is greatly enlarged compared with that of the normal horse in Fig. 2a. H&E. \times 2.6.

intermedia of the affected pony were positive for ACTH and β -End antisera. β -End and ACTH levels in plasma are elevated in horses with C's d [8]. These results suggest that the quality of cells in the pars intermedia may be altered in the pony. Orth and Nicholson [7] reported that bioreactive ACTH levels in the pars intermedia of horses with C's d are similar to those in normal horses, though immunoreactive ACTH levels in the pars intermedia are higher in horses with C's d than normal. In the present study, however, the adrenal gland as well as the pars intermedia was greatly enlarged in the affected pony compared with those in the normal horse. These findings suggest that immunoreactive ACTH in the pars intermedia of this pony may be biologically active. It has been reported that the plasma ACTH level is greatly elevated in horses with C's d [7]. In the pony, showing clinical signs of C's d, the plasma ACTH level was also elevated. Thus, C's d in this pony may be caused by the hypersecretion of ACTH from hyperplasia or adenoma of the pars intermedia. Plasma cortisol and ACTH levels in this pony are in good agreement with the report of the horse with C's d by van der Kolk *et al.*[10], that the

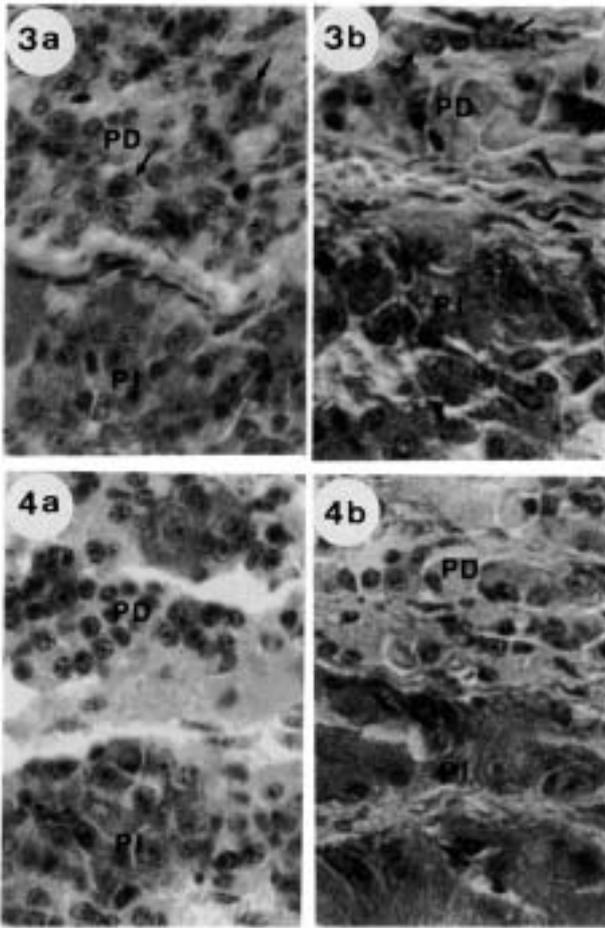


Fig. 3. Pituitary gland stained with anti ACTH serum. a; normal horse: ACTH immunoreactive cells are observed in the pars distalis (PD) and the pars intermedia (PI). Some of ACTH cells in the PD are shown by arrows. The cells are more numerous in the PI than in the PD. $\times 330$. b; the pony with Cushing's disease: ACTH immunoreactive cells are found in both pars distalis (PD) and pars intermedia (PI) as in normal horse. Some of their cells in the PD are shown by arrows. The ACTH cells are hypertrophied and reacted more strongly compared with those in normal horse in Fig. 3a. $\times 330$.

Fig. 4. Pituitary gland stained with anti β -End serum. a; normal horse: β -End immunoreactive cells are observed in the pars distalis (PD) and the pars intermedia (PI). Some of β -End cells in the PD are shown by arrows. The cells are more numerous in the PI than in the PD. In the PI, β -End cells are less observed than the ACTH cells. $\times 330$. b; the pony with Cushing's disease: Few β -End immunoreactive cells are found in the pars distalis (PD). In the pars intermedia (PI), the β -End immunoreactive cells are hypertrophied and reacted more strongly compared with those in normal horse in Fig. 4a. $\times 330$.

basal plasma cortisol level is lowered but the basal ACTH level is greatly elevated. Dybdal *et al.* [2] reported that normal diurnal rhythm in plasma cortisol, high in the morning and low in the evening, is absent in horses with C's d and that the maximum value in the cortisol is the same in horses between C's d and normal. While equine C's d is defined as pituitary-dependent hyperadrenocorticism

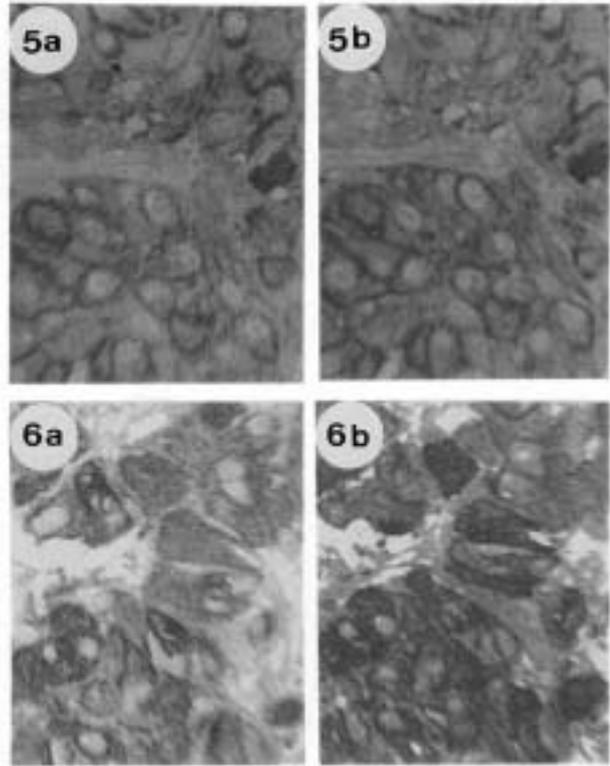


Fig. 5. Serial sections of the pars intermedia in normal horse. The cells immunoreactive with both antisera to ACTH and β -End are observed. a; Stained with anti ACTH serum. $\times 330$. b; Stained with anti β -End serum. $\times 330$.

Fig. 6. Serial sections of the pars intermedia in the pony with Cushing's disease. The cells immunoreactive with both antisera to ACTH and β -End are more numerous and larger compared with the normal horses in Fig. 5. a; Stained with anti ACTH serum. $\times 330$. b; Stained with anti β -End serum. $\times 330$.

[4], the present results that in the pony with C's d, plasma cortisol level was not elevated except for shortly before death are the characteristics in C'd in this species. Taken together, the biological effects of ACTH hypersecreted in this pony may result in adrenal hyperplasia rather than hyperfunction.

In the present study, the cells of the pars intermedia immunoreactive with both ACTH and β -End were more numerous in the pony with C's d than in the normal horses. This result indicates that hypersecretion of β -End in addition to ACTH may be one of the factors inducing equine C's d. This notion is supported by the following reports: β -End levels in plasma and in cerebrospinal fluid are much higher in horses with C's d than in normal horses [5]; such demeanours as lethargic, docile, and dull in Cushing's disease may be a result of raised level of β -End [4]. The present observation that β -End immunoreactive cells in the pars distalis were reduced in our pony is well aligned with that of Millington *et al.* [5] in Cushing's horses.

Consequently, the present study recorded the equine C's d which was first found in Japan and showed that equine C's d may be induced by the hypersecretion of ACTH and



Fig. 7. Adrenal gland in a normal horse (a) and a pony with Cushing's disease (b). The adrenal gland in the pony with Cushing's disease is hypertrophied compared with that in normal horse. H & E. $\times 7.0$.

β -End from an enlarged pars intermedia of the pituitary gland.

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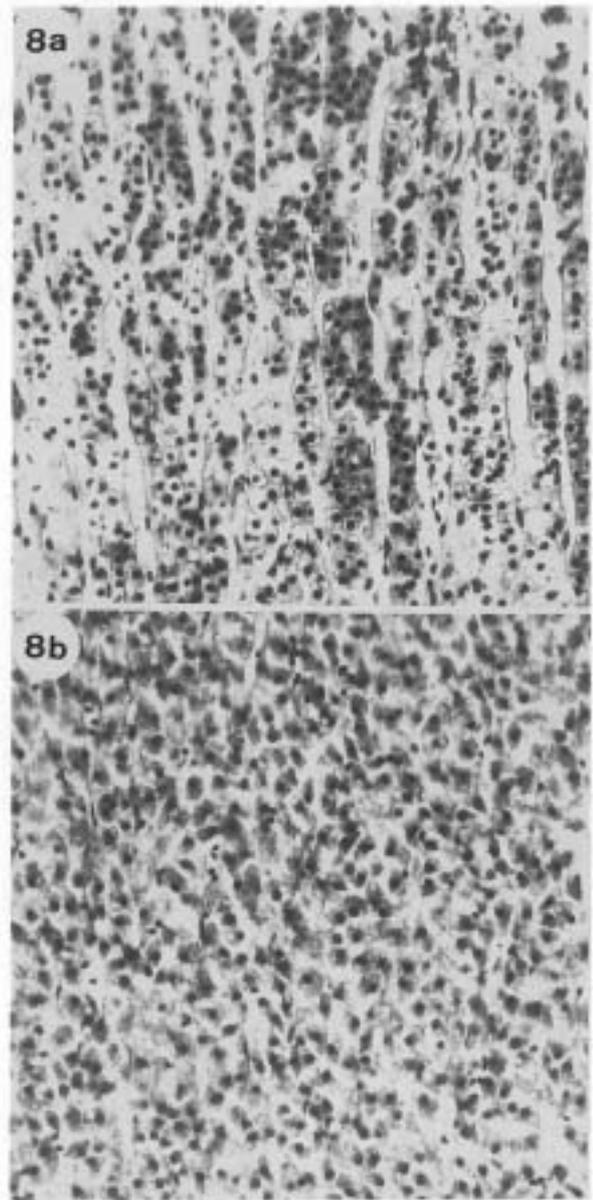


Fig. 8. Zona fasciculatae in a normal horse (a) and a pony with Cushing's disease (b). Zona fasciculatae shows palisades in normal equine but grow abnormally to disturb the arrangement in the pony with Cushing's disease. H & E. $\times 85$.

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