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II. Oxidative Enzymes of the Anterior Pituitary Gland in the Pig

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Summary

The anterior pituitary gland in the pig was studied histochemically for the activities of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), β -hydroxybutyrate dehydrogenase (β -HDH), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PDH), NADH diaphorase and NADPH diaphorase. These enzyme activities showed similar intracellular localization, but the intensity of the activities varied with the enzymes and the cell types. In the reactions except β -HDH, gonadotrophs and thyrotrophs showed the most activity, somatotrophs and mammatrophs had less activity than gonadotrophs and thyrotrophs, while follicular cells had the least activity.

In the castrated males, the enlarged-activated gonadotrophs and the enlarged-degenerated gonadotrophs showed intensive and very weak activity, respectively. The activity of former cells was augmented in order of G-6-PDH, NADH diaphorase > NADPH diaphorase > SDH, LDH.

In 1-month-old pig, the activity of NADH and NADPH diaphorase, G-6-PDH, SDH and β -HDH was observed. In 3-months-old pig, the activity of all enzymes studied was observed. The activity of NADH diaphorase and G-6-PDH was considerably augmented in gonadotrophs and thyrotrophs.

These results and a relationship between the activity of these enzymes and the secretory activity were discussed.

In the previous study (1), we classified the cells of the anterior pituitary gland in the pig into seven types by light microscopy. A relationship between the morphological changes of the cells and their secretory functions, and that between sexes and ages of the animals, and also the occurrence and distribution of various cell types were investigated. The secretory activity of these cell types was difficult to understand by the tinctorial quality and the cell morphology except for gonadotrophs which performed considerable functional changes after castration. The large gonadotrophs which appeared after castration were classified functionally into the enlarged-activated gonadotrophs and the enlarged-degenerated gonado-

trophs.

We attempted to investigate the oxidative enzymes of the anterior pituitary gland in the pig, in the expectation that the enzyme histochemical approach would yield a suitable interpretation of the problem mentioned above. Namely, a relationship between the secretory condition and the intermediary metabolism of the cell types identified by light microscopy might be indicated by these enzyme activity.

Materials and Methods

The pituitary glands of 17 Yorkshire/Landrace hybrid pigs were used for the present study. They consisted of four each of male, female and castrated male of 6-months old, and one each of female of 1-, 2-, 3-, 4- and 5-months old. The castration was performed within one month after birth by the conventional technic. The ovaries of the animals were grossly observed at the same time.

The pituitary glands were removed immediately after slaughter. They were cut into two halves at the median plane, rinsed in 0.1M cacodilate buffer (0°C–4°C) of pH 7.2. Two serial sections, 6 μ thick, were made in a cryostat. One of them was used for the histochemical demonstration of one of the following enzymes: SDH, LDH, β -HDH, GDH, G-6-PDH, NADH diaphorase and NADPH diaphorase. The other was fixed in 10% formolsaline (neutral formalin 1: Ringer's solution 9) for 18 hours and stained with PAS (2), aldehyde fuchsin (3) or erythro-sine-orange G-aniline blue method (1), respectively. All enzymes were demonstrated by the technic of Barka-Anderson (4) and incubated for 40 minute at 37°C. Control sections were incubated in the media which lacked specific substrates.

Counts of the cells with enzyme activity were determined by the method of Inoue (5). Enzyme activity was roughly expressed as zero to four plus scales.

Results

In the anterior pituitary glands of 6-months-old pigs, all enzyme activities examined were found in the cytoplasm of the gland cells. The activity was intense in NADH and NADPH diaphorase, G-6-PDH, SDH and LDH, but was weak in GDH and β -HDH (Table 1). The cells with the positive enzyme activity were distributed throughout the anterior pituitary gland. The cells with the most activity (G-6-PDH and NADH diaphorase: four plus scale, NADPH diaphorase, SDH and LDH: three plus scale, β -HDH: two plus scale, GDH: one plus scale) on individual enzyme reactions were almost limited to the zona tuberalis.

1. Identification of the Cell Types with Enzyme Activity

The cells with the most activity on various enzyme reactions were weakly stained with PAS. A number of them were also weakly stained with aldehyde fuchsin. These cells were classified into two types. One of them was medium or

TABLE 1. *Distribution of Oxidative Enzyme in the Anterior Pituitary Gland Cells (6-months-old Male Pigs)*

Enzymes	Gonadotrophs and Thyrotrophs	Somatotrophs and Mammatrophs	Follicular cells
Glucose-6-phosphate dehydrogenase	###	##	±
Succinate dehydrogenase	##	##	+
Lactate dehydrogenase	##	##	+
β -Hydroxybutyrate dehydrogenase	+	##	±
Glutamate dehydrogenase	+	+	0
NADH diaphorase	###	##	+
NADPH diaphorase	##	##	+

Enzyme activity was evaluated arbitrarily on a zero to four plus scale.

large in size, oval or polygonal in shape and was distributed along sinusoids throughout the gland. The other was medium in size, polygonal or irregular in shape and was distributed along the sinusoid in zone tuberalis, but not in other parts of the gland. These cells were degranulated and their Golgi apparatus were fully developed. The two cell types just stated corresponded to gonadotrophs and thyrotrophs of the activated type (Figs. 1-4) which we classified in the previous study (1). Gonadotrophs (Figs. 1, 2) and thyrotrophs with relatively weak enzyme activity were uniformly and intensively stained with PAS or aldehyde fuchsin.

Somatotrophs and mammatrophs, stained with erythrosine and/or orange G which distributed to pars distalis except the zona tuberalis showed moderate activity in all enzymes studied (Table 1). The degree of activity in these cells did not become so intense as the gonadotrophs and thyrotrophs even though the cells were degranulated and their Golgi apparatus were fully developed (Figs. 5, 6). Follicular cells were the least active of all enzymes studied (Fig. 5).

2. Sex Difference in Enzyme Activity

The cells with positive enzyme activity were more in females and castrated males than in males. The cells with the high activity increased in number in females and castrated males, while those with low activity increased in number in males. The cells which augmented considerably the activity in NADH diaphorase and G-6-PDH reactions were frequently observed in castrated males (Fig. 7), but were hardly observed in females and males.

In castrated males, the cells with the most activity in individual reactions varied in their frequency. In general, these cells were large in size, oval or polygonal in shape, and stained weakly with PAS or aldehyde fuchsin. They possessed developed Golgi apparatus and a small number of vacuoles. In these cells, enzyme

TABLE 2. *Relationship of Oxidative Enzyme Activity and Ages in the Anterior Pituitary Gland Cells (Female Pig)*

Enzyme	Age (month)	1	2	3	4	5	6
Glucose-6-phosphate dehydrogenase		++	++	###	###	###	###
Succinate dehydrogenase		++	++	##	##	##	##
Lactate dehydrogenase		—	++	##	##	##	##
β -Hydroxybutyrate dehydrogenase		+	+	+	+	+	+
Glutamate dehydrogenase		—	±	+	+	+	+
NADH diaphorase		##	##	###	###	###	##
NADPH diaphorase		##	##	##	##	##	###

Enzyme activity was evaluated arbitrarily on a zero to four plus scale.

activity was augmented in order of G-6-PDH, NADH diaphorase > NADPH diaphorase > SDH, LDH. A number of the enlarged cells with lower activity were observed in G-6-PDH, NADH and NADPH diaphorase, SDH and LDH reactions in castrated males. In these cells, the activity of β -HDH and GDH was very weak or absent. The cells were polygonal or irregular in shape and possessed enlarged vacuoles. They were extremely hypertrophied and were stained both with PAS and aniline blue, or were stained intensely with aniline blue.

3. *Age Difference in Enzyme Activity*

No changes according to age were observed in the intracellular localization of the enzyme activity and the distribution of the gland cells with special enzyme activity up to 6 month old in females. The change of enzyme activity according to age was summarized in Table 2. In 1-month-old pig, the cells with enzyme activity were observed throughout the gland in NADH and NADPH diaphorase, G-6-PDH, SDH and β -HDH reactions. A small number of the cells with the most activity of NADH and NADPH diaphorase were distributed in the zona tuberalis (Fig. 8). Enzyme activity was moderate in G-6-PDH and SDH reactions (Fig. 9), very weak in β -HDH reaction and not demonstrated in LDH and GDH reactions. In 2-months-old pig, the enzyme activity of LDH appeared throughout the gland (Fig. 10). In 3-months-old pig, very weak enzyme activity of GDH was demonstrated (Fig. 11). The cells with positive enzyme activity as well as the most enzyme activity increased in number. The cells with the most activity were observed in all enzymes studied. They were numerous in the zona tuberalis. In the ovary, ripe follicles were observed grossly. The degree of enzyme activity was the same as that of 6-months-old pig. The cells with positive enzyme activity increased considerably in number and the activity became very intense in G-6-PDH and NADH diaphorase.

Discussion

Lojda (6), Lojda and Schreiber (7), and Bleicher *et al.* (8) reported the histochemical activity of the oxidative enzymes in the rat pituitary gland. Lojda and Schreiber (7) found no appreciable difference in the enzyme activity between cell types. Bleicher *et al.* (8) demonstrated that the highest activity was in the cells characterized as gonadotrophs, and that the level of the activity in these cells was considerably augmented after castration. Within 3 to 4 days following castration, histochemical reactions were intensified in these cells (TPND > G-6-PDH > G-3-PDH > DPND) although other pituitary components were unaltered.

On our own study, the most activity was demonstrated in gonadotrophs and thyrotrophs. In castrated males, the enlarged gonadotrophs with increased or decreased activity were observed. In the former, the activity was remarkably augmented in G-6-PDH and NADH diaphorase. G-6-PDH is the enzyme of hexose monophosphate shunt, which is a NADPH-generating system that provides NADPH that appears to be important to synthetic mechanisms taking place in the cells of endocrine glands, such as adrenals (9-13), islets of Langerhans (14) and pituitary gland (15). Therefore, it is considered that the cells with the most activity of G-6-PDH are in very active stage of hormone synthesis and/or release.

In castrated males, the enlarged-activated gonadotrophs were intense, and the enlarged-degenerated gonadotrophs were very weak in the enzyme reactions studied. This supported the validity of our classification of the two types on the enlarged-gonadotrophs by their morphology and tinctorial characteristics (1). It seems that the variation of frequency of the enlarged-activated gonadotrophs arises from the variable reaction depending on individuals following castration.

The oxidative enzymes including those of the glycolytic pathway, the hexose monophosphate shunt and citric acid cycle were selected for the present studies. The citric acid cycle is of importance as a system that provides ATP that essential as the energy of synthetic mechanisms (16, 17). In the pig anterior pituitary gland, gonadotrophs and thyrotrophs showed the most activity in all enzyme reactions. Somatotrophs and mammatrophs had less activity than gonadotrophs and thyrotrophs, and follicular cells were the least reactive. The degree of the enzyme activity in the former cells did not show remarkable changes, even though the cells were degranulated and their Golgi apparatus were fully developed. Balogh and Cohen (15) and Pearse (18) reported in the human anterior pituitary that basophiles were the most active, the acidophiles were intermediate, and chromophobes were the least active in several oxidative enzymes studied. It is considered that gonadotrophs and thyrotrophs with the most activity of the oxidative enzymes of the citric acid cycle necessitate more energy than somatotrophs and mammatrophs do in synthesizing their hormones.

It was reported that growth hormone and thyroid stimulating hormone are

synthesized during fetal and neonatal life in the rat anterior pituitary gland (19-21). In this pig anterior pituitary gland, the relatively intense activity of G-6-PDH, SDH and NADH, and NADPH diaphorase were observed in zona tuberalis of 1-months old pig. At this time, the zona tuberalis was indistinct and contained numerous immature, non-granulated cells (1). In 3-months-old pig, the activity of G-6-PDH, SDH and NADH diaphorase became intense in gonadotrophs and thyrotrophs, and the ovaries contained ripe follicles. The zone tuberalis was more extensive and contained a number of mature granulated cells of gonadotrophs and thyrotrophs (1). The above facts suggest that, in pig anterior pituitary gland, LH, FSH and TSH are synthesized in as early as one month after birth, and that the activity of the enzymes parallels the formative function of the secretory granules in these cell types.

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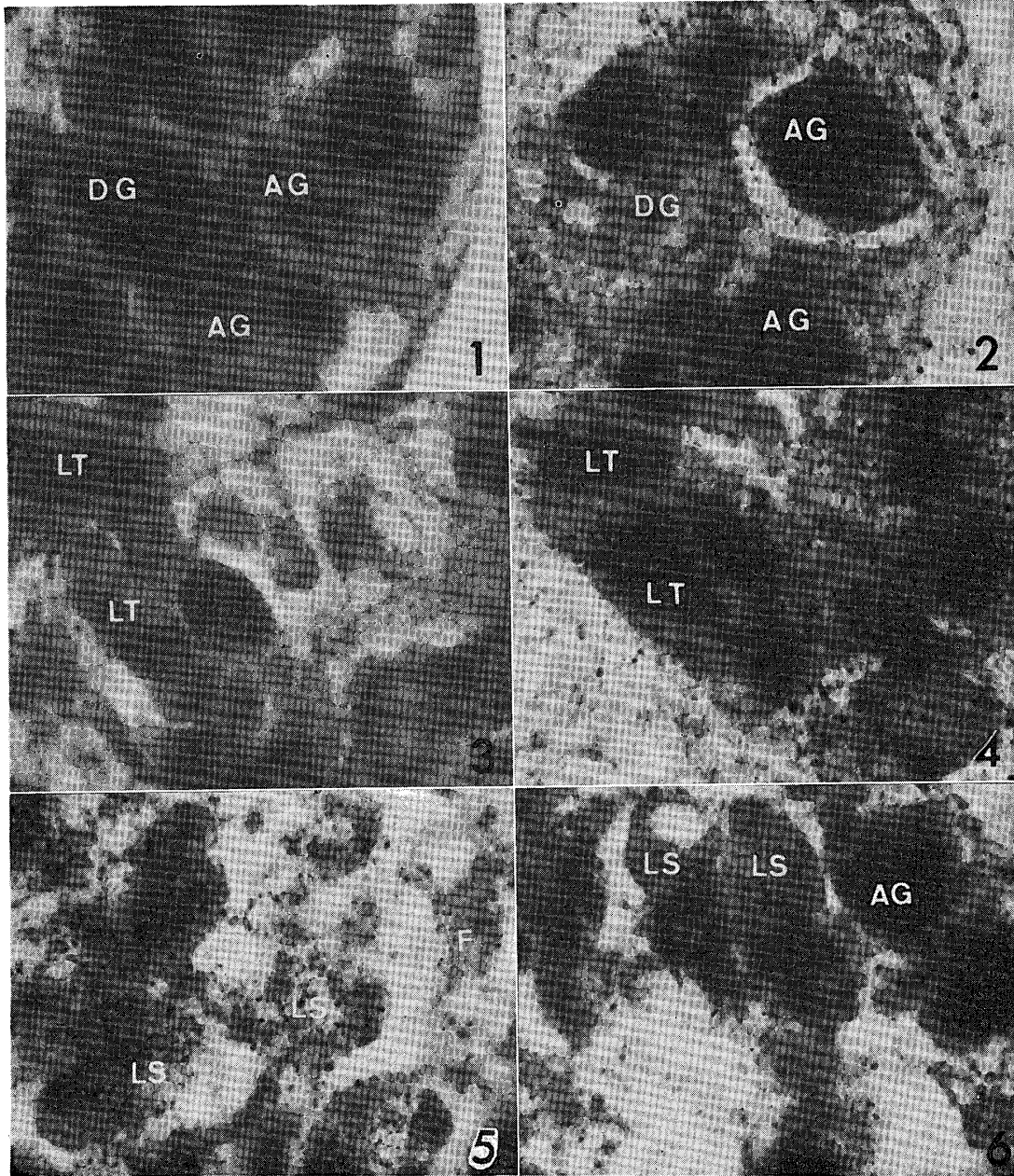
Explanation of Figures

All photomicrographs except Figs. 1 and 3 were taken from preparations of enzyme histochemistry. Figs. 1 and 3 were stained with the combined method of PAS and erythrosine-orange G-aniline blue method.

Plate 1

Explanation of Figures

- FIGS. 1-2. Two serial sections. Enlarged-activated gonadotrophs (AG), stained weakly with PAS (Fig. 1) showing marked NADH diaphorase activity (Fig. 2). Enlarged-degenerated gonadotrophs (DG), stained with PAS and aniline blue (Fig. 1) showing low activity (Fig. 2). Castrated male. $\times 1000$.
- FIGS. 3-4. Two serial sections. Large thyrotrophs (LT), stained relatively weak with PAS (Fig. 3) showing marked NADH diaphorase activity (Fig. 4). Castrated male. $\times 1000$.
- FIGS. 5-6. Large somatotrophs (LS) showing moderate NADH diaphorase activity and Follicular cells (F) showing very weak activity. Castrated male. $\times 1000$.



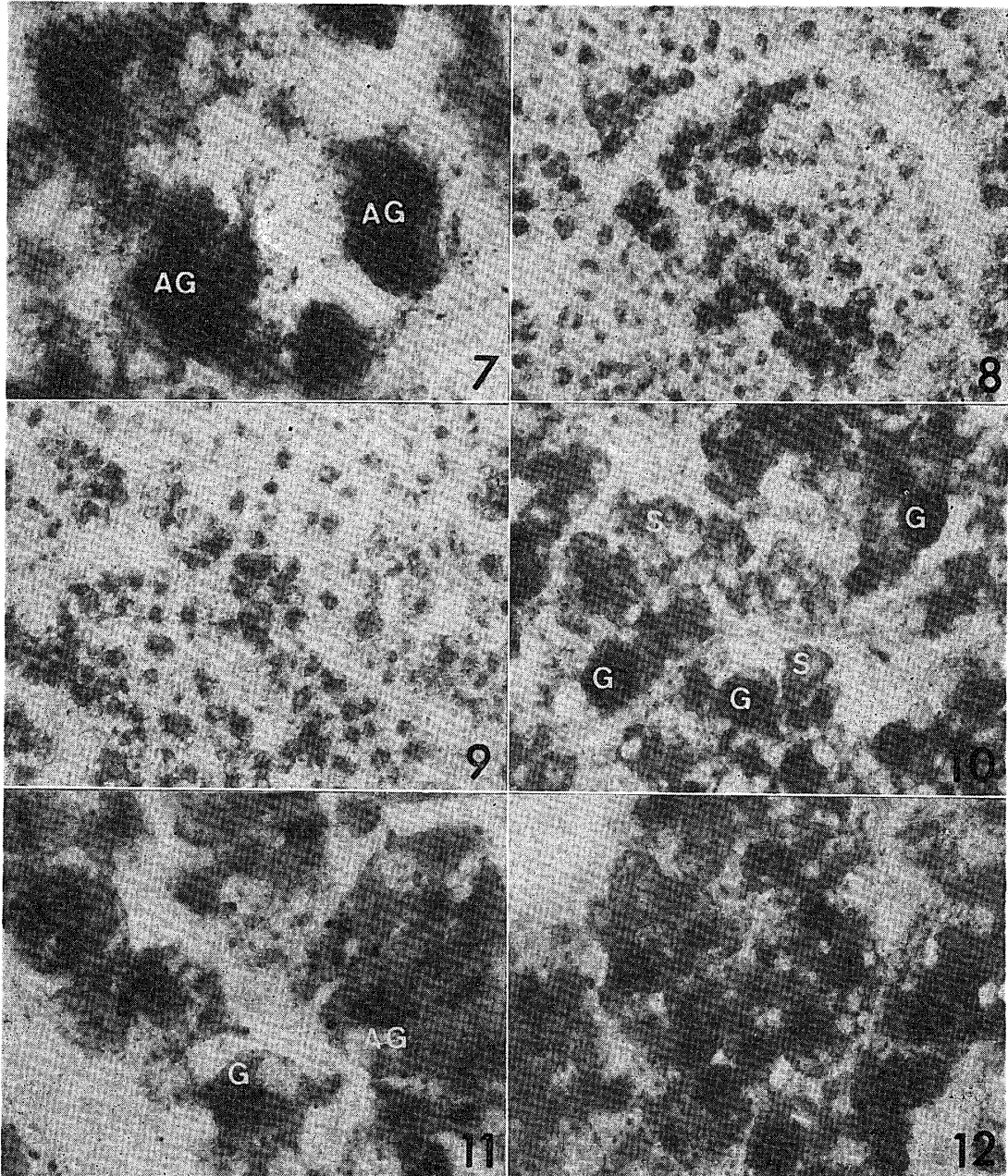


Plate 2

Explanation of Figures

- FIG. 7. Enlarged-activated gonadotrophs (AG) showing strikingly strong G-6-PDH activity. Castrated male. $\times 1000$.
- FIG. 8. A cluster of gonadotrophs in the zona tuberalis showing strong NADH diaphorase activity in 1-month-old pig. Female. $\times 100$.
- FIG. 9. A cluster of cells in the zona tuberalis showing moderate G-6-PDH activity in 1-month-old pig. Female. $\times 100$.
- FIG. 10. LDH activity in 2-months-old pig. Gonadotrophs (G) showing the strong activity compared with somatotrophs (S). Female. $\times 400$.
- FIG. 11. GDH activity in 3-months-old pig. Gonadotrophs (G) and enlarged activated gonadotrophs (AG) showing the moderate activity. Female. $\times 1000$.
- FIG. 12. LDH activity in 3-months-old pig. A cluster of gonadotrophs with the strong activity in the zona tuberalis. Female. $\times 400$.