

PHYSIO-HISTOLOGICAL STUDIES ON THE PHYSIOLOGICAL OBESITY OF MEAT PIGS, (REPORT IX), ESPECIALLY ON THE OCCURRENCE OF GLYCOGEN WITHIN THE NUCLEI AND CYTOPLASMS OF THE ZONA RETICULARIS IN THE ADRENALS OF WILD BOARS AND LANDRACE MEAT PIGS IN COMPARISON WITH THAT OF YORKSHIRE-AND LANDRACE F_1-MEAT PIGS, YORKSHIRE PIGLETS, YORKSHIRE PIGS IMMUNIZED WITH HOG-CHOLERA VIRUS, AND OTHER PIGS

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By

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Introduction

Physio-histological studies on the mechanism of adipositas and the effect of fattening have been done histochemically on various organs of Yorkshire- and Landrace FI-pigs used for experiments in feeding on meat pigs. According to previous studies (1, 2, 3, 4), there was an occurrence of glycogen in the nuclei and cytoplasms of the zona reticularis in the adrenals. Previously no one has found glycogen in the nuclei of the adrenals. For the physiological meaning of this phenomenon, we must note previous reports (5, 6, 7, 8, 9, 10, 11). In these reports the following points on the mechanism of obesity have been clarified: hyperadreno-corticism accelerated hyperinsulinism to increase the fat metabolism from the

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points of a relatively high concentration of glycogen in the zona fasciculata and zona reticularis, a remarkable synthesis and secretion of corticosteroids in the adrenocortex, and activation of phosphorylase and phosphatases, an increase of glucose-6-phosphate by the accelerated glycogenolysis, the presence of a very active hexose-monophosphate shunt in the adrenal tissue by the production of more TPNH, the splitting of the cholesterol side-chain and the reduction of certain steroids by this cofactor and Δ^5 -3 β -dehydrosterol-dehydrogenase in the adrenocortex, the production of more insulin by the stimulation in the β -cells of the pancreas, and the inhibition of epinephrine production in the adrenomedulla. Also, it has been clarified that the increased food-intake tended to produce hyperinsulinism and hyperadrenocorticism, which could in turn significantly further the obesity; and that hyperadrenocorticism accelerated hyperinsulinism to increase the fat metabolism.

According to Sutherland (12, 13) and Kondo (14), it has been established biochemically that the regulation of the intracellular reaction is by hormone. Thus it is a possible working hypothesis. Williams (15) (1962), classified how recent investigations have helped to clarify the mechanisms involved in the synthesis and release of corticosteroids in the chapter of obesity. Our previous morphological reports (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11) seemed to be inaccorded with those biochecmial opinions.

If the pigs are considered to be physiological adipositas it might be important to observe the relationship between the occurrence of abundant glycogen in the nuclei and cytoplasms of the adrenocortical cells and the deposition of fat and corticosteroid in the cytoplasms of the adrenocortical cells.

In the present investigation special attention was paid to the presence of glycogen in the nuclei and also in the cytoplasms of the adrenals of wild boars and Landrace meat pigs in comparison with that in Yorkshire- and Landrace-Fi-meat pigs, hog-cholera virus-immunized Yorkshire pigs, healthy piglets and other conditioned pigs.

Materials and Methods for Studies

The pigs used for these studies were 70 Yorkshire pigs, 26 Landrace pigs, 50 Landrace-F1 pigs, 29 Yorkshire pigs immunized with hog-cholera virus, 3 Yorkshire pigs affected with steatitis (yellow fat disease), 6 Landrace-F1 pigs inoculated with iodo-casein, 23 piglets at 45 days after birth, and 20 wild boars collected among the moutains. Adult pigs were used at the age of 190 to 200 days after birth. The 70 Yorkshire pigs consisted of 50 used for studies on the feeing standard in the Miyagi Prefectural Agricultural Experiment Station and 20 from the Matsuzaka slaughter house. The 26 Landrace pigs consisted of 20 from the Matsuzaka slaughter house and 6 used for studies on the feeding standard in the Ibaragi National Swine Stockyard. The 50 Landrace-F1 pigs consisted of 30 from the feeding standard study in Miyagi Prefectural Agricultural Experiment station and 20 from the Matsuzaka

slaughter house. The 29 Yorkshire pigs immunized with hog-cholera virus and the 23 piglets were presented by the National Institute of Animal Health in Tokyo. The 3 Yorkshire pigs affected with steatitis were from the Miyagi Agricultural College in Sendai studied, and the Landrace-F1 pigs inoculated with iodo-casein were slaughtered in Nagoya. The 20 wild boars were collected by hunters in Oita Prefecture.

The total adrenals from these pigs were fixed in buffered formol fluid, embedded in paraffine, and cut into 6μ sections. The stains employed were as follows: Hematoxylin eosin stain for general histology and PAS-hematoxylin stains with or without saliva digestion for glycogen.

Table 1. Occurrence of glycogen within the nuclei and cytoplasms of the adrenocortical cells of the wild boars

| | Gl | ycogen | (| Glycogen deposition (Cell count/1 section) | | | | | | |
|---------------------------|--------------------|-----------|--------------------------|--|--|----------------------------|--------------|--|--|--|
| Sex | Name | Age | Intra- nuclear (a) | Intna- nuclear & intra- cytoplasm. (b) | Intra- nuclear vacuoliza- tion (c) | Intra- cytoplas- mic | Total (a+b+c | | | |
| | 150 | 3 yrs. | 3 | 0 | 3 | 100 | 6 | | | |
| | 152 | 3 yrs. | 0 | - | i | | 1 | | | |
| | 153 | 3 yrs. | Ŏ | 1 | | t . | | | | |
| | 154 | 1 yr. | ő | F . | | | 0 | | | |
| Male | 157 | | ŏ | _ | | | 1 | | | |
| | 159 | 1 yr. | 1 | | _ | | 0 | | | |
| | 160 | 9 TTTG | 3 | | _ | | 0 | | | |
| | 162 | 2 yrs. | | _ | | | 1 | | | |
| | 163 | 4 yrs. | 0 | | _ | | 0 | | | |
| | ì | l yr. | 0 | | | 94 | 4 | | | |
| | 164 | 1 yr. | 0 | Intra-nuclear & intra-nuclear & intra-cytoplasm. (b) | 0 | | | | | |
| Female | 155 | 5 yrs. | 0 | 0 | 0 | 40 | 0 | | | |
| | 158 | 1 yr. | 0 | 0 | 0 | | Ö | | | |
| | 168 | l yr. | 0 | 0 | 4 | _ | 4 | | | |
| | 151 | | 0 | 0 | 2 | 46 | 2 | | | |
| | 156 | | 0 | 0 | (| | ī | | | |
| | 161 | | 0 | 0 | - 1 | | 0 | | | |
| Unknown | 165 | 1 yr. | 0 | - 1 | | | 1 | | | |
| | 166 | 1 yr. | 0 | - | | | | | | |
| | 167 | - J | 1 | 1 | | | 1 | | | |
| | 169 | _ | 0 | - | | | 5 0 | | | |
| | Male (10 d | cases) | 7 | 0 | ß. | EKO | 10 | | | |
| | Female (3 | | o | - 1 | | | 13 | | | |
| Total | Unknown | (7 cases) | i | | | | 4 10 | | | |
| | Total (20 | cases) | 8 | 0 | 19 | 1939 | 27 | | | |
| | Male | | 0.7 | 0 | 0.6 | 55.3 | 1.3 | | | |
| | \mathbf{Female} | | 0 | 0 | 1 | I | 1.3 1.3 | | | |
| Average | Unknown | | 0.1 | 0 | | | 1.3 | | | |
| | Total | | 0.1±0.3 | 0 | 1.3±1.4 | 157±119 | 1.3±1.4 | | | |
| Glycogen-b n total cas | earing perc ses | entage | 40 % | 0 % | 95 % | 100 % | 55 % | | | |

Results

1. Glycogen occurrence within the nuclei and cytoplasms of the adrenocortical cells of the wild boars.

The wild boar used for the studies consisted of 9 male, 3 female and 7 sex unknown animals.

It was noticed that a remarkably small amount of glycogen within the nuclei and cytoplasms of the swine adrenals showed a strong positive to PAS-stain. The average glycogen deposition in the nuclei was shown in the wild boars at 40 %, intranuclear and intracytoplasmic glycogen at 95 %, and glycogen deposition in the cytoplasm at 100 %. The results are shown in Table 1, where the mean value and mean error of cell counts of the glycogen-laden nuclei in one section are 0.1 ± 0.3 in glycogen-laden nuclei and cytoplasms 0, and vacuolized nuclei with few or no glycogen 1.3 ± 1.4 . The presence of glycogen in the cytoplasms and nuclei seemed to have no relation to the sex except for a little more intensive appearance in the males than in the females.

2. Glycogen occurrence within the nuclei and cytoplasms of the adrenocortical cells of the Landrace meat pigs.

The Landrace meat pigs used for these studies consisted of 5 male boars, 2 hogs and 13 sows from the Matsuzaka-slaughterhouse, and 3 hogs and 3 sows from Ibaragi. The occurrence of glycogen in the nuclei and cytoplasms of the adrenals of the Landrace meat pigs is indicated in Table 2.

It was noticed that a remarkably small amount of glycogen within the nuclei and cytoplasms of the adrenocortical cells of Landrace pigs showed a strong positive to PAS-stain. The average glycogen deposition in the nuclei was shown in the Landrace meat pigs of Matsuzaka at 65 % and that of Ibaragi at 50 %; glycogen deposition in both nuclei and cytoplasms in Matsuzaka's Landrace at 40 % and in Ibaragi's Landrace at 17 %; intranuclear vacuolization without glycogen in Matsuzaka's Landrace at 55 % and Ibaragi's Landrace at 33 %; and the glycogen deposition in the cytoplasms of Matsuzaka's Landrace at 100 % and that of Ibaragi's Landrace at 100 %. The more intensive appearance of glycogen in the nuclei and cytoplasms of the Matsuzaka Landrace might be related to the feeding and breeding in the rural private farms over against the Ibaragi Landrace which were raised in a Governmental farm.

As the results in Table 2 show, the cell counts (mean value and mean error) of the glycogen-laden nuclei in one section are 2 ± 2 in Matsuzaka's Landrace-pigs and 0.8 ± 1.3 in Ibaragi's, that of the vacuolized nuclei with few or no glycogen 1 ± 2 in Matsuzaka's and 0.4 ± 0.3 in Ibaragi's, and that of the glycogen-laden cytoplasms 860 ± 572 in Matsuzaka's and 92 ± 118 in Ibaragi's. Also the total cell counts (mean value and mean error) of the glycogen deposition in the nuclei, in

Table 2. Occurrence of glycogen within the nuclei and cytoplasms of the adrenocortical cells of the Landrace meat pigs.

| · · · · | | Glycogen | 1 | ycogen depos | | | tion) |
|---------------------|--|--|--|--|---|---|--|
| Place | Sex Name | | Intra- nuclear (a) | Intra- nuclear & intra- cytoplasm. (b) | Intra- nuclear vacuole (c) | Intra- eytoplas- mic | Total (a+b+c) |
| | Male boars | 178 182 183 185 187 | 0 1 0 4 2 | 0 1 0 0 0 | 1 4 0 8 2 | 152 112 183 1237 1135 | 1 6 0 12 4 |
| | Hogs | 176 184 | 1 4 | 3 2 | 0 | 1728 475 | 4 6 |
| Matsuzaka | Sows | 171 172 173 174 175 177 179 180 181 186 188 189 | 5 2 0 0 13 0 1 2 0 7 5 0 4 | 6 0 0 4 0 0 2 0 3 0 0 2 | 0 0 0 0 55 0 2 3 0 3 5 1 | 0 2378 0 531 0 301 55 389 0 336 2 745 3 1023 0 489 3 1075 5 775 1 430 | 11 2 0 0 72 0 3 7 0 13 10 1 |
| ĺ | Total | Male bore (5) Hogs " (2) Sows " (13) Total " (20) | 7 5 49 61 | 1 5 17 | 15 0 70 | 2819 2203 10712 15734 | 23 10 136 169 |
| | Mean value and mean error. | | 2±2 | 1±2 | 1±2 | 860±572 | 5±4 |
| | Glycogen-bearing percentage in total cases | | 65 % | 40 % | 55 % | 100 % | 75 % |
| | Glycoge tage in | n-bearing percen- total cases | 50 % | 17 % | 33 % | 100 % | 67 % |
| | Hogs | 130 131 129 | 1 3 15 | 0 0 1 | 0 0 3 | 290 78 10 | 1 3 19 |
| Ibaragi | Sows | 119 118 117 | 0 0 0 | 0 0 0 | 0 0 2 | 12 50 20 | 0 0 2 |
| | Total | Hogs (3) Sows (3) | 19 0 | 1 0 | 3 2 | 378 82 | 23 2 |
| | | Total (6) | 19 | 1 | 5 | 450 | 25 |
| | Mean v | alue and mean | 0.8±1.3 | 0 | 0.4±0.3 | 92±118 | 1.2±1.3 |

both nuclei and cytoplasms and intranuclear vacuoles without glycogen, are 5 ± 4 in Matsuzaka's Landrace pigs and 1.2 ± 1.3 in Ibaragi's. The presence of glycogen in the cytoplasms and nuclei seemed to have no relation to the sex and body weight. However it might be related to the administration and breeding since there was a more intensive appearance in the Matsuzaka's Landrace pigs raised on rural private farms than in the Ibaragi pigs raised in the National Government farms.

3. Glycogen occurrence with the nuclei and cytoplams of the the adrenocortical cells of the Yorkshire meat pigs

The Yorkshire meat pigs used for these studies consisted of 20 from the second experiment of Miyagi's Prefectural Agricultural Experiment Station, 14 from the third experiment, 16 from the fourth experiment, and 20 from the Matsuzaka Slaughter house. With the exception of those from Matsuzaka and Miyagi's fourth experiment, the occurrence of the glycogen deposition in the nuclei and cytoplasms of the glycogen deposition in the nuclei and cytoplasms of the adrenocortical cells of the Yorkshire meat pigs were described in the previous reports (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11). The results for the Matsuzaka and the Miyagi fourth experiment pigs are indicated in Table 3.

Table 3. Occurrence of glycogen within the nuclei and cytoplasms of the adrenocortical cells of the Yorkshire meat pigs

| | | | Gly | cogen deposit | tion (Cell co | unts/1 section | on) |
|--|------------------------|--------------|----------|--|-------------------------------|----------------------------|-----------|
| | | Glycogen | | Intra- nuclear & intra- cytopl. | Intra- nuclear vacuoles | Intra- cytoplas- mic | Total |
| Group | | Name | (a) | (b) | (c) | | (a+b+c) |
| Second experiment Miyagi's cases (20 cases) | | $3{\pm}4$ | 37±34 | 47±38 | 530±637 | 47±38 | |
| Third ex Miyagi's | cperiment cases (14 | cases) | 48±45 | 33±36 | 6±11 | 613±309 | 87±75 |
| | 4- 1 | | 324 | 12 | 10 | 505 | 346 |
| | | 4-2 | 108 | 26 | 5 | 761 | 139 |
| . | | 4-3 | 194 | 3 | 16 | 88 | 216 |
| es es | Hogs | 4-4 | 14 | 6 | 7 | 202 | 27 |
| srimer | | 4-5 | 44 | 0 | 2 | 158 | 46 |
| bel s | | 4-6 | 84 | 14 | 11 | 273 | 109 12 |
| Fourth experiment of Miyagi's cases (16 cases) | | 4- 7 4- 8 | 7 122 | 1 3 | 4 1 | 451 466 | 126 |
| urtl Miy 3 ca | | 4-11 | 28 | 51 | 0 | 491 | 79 |
| Fou of 1 (16 | | 4-12 | 278 | 15 | 11 | 125 | 304 |
| | | 4-13 | 46 | 1 | 3 | 20 | 50 |
| | | 4-14 | 12 | 7 | 0 | 99 | 19 |
| | Sows | 4-15 | 351 | 65 | 5 | 1850 | 421 |
| | | 4-16 | 129 | 0 | 7 | 17 | 136 |
| | | 4-17 | 89 | 3 | 4 | 213 | 96 |
| | | 4-18 | 427 | 13 | 0 | 94 | 440 |

Table 3. Continued

| | Mean value mean error | | 141±134 | 14±19 | 5±5 | 363±96 | 160±5 |
|------------------------------|-----------------------------------|--|---------|----------|-------|---------|--------|
| | | M 203 | 12 | 45 | 6 | 794 | 63 |
| | | M 204 | 15 | 26 | 14 | 554 | 55 |
| | | M 206 | 10 | 7 | 6 | 286 | 23 |
| | Hogs | M 207 | 15 | 31 | 3 | 584 | 49 |
| | | M 202 | 3 | 3 | 2 | 45 | 8 |
| | | M 195 | 9 | 1546 | 5 | 1031 | 1560 |
| | | Average | 11 | 276 | 6 | 549 | 293 |
| • | | M 205 | 12 | 12 | 10 | 323 | 34 |
| Matsuzaka's cases (20 cases) | | M 208 | 83 | 16 | 36 | 130 | 135 |
| | | M 209 | 161 | 9 | 42 | 280 | 212 |
| | Sows | M 210 | 29 | 46 | 10 | 858 | 85 |
| | | M 199 | 30 | 5 | 15 | 23 | 50 |
| | | M 200 | 1 | 146 | 9 | 1026 | 156 |
| | | M 201 | 4 | 0 | 51 | 23 | 55 |
| | | M 196 | 13 | 898 | 6 | 598 | 917 |
| | | M 197 | 13 | 84 | 27 | 882 | 124 |
| | | M 198 | 53 | 5 | 4 | 50 | 62 |
| | | M 191 | 11 | 3 | 7 | 201 | 21 |
| | | M 192 | 27 | 2 | 28 | 168 | 57 |
| | | M 193 | 73 | 3 | 16 | 191 | 92 |
| | | M 194 | 5 | 73 | 11 | 854 | 85 |
| | | Average | 37 | 93 | 19 | 400 | 149 |
| | | Total average, mean value & mean error (20 cases) | | 31±47 | 21±15 | 385±374 | 104±66 |
| G | lycogen-bearing total cases of | g percentage Miyagi | 100 % | 88 % | 81 % | 100 % | 100 % |
| | lycogen-bearing total cases of | | 100 % | 95 % | 100 % | 100 % | 100 % |

It was noticed that remarkably large amounts of glycogen within the nuclei and cytoplasms of the adrenocortical cells of Yorkshire pigs showed a strong positive to PAS-stain. The average glycogen deposition in the nuclei was 100 % in the second Miyagi's experiment, 92 % in the third Miyagi experiment, 100 % in the fourth Miyagi experiment, and 100 % in the Matsuzaka experiment. The glycogen depositions in both nuclei and cytoplasms were 100 % in the second Miyagi experiment, 92 % in the third Miyagi experiment, 94 % in the fourth Miyagi experiment, and 95 % in the Matsuzaka cases. The intranuclear vacuolizations without glycogen were 100 % in the second Miyagi case, 71 % in the third Miyagi case, 81 % in the fourth Miyagi case and 100 % in the Matsuzaka case. The glycogen depositions in the cytoplasms were 100 % in the second, third and fourth Miyagi experiments and in the Matsuzaka case.

Generally the presence of glycogen in the nuclei and cytoplasms might be related to the feeding groups, but the relationships between the occurrence of

glycogen and the feeding modus are unknown at present.

As the results show in Table 3, the cell counts (mean value and mean error) of the glycogen-laden nuclei in one section are 3 ± 4 in the second Miyagi case, 48 ± 45 in the third Miyagi case, 141 ± 134 in the fourth Miyagi case and 37 ± 45 in the Matsuzaka case. These cell counts are variable and different in each experiment. The presence of glycogen in both the cytoplasms and nuclei are 37 ± 34 in the second Miyagi case, 33 ± 36 in the third Miyagi case, 14 ± 19 in the fourth Miyagi case, and 31 ± 47 in the Matsuzaka case. Those values seemed to have no relation to the difference in feeding modus, but might be related to the administration and feeding because of a more intensive appearance in the Matsuzaka Yorkshire pigs from private farms than in those from the Miyagi Prefectural Experiment Station.

The cell-counts of the intranuclear vacuoles without glycogen are 47 ± 38 in the second Miyagi case, 6 ± 11 in the third Miyagi case, 5 ± 5 in the fourth Miyagi case and 21 ± 15 in the Matsuzaka case. The cell-counts of intracytoplasmic glycogen are second Miyagi 530 ± 637 , third Miyagi 613 ± 309 , fourth Miyagi 363 ± 76 and Matsuzaka 385 ± 374 . Generally the presence of glycogen in the cytoplasms did not vary in the different experiment.

4. Glycogen occurrence within the nuclei and cytoplasms of the adrenocortical cells of Landrace-F_I meat pigs

The Landrace-Fi meat pigs used for these studies consited of 15 from Miyagi's sixth experiment, 15 from Miyagi's seventh esperiment, 20 from Matsuzaka's slaughter-house and 3 from Nagoya. The former 30 pigs in Miyagi belonged to the YL-strain, and the later 23 pigs in Matsuzaka and Nagoya belonged to the LY-strain. However, the difference of Y1 and LY will be studied in detail in a future program. With the exception of the pigs from Matsuzaka and Nagoya, the occurrence of the glycogen deposition in the nuclei and cytoplasms of the adrenal cortical cells of the Landrace Fi-meat pigs was reported in the previous reports (1, The results in the Matsuzaka and Nagoya cases are indicated in Table 4. It was noticed that a remarkably large amount of glycogen within the nuclei and cytoplasm of the adrenocortical cells of Landrace pigs showed a strong positive to The average glycogen deposition in the nuclei was 92 % in the sixth Miyagi experiment, 93 % in the seventh Miyagi experiment, 40 in the Matsuzaka case and 100 % in the Nagoya cases. The glycogen depositions in both nuclei and cytoplasms were 100 % in the sixth experiment, 46 % in the seventh experiment, 95 % in the Matsuzaka case and 100 % in the Nagoya case. The intranuclear vacuolizations without glycogen were 77 % in the sixth Miyagi experiment, 66 % in the seventh Miyagi experiment, 80 % in the Matsuzaka case and 66 % in the Nagoya case. The glycogen depositions in the cytoplasms were 100 % in the sixth and seventh Miyagi experiments, and in the Matsuzaka and Nagoya cases.

As the results show in Table 4, the cell counts (mean value and mean error) of

Table 4. Occurrence of glycogen within the nuclei and cytoplasms of the adrenocortical cells of the Landrace F_1 -meat pigs

| | | | Gly | cogen deposit | ion (Cell co | unts/1 section | on) |
|--------------------------------------|---|---|--|---|---|--|---|
| | Sex | Glycogen Name | Intra- nuclear (a) | Intra- nuclear and intra- cytoplas- mic (b) | Intra- nuclear vacuoles (c) | Intra- cytoplas- mic | Total (a+b+c) |
| Matsuzaka | Hogs | M 211 M 214 M 215 M 216 M 217 M 218 M 221 M 223 M 224 M 227 M 228 | 0 2 0 0 0 0 0 0 0 499 0 | 6 6 0 6 7 8 5 6 22 4 34 | 0 3 16 3 1 0 0 2 10 5 8 | 83 413 119 375 666 378 108 88 173 220 1204 | 6 11 16 9 8 8 5 10 509 9 43 |
| Landrace-F ₁ in Matsuzaka | Sows Mean value 8 | M 212 M 213 M 219 M 220 M 222 M 225 M 225 M 226 M 229 M 230 Average | 46 0 2 18 3 0 5 0 256 0 32 | 3 28 178 15 51 2 2 5 4 32 | 4 0 7 3 2 1 4 3 1080 2 | 348 91 373 1806 253 1187 77 50 59 352 472 | 3 37 199 17 55 83 10 1340 6 |
| Landrace F ₁ in Nagoya | Iodo-casein group | I 13 I 12 I 14 I 15 I 16 I 17 | 10 11 8 3653 7 602 | 7 48 93 58 24 43 | 3 0 0 358 6 95 | 471 643 660 552 279 465 | 20 59 101 4069 37 740 |
| Glyo in to | cogen-laden perce otal cases of Mat | ntage | 40 % | 95 % | 80 % | 100 % | 120 |
| Glyo in to | cogen-laden perce otal cases of Nago | ntage oya | 100 % | 100 % | 66 % | 100 % | |
| Mea expe | n value & mean e eriment of Miyagi | rror Sixth (15 cases) | 11±13 | 8±10 | 8±12 | 378±217 | 26±28 |
| Seve | n value & mean e enth experiment c agi (15 cases) | error in of | 7±7 | 2±4 | $4{\pm}6$ | 288±290 | 13±13 |

the glycogen-alden nuclei in one section were sixth Miyagi experiment 11 ± 13 , seventh Miyagi experiment 7 ± 7 , and Matsuzaka cases 4 ± 6 . The glycogen-deposition in both nuclei and cytoplasms were sixth Miyagi experiment 8 ± 10 , seventh Miyagi experiment 2 ± 4 , and Matsuzaka case 35 ± 60 . The intranuclear vacuolizations were sixth Miyagi experiment 8 ± 12 ; seventh Miyagi experiment 4 ± 6 , and Matsuzaka case 3 ± 2 . The intracytoplasmic glycogen depositions were sixth Miyagi experiment 378 ± 217 , seventh Miyagi experiment 288 ± 290 , and Matsuzaka case 524 ± 635 .

5. Glycogen occurrence within the nuclei and cytoplasms of the adrenocortical cells of the Yorkshire piglets at 45 days after birth

Twenty-three Yorkshire piglets at 45 days after birth were used for these studies. The results are indicated in Table 5.

It was noticed that a remarkably scanty amount of glycogen within the nuclei

Table 5. Occurrence of glycogen within the nuclei and cytoplasms of the adrenocortical cells of the Yorkshire piglet at the 45 days after birth

| | | | Glycogen depos | sition (Cell o | count/l secti | on) |
|-----------------------------|----------------------------|-------------------|---|-------------------------------|----------------------------|--------|
| Glycogen | | Intra- nuclear | Intranuclear & intra- cytoplasmic | Intra- nuclear vacuoles | Intra- cytoplas- mic | Total |
| Group | Name | (a) | (b) | (c) | | (a+b+c |
| | PG 63 | 0 | 0 | 0 | 18 | 0 |
| | PG 64 | 0 | 0 | 0 | 20 | 0 |
| | PG 65 | 0 | 0 | 0 | 17 | 0 |
| | PG 66 | 1 | 0 | 0 | 5 | 0 |
| | PG 67 | 0 | 0 | 0 | 12 | 0 |
| | PG 68 | 1 | 1 | 0 | 18 | 2 |
| | PG 69 | 0 | 0 | 0 | 23 | 0 |
| | PG 41 A | 0 | 0 | 0 | 89 | 0 |
| | PG 41 B | 0 | 0 | 0 | 59 | 0 |
| Healthy | PG 42 B | 1 | 0 | 0 | 5 | 1 |
| Yorkshire | PG 43 A | 3 | 0 | 0 | 43 | 3 |
| $\mathbf{piglets}$ | PG 43 B | 0 | 0 | 1 | 308 | 1 |
| (23 cases) | PG 44 A | 3 | 1 | 1 | 72 | 5 |
| , | PG 44 B | 1 | 3 | 0 | 154 | 4 |
| | PG 45 A | 0 | 0 | 0 | 16 | 0 |
| | PG 45 B | 0 | 0 | 1 | 178 | 1 |
| | PG 46 A | 0 | 0 | 0 | 112 | 0 |
| | PG 46 B | 0 | 0 | 1 | 153 | 1 |
| | PG 47 A | 1 | 3 | 0 | 216 | 4 |
| | PG 47 B | 1 | 3 | 0 | 56 | 4 |
| | PG 48 A | 0 | 0 | 0 | 92 | 0 |
| | PG 48 B | 0 | 0 | 0 | 73 | 0 |
| | Mean value & mean error | 0.5±0.9 | 0.5±1.0 | 0.2±0.4 | 82±79 | 1.2±1. |
| Glycogen-bearin total cases | ng percentage | 3 5 % | 22 % | 18 % | 100 % | 48 % |

and cytoplasms of the adrenocortical cells of the Yorkshire piglets showed positive to PAS-stain. The average glycogen deposition in the nuclei indicated granular. The glycogen-bearing percentages were 35 % in the nuclei, 22% in both nuclei and cytoplasms, 18 % in the vacuolized nuclei, and 100 % in the cytoplasms. As the results show in Table 5, the cell counts (mean value and mean error) of the glycogen deposition in one section are 0.5 ± 0.9 in the nuclei, 0.5 ± 1.0 in both nuclei and cytoplasms, 0.2 ± 0.4 in the nucleus with vacuoles, and 82 ± 79 in the cytoplasms.

6. Glycogen occurrence within the nuclei and cytoplasms of the adrenocortical cells of the Yorkshire pigs immunized with hog-cholera virus, and in the Yorkshire pigs affected with steatitis

The animals used for these studies consisted of 29 Yorkshire pigs immunized with hog-cholera virus and Yorkshire pigs affected with steatitis. With the exception of 3 Yorkshire pigs affected with steatitis, the occrrence of the glycogen deposition in the nuclei and cytoplasms of the adrenocortical cells of the hog-cholera-immunized Yorkshire was indicated in the previous report (5).

| Table 6. Oc | rrence of glycogen within the nuclei and cytoplasms of the adrenocortical |
|-------------|---|
| cells | the immunized with hog-cholera virus and affected with steatitis |

| | | GI | Glycogen deposition (Cell counts/1 section) | | | | | | |
|----------------------|---------|-------------------|---|-------------------------------|----------------------------|---------|--|--|--|
| Glycogen Group Name | | Intra- nuclear | Intranuclear and cytoplasmic | Intra- nuclear vacuoles | Intra- cytoplas- mic | Total | | | |
| | | (a) | (b) | (c) | | (a+b+c) | | | |
| Pigs immunized w | | 0.4±0.7 | 2±3 | 10±10 | 29±27 | 12±12 | | | |
| | Y 3 | 1 | 10 | 2 | 870 | 13 | | | |
| Pigs affected | Y 4 | 2 | 3 | 1 | 855 | 5 | | | |
| with steatitis | Y 1 | 1 | 0 | 11 | 283 | 1 | | | |
| | Average | 1.3 | 4.4 | 4.7 | 669 | 6.3 | | | |

The result from the studies are indicated in Table 6. It was noticed that a remarkably small amount of glycogen was found within the nuclei and cytoplasms of swine adrenals of the above-mentioned immunized and affected pigs. According to the previous report (5), there existed a hypofunction of epinephrine in the physiological obesity and the hyperfunction of epinephrine in the cases immunized with hog-cholera virus. The inhibition of hyperglycogenolysis by the high activity of epinephrine production is possible.

7. Comparison of the glycogen-deposition within the nuclei and cytoplasms of the adreno-cortical cells of Yorkshire, Landrace, Landrace-FI, and wild boars

The glycogen-deposition within the nuclei and cytoplasms of the adrenocortical cells of 122 Yorkshire pigs. 20 from the second Miyagi experiment, 14 from the third Miyagi experiment, 20 from Matsuzaka, 29 hog-cholera-immunized pigs and 23 piglets 45 days after birth were studied for physiological obesity.

The results of the glycogen-deposition within the nuclei and cytoplasms of the adrenocortical cells of Yorkshire-pigs are indicated in Fig. 1. There were described four adrenals as the ellipus, and existed cortex in the peripheral portion and medullary in the centrum drawn by the oblique lines.

• shows the intranuclear glycogen deposition in the upper part of the cortex. indicates the cytoplasmic glycogen deposition in the lower part. Numerals in the upper and lower parts of the cortex show the mean value and mean error and the numbers of • and • expressed the ratio of the mean value of the intranuclear-and cytoplasmic glycogen deposition corrected the numbers as 100.

From Fig. 1 it is clear that a remarkable occurrence of the glycogen deposition, especially within the nuclei was found in all cases of the 2nd, 3rd and 4th

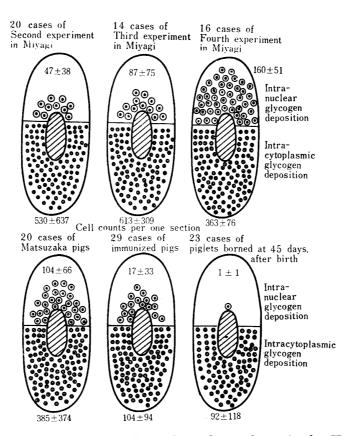


Fig. 1. Glycogen deposition within the nuclei and cytoplasms in the Yorkshire meat pigs.

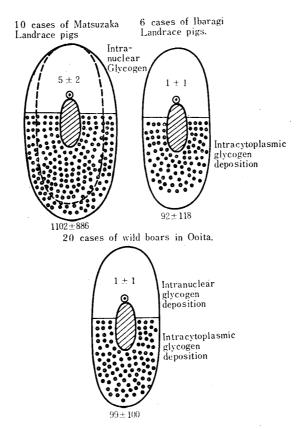


Fig. 2. Glycogen deposition within the nuclei and cytoplasms in the Landrace-pigs and wild boars.

experiments in Miyagi and in all Matsuzaka Yorkshire pigs. Also the occurrence of glycogen within the nuclei and cytoplasms decreased in the Yorkshire-pigs immunized with the hog-cholera virus, and most of the piglets contained no or few glycogen deposition within the nuclei. Accordingly Yorkshire-pigs had a large amount of glycogen deposition within the nuclei and cytoplasm as if it is a characteristic deposition. However these fact might be influenced by the immunization and young age. It is very important to study the occurrence of the glycogen deposition as it varies with age and feeding-formula.

Fig. 2 indicates the occurrence of the glycogen deposition within the nuclei and cytoplasms of the Landrace pigs and wild boars. Recently the breeding boars of Landrace have been imported from England, Sweden, Holland and U.S.A., and have become more wide-spread in Japan. The present authors chose the Landrace-pigs and wild boars as the fat-poor types for the studies on the physiological obesity and glycogen deposition.

From Fig. 2 it is clear that a small quantity of glycogen deposition within the nuclei was found in all cases of Matsuzaka and Ibaragi Landrace pigs. Wild boars were similar to the Landrace. The Ibaragi pigs received normal feeding in the National Farm of Breeding Swine. The Matsuzaka pigs were kept in farmhouses and the wild boars were in the mountains. Accordingly these animals

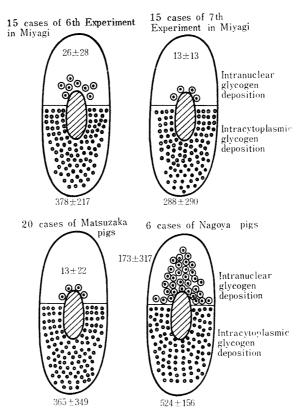


Fig. 3. Glycogen deposition within the nuclei and cytoplasms in the Landrace- F_1 meat pigs.

differed in feeding-modus. However, it was interesting that the degree of glycogen deposition in the nuclei of the Landrace pigs was similar to that in the wild boars.

Fig. 3 indicated the degree of the glycogen deposition within the nuclei of the Landrace-Fi. They were divided into the two types; those containing a small quantity of glycogen-deposition and those with a large amount of the glycogen-deposition within the nuclei. The Landrace-Fi of the sixth and seventh experiments in Miyagi Agriculture Experiment Station originated from a Landrace father and Yorkshire mother. The origin of the Landrace Fi in Matsuzaka and Nagoya were unknown. In future it will be important to compare the glycogen deposition in the Landrace F_1 pigs of Landrace A × Yorkshire A and Yorkshire A × Landrace A × Landrace

In fact, a small quantity of glycogen deposition was found in the Miyagi and Matsuzaka cases and a large amount in the Nagoya cases.

8. Resting and activated stages in the glycogen-synthesis and glycogenolysis of the adrenocortical cells of the various pigs.

The 227 pigs used for these studies consisted of 73 Yorkshire, 26 Landrace, 56 Landrace-F₁, 29 Yorkshire immunized hog-cholera virus, 23 Yorkshire-piglets, and 20 wild boars. Observing the modus of the glycogen deposition with the nuclei and cytoplasms of the adrenocortical cells in the 227 pigs, these seemed to exist

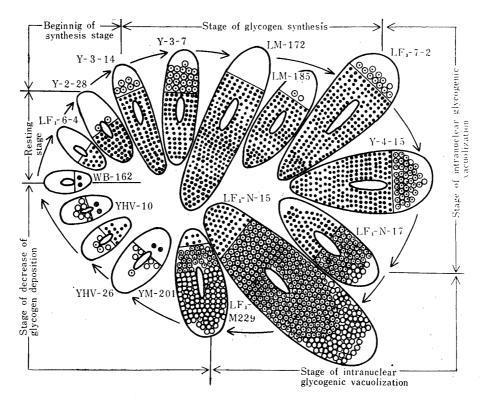


Fig. 4. Schematic figures of various stages of the glycogen synthesis, glycogenolysis, and resting in the glycogen deposition within the nuclei and cytoplasma of the adrenocortical cells of the Yorkshire-, Landrace-, Landrace F₁-meat pigs and wild boars. Remarks: sindicated glycogen deposition in the cytoplasm, oin the nuclei, and ointranuclear vacuolization. Whole ellipsoid size showed the transversal section of an adrenal and consisted of the cortex peripherally and medullary centrally. The numbers of s, o and ocrresponded to 1/10 of the real counts. The abreviate of the individual numbers showed the following: Y: Yorkshire. YM: Matsuzaka's Yorkshire, YHV: Yorkshires immunized with hog-cholera virus, LM: Matsuzaka's Landrace, LF₁: Landrace-F₁, M: Matsuzaka, N: Nagoya, and WB: Wild boars.

a resting and an activated stage in the glycogen-synthesis and glycogenolysis. Without regard to the strains and other conditions, they were arranged or classified from the view point of resting and activated types of glycogen-synthesis and glycogenolysis. The resting type and a small quantity of glycogen deposition within the cytoplasms. The activated types devided into several forms: 1) slight increase of glycogen deposition within the cytoplasms only, 2) a small glycogen deposition in the nuclei and a large amount in the cytoplasms, 3) slight increase of the nuclear glycogen and increase of cytoplasmic glycogen, 4) remarkable increase of the glycogen deposition in both nuclei and cytoplasms, 4) remarkable increase of intranculear glycogen and nuclear vacuolization and increase of cytoplasmic glycogen deposition, 6) remarkable increase of intranuclear vacuolization and decrease of glycogen deposition in both nuclei and cytoplasms, 7) decrease of the intranuclear vacuolization and cytoplasmic glycogen deposition. They fall into

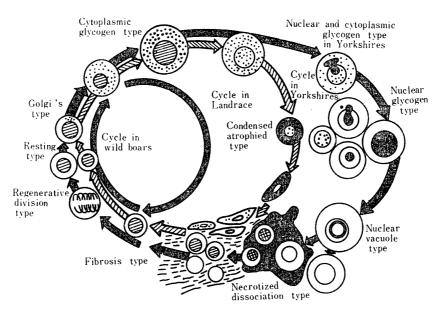


Fig. 5. Modus of glycogen deposition within the nuclei and cytoplasms of the adrenocortical cells of Yorkshire- meat pigs and wild boars.

the following groups: a) resting stage (1), b) glycogen-synthesis stage (2, 3, 4, 5, and 6) in the cytoplasms, c) beginning stage of appearance of intranuclear glycogen (3 and 4), d) stage of increased intranuclear glycogen deposition (5 and 6), e) stage of intranuclear vacuolization, f) stage of decreased glycogen deposition within the nuclei and cytoplasms. According to the present author's opinion, it might be existed that glycogen deposition in the adrenocortical cells would develop from the resting stage to the glycogen-synthesis stage through the glycogen deposition within the nuclei and cytoplasms, and feed-back from the glycogen synthesis stage to the resting stage through the glycogenolysis accompanied with intravacuolization and the decrease of cytoplasmic glycogen. Among the 227 adrenals the present authors extracted cases corresponding to the various forms of glycogen deposition from the Tables 1, 2, 3, 4, 5, and 6.

Table 7 shows the examples of the various forms of glycogen deposition for the sake of the establishment of a working hypothesis, e.g. the schematic cycles of the feed-back in the glycogen-synthesis and glycogenolysis in the adrenocortical cells.

c. Various of glycogen deposition within the nuclei and cytoplasm of the adrenocortical cells

There were three types of the feed-back cycles in the glycogen-synthesis and glycogenolysis. They were a) wild boar's cycle, b) Landrace's cycle and c) Yorkshire's cycle. These are shown schematically in Fig. 5. In the wild boar's cycle the glycogen deposition is remarkably simple. It contained only a resting type to cytoplasmic deposition which is confined to the cytoplasm. The modus of deposition

Table 7. The examples of the various forms of glycogen deposition

| | Glycogen deposition (Cell counts per 1 section of adrenal) | | | | | | |
|----------------------------|--|---------|--------------------|----------------------|---|-----------------------|--|
| | Glycogen | Intra- | Nuclear | Intra- nuclear | Intra- | Total | |
| Stages | | nuclear | & cyto- plasmic | vacuoli- zation | cyoplas- mic | | |
| | Name | (a) | (b) | (c) | | (a+b+c) | |
| Resting | PGL-42 | 0 | 0 | 0 | 5 | 0: 5 | |
| stage | YHV-22 | 0 | 0 | 0 | 17 | 0: 17 | |
| | WB-162 | 0 | 0 | 0 | 23 | 0: 23 | |
| | L-119 | 0 | 0 | 0 | 12 | 0: 12 | |
| Slight increase of | PGL-48 | 0 | 0 | 0 | 73 | 0: 73 | |
| cytoplasmic glycogen | YHV-2 | 0 | 0 | Ŏ | 70 | 0: 70 | |
| | WB-161 | 0 | 0 | 0 | 51 | 0: 51 | |
| | L-118 | 0 | 0 | 0 | 50 | 0: 50 | |
| | LF ₁ -6-4 | 0 | 0 | ő | 131 | 0:131 | |
| Slight increase of nuclear | L(I)-130 | 1 | 0 | 0 | 290 | 1:290 | |
| & cytoplasmic glycogen | Y-2-42 | 13 | Ö | 0 | 467 | 5:467 | |
| 3 2 30 | Y-2-28 | 14 | Ö | Ö | 463 | 14:463 | |
| | LF ₁ -7-8 | 1 | ı i | ŏ | $\begin{array}{c} 203 \\ 224 \end{array}$ | 2:224 | |
| | Y-3-6 | 8 | ō | $\overset{\circ}{2}$ | 228 | 10:228 | |
| Slight increase of | L(M)-180 | 2 | 2 | 3 | 1023 | 7:1023 | |
| nuclear glycogen & | L(M)-176 | 1 | 3 | 0 | 1728 | 4:1728 | |
| increase of cytoplasmic | L(M)-186 | 7 | 3 | 3 | 1075 | 13:1054 | |
| glycogen | L(M)-185 | 4 | 0 | 8 | 1237 | 12:1237 | |
| | L(M)-190 | 4 | 2 | 1 | 1248 | 7:1248 | |
| | L(M)-172 | 2 | 0 | 0 | 2378 | 2:2378 | |
| | LF ₁ -7-11 | 4 | 5 | 0 | 952 | 9: 952 | |
| Remarkable increase of | LF ₁ -7-6 | 116 | 0 | 11 | 1282 | 127:1282 | |
| glycogen in both nuclei | Y-3-4 | 130 | 92 | 10 | 938 | 232: 938 | |
| & cytoplasms | Y-3-5 | 104 | 5 | 41 | 189 | 150: 189 | |
| | Y-3-7 | 89 | 113 | 1 | 736 | 203: 736 | |
| | Y-3-2 | 61 | 28 | 4 | 924 | 93: 924 | |
| | Y-4-1 | 324 | 12 | 10 | 505 | 346: 505 | |
| | Y-4-12 | 278 | 15 | 11 | 125 | 304: 125 | |
| · | Y-4-15 | 351 | 65 | 5 | 1850 | 421:1850 | |
| | LF ₁ -7-2 | 36 | 60 | 7 | 2085 | 103:2085 | |
| Remarkable increase of | LF ₁ N-15 | 3653 | 58 | 358 | 552 | 4069: 552 | |
| nuclear glycogen and | LF ₁ N-17 | 602 | 43 | 95 | 465 | 740: 465 | |
| vacuolisation | LF ₁ N-229 | 256 | 5 | 1080 | 59 | 1341: 59 | |
| Increase of nuclear | Y-2-26 | 118 | 140 | 131 | 80 | 389: 80 | |
| glycogen and decrease | Y-2-4 | 478 | 27 | 62 | 21 | 567: 21 | |
| of cytoplasmic glycogen | Y-2-14 | 592 | 6 | 97 | 63 | 695: 63 | |
| Intranuclear vacuoliza- | YHV-10 | 0 | 0 | 74 | 16 | 74: 16 | |
| tion and decrease of | YHV-13 | 5 | 8 | 23 | 64 | 36: 64 | |
| cytoplasmic glycogen | Y-M-201 | 4 | 0 | 51 | 23 | 55 : 23 | |

| YHV-26 | 7 | 8 | 13 | 104 | 28: 104 |
|----------------------|--|---|---|---|--|
| LF ₁ -7-4 | 13 | 2 | 11 | 41 | 26: 41 |
| | 5 | 0 | 11 | 69 | 16: 69 |
| _ | 2 | 6 | 2 | 88 | 10: 88 |
| WB-152 | 0 | 0 | 1 | 17 | 1: 17 |
| Y-3-17 | 40 | 40 | 0 | 137 | 50: 137 |
| Y-4-11 | 28 | 28 | 0 | 491 | 79: 491 |
| LF,-N-14 | 8 | 8 | 0 | 660 | 101: 660 |
| | 11 | 11 | 0 | 643 | 59: 643 |
| Y-3-14 | 13 | 13 | 0 | 840 | 55: 840 |
| Y-3-15 | 14 | 14 | 0 | 556 | 80: 556 |
| | $\begin{array}{c} LF_1-7-4\\ LF_1-7-17\\ LF_1M-223\\ WB-152\\ \hline\\ Y-3-17\\ Y-4-11\\ LF_1-N-14\\ LF_1-N-12\\ Y-3-14\\ \end{array}$ | $\begin{array}{c cccc} LF_1-7-4 & 13 \\ LF_1-7-17 & 5 \\ LF_1M-223 & 2 \\ WB-152 & 0 \\ \hline \\ Y-3-17 & 40 \\ Y-4-11 & 28 \\ LF_1-N-14 & 8 \\ LF_1-N-12 & 11 \\ Y-3-14 & 13 \\ \hline \end{array}$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$ |

Table 1. Continued

Remarks: PGL: Yorkshre piglet, YHV: Yorkshire immunized with hog-cholera virus, WB: wild boar, L: Landrace, LF₁: Landrace-F₁, Y: Yorkshire, L(M): Landrace in Matsuzaka, L(I): Landrace in Ibaragi, Y(M): Yorkshire in Matsuza, LF₁M: Landrace-F₁ in Matsuzaka, LF₁N: Landrace-F₁ in Nageya, Y-2: Second experiment in Miyagi, Y-3: Third experiment in Miyagi, Y-4: fourth experiment in Miyagi, LF₁-6: Landrace-F₁ of Sixth experiment in Miyagi and LF₁-7: Landrace-F₁ of Seventh experiment in Miyagi.

tion is remarkably simple i.e. resting type to cytoplasmic deposition type. This cycle contained no nuclear deposition, vacuolization, necrotized dissociation, and fibrosis types.

In the Landrace's cycle there were found the resting types, Golgi's type, cytoplasmic deposition, nuclear and cytoplasmic deposition, condensed atrophied type, fibrosis, and regenerative division type. Both glycogenolysis and glycogensynthesis were slightly activated. Neverthless, no nuclear glycogen bodies were found in the nuclei, and there were only a small amount of granular deposition of glycogen in the nuclei. Condensed atrophied type was small in size and apparently indicated the increase of glycogen in the cytoplasm. These type seemed to be transformed to the spindle-like fibroblast with glycogen in the fibrosis.

In the Yorkshire's cycle, there were various stages such as the resting type, Golgi's type, cytoplasmic glycogen type, nuclear-cytoplasmic glycogen type, nuclear vacuole type, necrotized dissociation type immersed with polysaccharidic fluid, and fibrosis type. Glycogen deposition and vacuolization in the nuclei were characteristic in this cycle.

In short, it is important to observe the metabolic phases in the glycogen deposition. If the obesity would develop by the hyperadrenocorticism through glycogenolysis to steroid synthesis, the synthesis and decomposition of cytoplasmic glycogen, and glycogen-invasion to the nuclei might play an important role in hyperadrenocorticism. Moreover, to study cytologically the nature of the intranuclear glycogen deposition may become the subject for future investigation. Also the difference of the cycles of glycogen metabolic phase in the wild-boars, Landrace- and Yorkshire-meat pigs may develop to the inherited charactors for future investigation of the difference between wildness and domestication.

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