Histological Study of the Anterior Pituitary after Withdrawal of Methylthiouracil Administration in Mice

By

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Introduction

Recently, basophiles, especially beta cells, of the anterior pituitary have been generally accepted as being the site of TSH production (Zeckwer, '38a, b; Griesbach, '41; Griesbach and Purves, '45; Purves and Griesbach, '46a, 51a, b; Salter, '50; Halmi, '51, '52a, b; Halmi and Bogdanove, '51; Halmi and Gude, '54), although some authors are inclined to believe the acidophiles to be TSH-producer (Severinghaus, Smelser and Clark, '34a, b; Severinghaus, '37; Shigemori, '43; Adams, '46).

In our laboratory, Mase ('60) observed an increase in number and size of beta cells with hyaline vacuoles in methylthiouracil administered mature mouse pituitary gland, and suggested that so-called thyroidectomy cells are derived from beta cells. Uwadoko ('60) reported the occurrence of large-sized cells which were thought to be derived from beta cells and to represent one of their functional phases in methylthiouracil injected immature mouse pituitary. In the present study, the authors observed the histological changes occurring in immature mouse anterior pituitary after withdrawal of methylthiouracil administration in order to search to TSH-producer.

Material and Methods

Thirteen male and 14 female immature mice were used. From 10 days of age on, these mice were injected 20 times every day
with 0.02 mg of methylthiouracil per gram of body weight. They were sacrificed at 1, 5, 10, 15 and 20 days after withdrawal of methylthiouracil, while 10 mice of both sexes used as injection control animals were injected with saline-solution of the same dose as in the experimental group. Also, 10 mice were kept untreated and were used as normal control animals. Materials were fixed with Helly solution and embedded in paraffin. They were serially sectioned at 4 micra in thickness in the sagittal plane. Hematoxylin-eosin stain, Heidenhain's azan stain and periodic acid-Schiff reaction were employed.

Observations

At 1 day after withdrawal of the administration: Acidophiles are observed as frequently as in the control, but indicate a slight increase in size (Fig. 3). The majority of the cytoplasm of the acidophiles are filled with more numerous fine granules than in the control, although granules in small numbers are contained in some acidophiles. Beta cells did not show any remarkable change in number when compared with those of the control. An increase in size and irregularity in shape of beta cells are observed more frequently than in the control. PAS positive granules in the cytoplasm of beta cells rather decrease in number (Fig. 2 and 4). Delta cells show a normal appearance in cytological detail. Chromophobes indicate no change from those of the control.

At 5 days after withdrawal of the administration: Acidophiles indicate an increase in number and cytoplasmic granulation, but their sizes are rather smaller than in the previous stage. In some cases, small acidophiles with picnotic nuclei are observed numerously in the central portion of the anterior lobe. Moreover, small acidophiles with dark nuclei are relatively often seen. Beta cells increase somewhat in size and are irregular in shape. Some show a decrease in cytoplasmic granulation (Fig. 5). Delta cells indicate no change. Chromophobes decrease in number.

At 10 days after withdrawal of administration: Acidophiles are observed more frequently in number than in the control. They are rather smaller than those of the control. Generally, the cytoplasm of acidophiles is filled more densely with fine granules than in the control. In beta cells, PAS positive granules in the cytoplasm are observed to be less in number than in the control (Fig.
6). Beta cells as a whole indicate an increase in size, but their number and irregularity in shape scarcely differ from those of the control. Delta cells do not differ in cytological detail from the control. Chromophobes show a decrease in number.

At 15 days after withdrawal of administration: Acidophiles show slight increase in occurrence when compared with the control. The size and cytoplasmic granulation of these cells do not indicate remarkable change. Beta cells do not differ in number from those of the control. Their size indicates rather a decrease, and a decrease in cytoplasmic granulation is still found (Fig. 7). Delta cells are almost the same in cytological detail as in the control animals. Chromophobes show a slight decrease in number.

At 20 days after withdrawal of administration: In this stage, acidophile, delta and chromophobe cells show an almost normal appearance in cytological detail. Beta cells indicate a slight increase in size and a slight decrease in cytoplasmic granulation, although not so remarkably different from those of the control (Fig. 8).

Discussion

Acidophiles: In thiouracil administration, many investigators reported a decrease in number of acidophiles (Mackenzie and Mackenzie, '43; Salter, Cortell and McKay, '45). On the other hand, an increase in number and size of acidophiles (Inaba, '49; Suzuki, '50) were also described. Tajima ('55) administered methythiouracil in male rabbits and observed in the early stage an increase in number of acidophiles and cytoplasmic granulation, and a decrease after prolonged administration. Recently, in our laboratory, Mase ('60) found that in the anterior pituitary of mature mouse injected with methylthiouracil acidophiles decreased gradually in number and cytoplasmic granulation with progress of experiment. Uwadoko ('60) reported that in mouse anterior pituitary injected with large doses of methylthiouracil, in the early stage acidophiles indicate a decrease in number, and with progress of the experiment these cells recovered gradually in number, but that morphological changes in size and cytoplasmic granulation of acidophiles were still recognized after injection for more than 15 times. In our experiment, also, it is assumed that, because of removal of methylthiouracil, acidophiles indicate a reactionary increase in number.
Beta cells: On the histological changes of beta cells in thiouracil administered animals, many authors have reported an increase in number and size of basophiles and an appearance of thyroidectomy cells (MacKenzie and MacKenzie, '43; Griesbach and Purves, '43; Salter, Cortell and McKay, '45; Higgins, '45), but others have described a decrease of basophiles in number (Iida, Sano and Kohata, '58) and no thyroidectomy cell (D'Angelo, '50). Tajima (55) reported in methylthiouracil administered rabbit that an increase of beta cells in number and cytoplasmic granulation were seen in the early stage but after prolonged administration beta cells were scarcely found and thyroidectomy cells appeared. On the other hand, Suzuki ('50) observed histologically no change of basophiles in methylthiouracil injected rabbit pituitary.

In our laboratory, Maese ('60) reported that methylthiouracil administration caused a decrease of beta cells in mature mice. Uwadoko ('60) examined the anterior pituitaries of methylthiouracil injected mice, and observed that in the early stage a marked decrease of beta cells and a slight increase of PAS and AF positive granules in their cytoplasm, and that after prolonged administration the occurrence of beta cells was almost similar to that in the controls, but hypertrophy of the cytoplasm and irregularity in shape were found. Further, he noted the occurrence of large-sized cells which had probably originated from acidophiles at early stage and represented one of their functional phases. In the present data, beta cells did not show so marked difference in occurrence throughout the experimental period, but a day after withdrawal of methylthiouracil administration hypertrophy of the cytoplasm and a decrease of PAS positive granules were seen, and beta cells in 20 days after withdrawal of administration still indicated a decrease in granulation of cytoplasm. From these findings, it is suggested that the morphological changes in beta cells continue for a relatively long period after withdrawal of methylthiouracil administration.

On TSH-producer, there have been two hypotheses: one considers acidophiles as TSH-producer, and the other basophiles. But recently, the latter is generally accepted from the results that beta cells responded sharply to thyroxine and that histological changes in beta cells had a correlation with the TSH content of the pituitary (Griesbach and Purves, '43, '45; Purves and Griesbach, '46a). In the present data, it is considered that a change of PAS
positive granules in beta cells indicates the phases of TSH secretion. On acidophiles, Purves and Griesbach ('46a) reported that want of thyroxine, a degree which does not effect acidophiles, caused an increase in number and size of basophiles and an increase of TSH secretion, and that a degranulation of acidophiles in thyroidectomy would be caused by failure of pituitary function due to a lack of thyroxine. In our data, also, acidophiles indicated an increase in size and cytoplasmic granulation, but it cannot be said from the present findings that acidophile is the TSH-producer, because acidophile is very sensitive to various preparations.

Delta cells: Purves and Griesbach ('51) showed in rats that delta cells are scarcely influenced by thyroidectomy but may increase in number at a late stage. In methylthiouracil administration, delta cells in immature mice did not indicate any remarkable change (Uwadoko, '60) as in this data, although in mature mice they decreased in number, size and cytoplasmic granulation (Mase, '60).

Theta cells: Theta cell was recently described by Sano ('58) and it was suggested that it secretes a hormone which may regulate the corpus luteum function from data at our laboratory (Kato, '56; Yamada, Sano, Kato and Mizutani, '56; Yamada, Sano, Okumura and Sakakura, '60). In the present paper, theta cells were occasionally found regardless of the experimental stage.

Chromophobes: In methylthiouracil administration, chromophobe cells increased in number in immature (Uwadoko, '60) and mature mice (Mase, '60). In the present study, a decrease in number of chromophobes showed a relation with increase in number of acidophiles. In our laboratory, it was pointed out for the mouse that chromophobes imply degranulated forms of chromophiles besides undifferentiated cells (Yamada, Sano and Ito, '57). The decrease of chromophobes suggests a decrease in number of such degranulated forms in proportion to the increase in number of acidophiles.

### Summary

From 10 days of age on, mice were injected 20 times every other day with 0.02 mg of methylthiouracil per gram of body weight, and at 1, 5, 10, 15 and 20 days after withdrawal of administration of
methylthiouracil their anterior pituitaries were histologically investigated.

1. Acidophiles showed a slight increase in size a day after withdrawal of administration, but at 5 days after rather decreased in size, while an increase in number and cytoplasmic granulation was seen. Thereafter these changes gradually disappeared, and at 20 days acidophiles showed an almost normal appearance in cytological detail.

2. Beta cells showed an increase in size and a decrease in cytoplasmic granulation at a day after withdrawal of administration, and at 10 days after withdrawal of administration an increase in size of beta cells was seen. However, granulation of beta cells remained decrease at 20 days after withdrawal of administration.

3. Delta cells did not show any appreciable change.

4. Theta cells were occasionally found regardless of administration.

5. Chromophobes showed a decrease in number at 5, 10 and 15 days after withdrawal of administration in relation to increase in number of acidophiles.

From these findings, it is considered that morphological changes of beta cells continue for a relatively long period after withdrawal of methylthiouracil administration.

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**Explanation of figures**

1. A male pituitary of the control group. Beta cells are well-granulated. PAS reaction ×1000
2. A male pituitary at a day after withdrawal of administration. Beta cells indicate a decrease in cytoplasmic granulation. PAS reaction ×1000
3. A male pituitary at a day after withdrawal of administration. Acidophiles show an increase in size. PAS reaction ×1000
4. A female pituitary at a day after withdrawal of administration. Beta cells indicate an increase in size and a slight decrease in cytoplasmic granulation and these cells are rather atrophic. PAS reaction ×1000
5. A female pituitary at 5 days after withdrawal of administration. Beta cell is slightly degranulated. PAS reaction ×1000
6. A male pituitary at 10 days after withdrawal of administration. Cytoplasmic degranulation of beta cells still seen. PAS reaction ×1000
7. A male pituitary at 15 days after withdrawal of administration. Beta cells are still degranulated. PAS reaction ×1000
8. A female pituitary at 20 days after withdrawal of administration. Beta cells are well-granulated. PAS reaction ×1000
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