
原 著

EXPERIMENTAL PRODUCTION OF PITUITARY ADENOMAS BY THE LONG TERM INGESTION OF METHYLTHIOURACIL COMBINED WITH CASTRATION OR THYROIDECTOMY

by

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INTRODUCTION

As for the mechanism of the development of the pituitary adenoma, there are many controversies and it is even questionable whether it is a true neoplasm or not. According to KRAUS³¹⁾ (1914), the adenoma involves the hypophysis in 30 per cent of persons from 50 to 60 years of age, in 15 per cent of persons from 30 to 50 and only in 6 per cent of persons below 20 years of age. For the explanation of the mechanism of the hypophyseal tumorigenesis of man, therefore it is of great help if the pituitary adenoma of animal could be produced experimentally by endocrine disturbances, e.g., by induced hypersecretion of trophic hormones from the anterior pituitary. Surgical castration or thyroidectomy might surely yield hormonal disorders in animals, but not cause tumor growth in the pituitary, though WOOLLEY and DICKIE¹¹⁾⁵⁶⁾ reported that they produced pituitary adenomas in castrated mice of a suprarenal tumor-susceptible strain, because sexual hormones are secreted not only by the testis or ovary but also by the suprarenal²⁶⁾⁴⁴⁾, and likewise thyroxine is synthesized not only in the thyroid gland but to some extent without it.⁵⁴⁾ MOORE et al.³⁵⁾ in 1953 stated that the pituitary adenoma could not be produced by surgical thyroidectomy, because hypertrophy of the residual thyroid tissue remaining after the operation kept the animal in the normal euthyroid state. On the other hand the long term administration of estrogenic hormones⁸⁾¹⁰⁾¹⁶⁾¹⁷⁾³⁴⁾³⁷⁾⁵⁰⁾⁵²⁾⁵⁷⁾, radioactive iodine (I^{131})¹⁴⁾¹⁵⁾¹⁹⁾²⁰⁾²¹⁾²²⁾ or thiouracil derivatives⁹⁾³⁵⁾⁴⁷⁾⁴⁹⁾ might induce the hormonal disturbances due to the hypofunction of some endocrine organs, and would be used as a method of the experimental production of the pituitary tumor.

HISTORICALS

SEIFTER⁴⁷⁾ et al. in 1949 reported on a greatly enlarged pituitary in a rat thiouracil-treated for two years. However it is generally accepted that spontaneous

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hypophyseal adenomas in old rats are so frequently met with⁽³⁸⁾⁽⁴⁵⁾⁽⁴⁶⁾⁽⁵⁵⁾ that any one can not decide whether the adenoma in SEIFTER's experiment was due to chronic feeding of the compound or developed spontaneously. In senile mice the spontaneous pituitary adenomas seem to be observed in only rare instances⁽⁵¹⁾. However DALTON, MORRIS and DUBNIK⁽⁹⁾ in 1948 reported on experimental pituitary tumors in two cases of C3H strain mice fed thiouracil diet for more than 464 days, and MOORE, BRACKNEY and BOCK⁽³⁵⁾ in 1953, presenting data on 4 mice of A strain fed 0.8 per cent propylthiouracil for 18 months, reported that three of them had chromophobe adenomas of the anterior pituitaries. In the first case it was hemorrhagic and about 5 mm in diameter, while in the second approximately 3 mm in diameter. In the third mouse it was 2 to 3 times the normal size. It is generally accepted that both C3H and A strain mice, which were studied by DALTON et al. and MOORE et al., are mammary tumor-susceptible strains⁽⁵¹⁾⁽⁶⁾. However in dd strain mice, which are most frequently used for experiment in this country, it is not known whether feeding of goitrogenic diet will be effective on bringing about tumorous enlargements of the pituitary or not. Some authors⁽²⁾⁽²⁷⁾ have regarded the basophil cells as the source of thyrotrophic hormone (TSH), because hypersecretion of TSH and at the same time proliferation of the cells could be observed in the anterior lobe after chronic administration of thiouracil⁽⁴⁾⁽⁷⁾⁽³³⁾⁽³⁹⁾. Although it was suggested by these data that the experimental adenoma was of basophil origin, SEIFTER et al.⁽⁴⁷⁾, DALTON et al.⁽⁹⁾ as well as MOORE et al.⁽³⁵⁾ regarded the adenoma as chromophobe. Likewise GRIESBACH⁽²³⁾ concluded that so called thyroidectomy cells yielded by goitrogenic feeding were originated from chromophobe cells. Which cell is the true origin of the experimental adenoma? May the development of the pituitary tumor be accelerated by endocrine disorders, or not? The present study was carried out to answer these questions.

MATERIALS AND METHODS

(1) About 250 male dd strain mice, 4 to 6 weeks old, were divided into 6 groups. The mice of the I, II and III group were fed normal diet. The mice of the IV, V and VI group were fed 0.5 per cent methylthiouracil (МЕТИОЦИЛ) in NM (an animal food by the ORIENTAL YEAST Co. Ltd.). The mice of the II and V group were castrated surgically, and those of the III and VI group were thyroidectomized, while those of the I and IV group were left without any surgical procedures (Table 1).

Table 1

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
Whole number of the mice at the beginning of experiment	50	45	24	50	50	30
Number of the mice available for examination	23	14	10	27	31	21
Surgical procedures	Without	Castration	Thyroidectomy	Without	Castration	Thyroidectomy
Administration of methylthiouracil	No	No	No	Yes	Yes	Yes

(2) The temperature in animal cages was occasionally as high as over 30°C in summer, however it was maintained continuously not below 10°C even in winter. Many experimental animals died in winter, because of the hypothyroidism induced by the continued goitrogenic treatment⁴⁸. Methylthiouracil urinary stones were discovered in some of them³⁶.

(3) Thyroidectomy was carried out with the aid of a magnifying glass for delicate dissection so as to remove all thyroid tissue as completely as possible. Whether the operations were radical or not, was verified histologically. The operative mortality in 4 to 5 weeks old mice was about 30 per cent, on the other hand thirty thyroidectomized mice one year old and over had all died in a short time after the surgical procedures³⁰. At autopsies performed one or two years later, residual functioning thyroid tissue could not be found in all instances except one, which were not treated with methylthiouracil. However in each animal fed goitrogenic diet, distinct regeneration of the remaining thyroid tissue was discovered microscopically.

(4) These mice were sacrificed successively as shown in Tables 2 to 8, but those which expired after the 326th day of the treatment, were autopsied soon after the death. Body weight, exophthalmos, if present, size of the thyroid and pituitary were measured (Tables 2 to 8).

Table 2

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
Number of the mice sacrificed	23	12	10	25	25	19
Number of the mice autopsied after death	0	2	0	2	6	3
Total	23	14	10	27	31	21

(5) The pituitaries were fixed in Bouin solution modified by HALMI²⁴, embedded in paraffin, and their frontal sections 4 μ thick (Fig. 1) were stained by azan, aldehyde fuchsin azan method²⁴ and examined in PAS (periodic acid SCHIFF) reaction.

(6) The anterior pituitary cell distribution: The anterior pituitary cells were calculated by RASMUSSEN's method^{29,43}. In this procedure three frontal sections in each hypophysis (therefore one section from every one third of it, viz. cephalic, medial and caudal), were taken. All the cells of each microscopic field, with the aid of oil immersion, were arranged in three kinds, i.e. eosinophilic, basophilic and chromophobe (Fig. 2).

(7) The measuring of dimensions in frontal planes of the pituitary: The frontal sections were enlarged photographically under a microscope and their dimensions were estimated with the aid of a planimeter.

RESULTS

The widest dimensions of the frontal sections of the anterior lobes and the body weights of the experimental animals are shown in Tables 3, 4, 5, 6, 7 and 8.

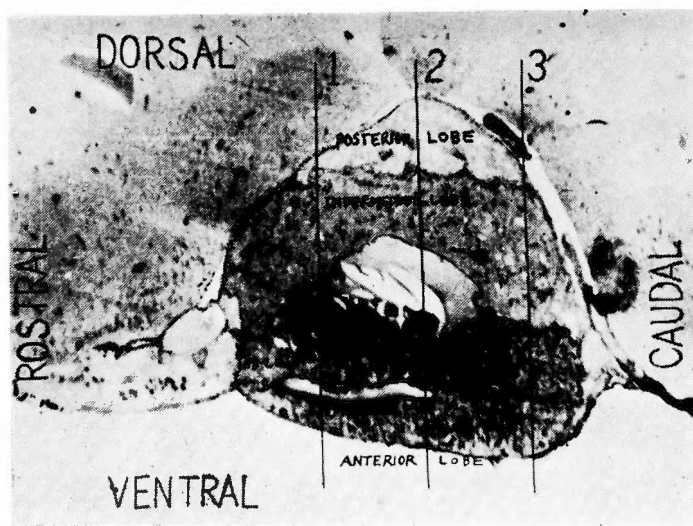


Fig. 1 Median-sagittal section of the mouse pituitary. Frontal sections for cell counting are made at levels 1, 2 and 3.

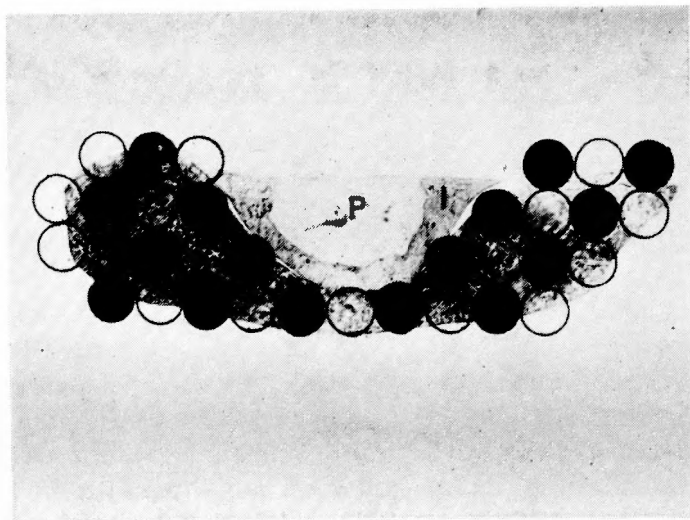


Fig. 2 Frontal section of the mouse pituitary.
 ○ : Microscopic field to be counted.
 ● : Microscopic field not to be counted.
 I : Intermediate lobe.
 P : Posterior lobe.

(1) The goiter and exophthalmos : Hypothyroid goiters were observed in all instances of the group IV and V fed methylthiouracil.

Some of them were histologically malignant²³⁾. Exophthalmos in addition were observed in half the mice of the group IV, V and VI respectively²⁸⁾ (Fig. 3).

(2) Senile changes of the normal anterior lobe in the mice (group I)

(i) Change in size: Change in size of the pituitary of the untreated mice could not be recognized (Table 3). The mean dimension of the widest frontal sections in the normal adult dd strain mice was 0.93 millimeter square (standard deviation 0.17, standard error 0.04).

(ii) Histological change: It is remarkable that in the lateral portions of some senile anterior pituitaries, localized proliferations of chromophobe cells were discovered. Though such proliferations are not to be regarded as adenomas (Fig. 4), the fact that the experimental adenoma in-

duced by the continued feeding of the methylthiouracil diet originated in the lateral part of the anterior lobe, where the localized chromophobe cell proliferation was discovered in the untreated old mice, is of great consequence in discussing the mechanism of the pituitary tumorigenesis.

(iii) Cell distribution of the normal anterior pituitary is illustrated in Table 9.



Fig. 3 Conspicuous exophthalmos observed in a mouse, which had been treated with methylthiouracil (No. 94).
Right : A control mouse.

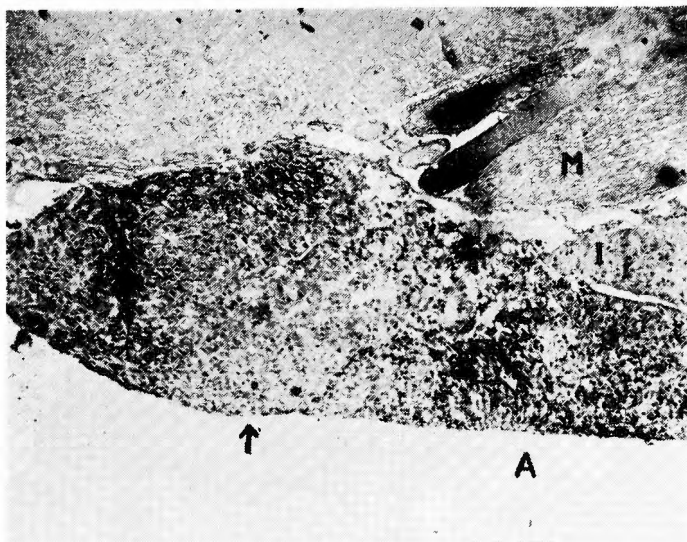


Fig. 4 A localized proliferation of chromophobe cells in the lateral area of the anterior pituitary in a normal senile mouse (No. 19).

A : Anterior lobe.

I : Intermediate lobe.

M : Mammillary body.

Halmi-Bouin fix., paraffin section, 4μ , azan stain, $\times 10 \times 5$.

The proportion of the chromophobe cells increased with age, while that of eosinophilic cells decreased.

(3) Changes of the anterior pituitary in the mice fed methylthiouracil and treated not surgically (group IV)

(i) Change in size : Enlargements of the dimensions of the frontal sections of the senile anterior lobes were observed frequently (Table 6). Macroscopically it is apparent in Figs. 5 and 8. The enlargement of the pituitary with age was mainly in the rostrocaudal and ventrodorsal direction, but not conspicuously in the lateral direction. In experiments generally the anterior pituitary of more than six times the normal weight seems to be regarded as adenomas, therefore in this study the anterior pituitary whose frontal section was over $6^{2/3}$ ($=3.31$) times the normal dimension ($=3.08$ millimeter square) was defined as macroscopic adenoma (gross tumor), and over $2^{2/3}$ ($=1.58$) times the normal dimension ($=1.48$ millimeter square) as macroscopic hypertrophy. According to this criterion the macroscopic hypertrophy of the pituitary in the group IV was first observed

Table 3

I GROUP (the mice without surgical procedures and without methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 1	30 days	33.3	0.87	
No. 2	34 days	32.5	1.03	
No. 3	71 days	37.0	1.02	
No. 4	103 days	31.0	0.85	
No. 5	103 days	30.5	1.07	
No. 6	134 days	32.5	0.86	
No. 7	134 days	32.0	0.81	
No. 8	186 days	33.0	1.23	
No. 9	196 days	33.0	1.08	
No. 10	196 days	28.0	0.84	
No. 11	196 days	28.0	0.89	
No. 12	209 days	26.0	1.13	
No. 13	209 days	22.0	1.15	
No. 14	230 days	21.0	0.79	
No. 15	230 days	26.0	0.59	
No. 16	230 days	28.0	0.95	
No. 17	276 days	32.0	0.86	
No. 18	276 days	34.5	0.76	
No. 19	476 days	40.5	1.23	*
No. 20	631 days	31.0	0.63	
No. 21	647 days	25.5	0.61	*
No. 22	647 days	31.0	1.19	*
No. 23	699 days	33.0	0.95	*

* Localized proliferation of chromophobe cells in the lateral area of the anterior pituitary.

Table 4

II GROUP (the mice with castration and without methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 24	26 days	38.0	0.82	
No. 25	30 days	32.5	0.90	
No. 26	48 days	22.0	0.98	
No. 27	68 days	29.0	0.88	
No. 28	96 days	34.0	1.01	
No. 29	125 days	28.5	0.67	
No. 30	171 days	28.5	0.96	
No. 31	259 days	34.0	1.25	
No. 32	259 days	32.0	0.94	
No. 33	332 days †	35.5	0.87	
No. 34	363 days †	37.0	1.23	*
No. 35	538 days	40.0	1.25	*
No. 36	589 days	28.0	0.82	*
No. 37	589 days	29.5	1.02	

† The mouse which expired in an animal-cage.

* Localized proliferation of chromophobe cells in the lateral area of the anterior pituitary.

Table 5

III GROUP (The mice with thyroidectomy and without methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 38	29 days	26.5	0.66	
No. 39	65 days	31.0	0.59	
No. 40	74 days	33.5	0.20	
No. 41	93 days	33.0	0.98	
No. 42	297 days	25.0	0.89	
No. 43	451 days	33.0	0.98	§
No. 44	660 days	25.0	0.68	
No. 45	660 days	24.0	1.05	
No. 46	660 days	31.0	0.65	*
No. 47	706 days	31.0	0.94	*

§ Residual functioning thyroid tissue was verified.

* Localized proliferation of chromophobe cells in the lateral area of the anterior pituitary.

Table 6

IV GROUP (The mice without surgical procedures and with methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 48	30 days	30.0	1.16	
No. 49	60 days	28.0	1.12	
No. 50	62 days	25.0	0.89	
No. 51	89 days	25.0	1.15	
No. 52	106 days	34.0	0.70	§§
No. 53	126 days	38.0	1.03	§§
No. 54	154 days	34.5	1.11	§§
No. 55	191 days	32.5	0.20	§§
No. 56	223 days	29.0	0.71	§§
No. 57	254 days	20.0	0.93	§§
No. 58	254 days	21.0	0.94	§§
No. 59	300 days	26.0	1.46	§§
No. 60	300 days	24.0	0.97	§§
No. 61	308 days	27.0	1.18	§§
No. 62	308 days	29.0	1.20	§§
No. 63	320 days	26.0	1.49	§§
No. 64	340 days	22.0	1.00	§§
No. 65	340 days	34.0	1.34	* §§
No. 66	342 days	33.0	1.11	§§
No. 67	342 days †	27.0	1.72	§§
No. 68	350 days	33.5	1.72	§§
No. 69	381 days	33.0	1.06	● §§
No. 70	546 days	29.0	1.41	● §§
No. 71	563 days	37.0	0.97	● §§

No. 72	587 days	37.5	2.00	* §§
No. 73	638 days †	30.0	0.90	§§
No. 74	638 days	28.0	3.35	● ** §§

† The mouse which expired in an animal-cage.

§§ Hypothyroid goiter was present.

● Exophthalmos was observed distinctly.

* Localized proliferation of chromophobe cells in the lateral area of the anterior pituitary.

** Histological adenoma.

Table 7

V GROUP (The mice with castration and with methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 75	30 days	30.0	0.65	
No. 76	48 days	34.0	0.99	
No. 77	68 days	33.0	1.25	
No. 78	96 days	33.0	0.93	
No. 79	122 days	27.0	0.74	§§
No. 80	155 days	36.0	0.86	§§
No. 81	196 days	26.0	0.89	§§
No. 82	196 days	22.5	1.16	§§
No. 83	201 days	34.0	1.47	§§
No. 84	251 days	25.0	1.20	§§
No. 85	292 days	28.0	1.51	§§
No. 86	305 days	31.0	1.74	§§
No. 87	305 days	28.0	1.76	§§
No. 88	326 days †	28.5	2.30	§§
No. 89	326 days	32.0	1.05	§§
No. 90	332 days	21.0	2.75	§§
No. 91	332 days	31.0	2.74	* §§
No. 92	368 days	30.5	1.09	§§
No. 93	497 days †	35.0	4.35	** §§
No. 94	521 days	33.0	0.91	● §§
No. 95	521 days	31.0	1.14	● * §§
No. 96	538 days	31.0	2.52	** §§
No. 97	558 days	27.0	1.71	● * §§
No. 98	562 days	26.0	1.04	§§
No. 99	567 days †	30.0	1.74	● §§
No. 100	570 days †	26.0	0.82	● * §§
No. 101	573 days	37.5	0.76	● * §§
No. 102	573 days †	28.0	3.82	** §§
No. 103	573 days	25.0	1.74	** §§
No. 104	585 days †	26.0	1.23	* §§
No. 105	589 days †	27.0	1.07	* §§

† The mouse which expired in an animal-cage.

§§ Hypothyroid goiter was present.

● Exophthalmos was observed distinctly.

* Localized proliferation of chromophobe cells in the lateral area of the anterior pituitary.

** Histological adenoma.

Table 8

VI GROUP (the mice with thyroidectomy and with methylthiouracil administration)

	Interval between the onset of experiment and the autopsy	Body weight (gramme)	Widest frontal dimension of the anterior pituitary (millimeter square)	Remarks
No. 106	31 days	31.0	0.75	
No. 107	65 days	27.5	0.72	
No. 108	89 days	24.0	1.22	
No. 109	89 days	24.5	1.14	
No. 110	90 days	28.5	1.34	
No. 111	122 days	36.0	1.27	§§
No. 112	139 days	20.5	1.44	
No. 113	139 days	28.5	1.45	§§
No. 114	159 days	33.5	1.12	
No. 115	180 days	23.0	1.87	§§
No. 116	235 days	24.0	1.42	
No. 117	243 days	19.0	1.95	
No. 118	276 days	29.0	3.38	§§
No. 119	276 days	23.0	0.98	§§
No. 120	283 days	29.0	2.28	
No. 121	289 days	19.0	2.12	§§
No. 122	289 days	23.0	1.84	§§
No. 123	338 days †	27.0	1.72	§§
No. 124	481 days †	29.0	3.60	● ** §§
No. 125	498 days †	24.0	2.43	● §§
No. 126	503 days	25.0	1.87	** §§

† The mouse which expired in an animal-cage.

§§ Goiter developed from the residual thyroid tissue.

● Exophthalmos was observed distinctly.

** Histological adenoma.

Table 9

I GROUP (The mice without surgical procedures and without methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 2	34 days	62.2 %	3.6%	34.2 %
No. 7	134 days	53.4 %	4.3%	42.3 %
No. 19	476 days	37.8 %	4.0%	58.2 %
No. 23	699 days	10.5 %	2.0%	87.5 %

on the 320th day after the onset of the experiment, and the gross tumor on the 638th day (Table 24).

(ii) Histological change: As will be mentioned later, a localized proliferation of the same type of cells over 1 millimeter in diameter should be called histological adenoma (microtumor). The microtumor was first recognized on the 638th day of the experiment. Before the development of the adenoma, three stages were recognized histologically.

I stage (stage of proliferation and hypertrophy)

Two or three weeks after the beginning of the methylthiouracil administration, basophilic cells in the anterior lobe gained in number as well as in size. Besides the typical fine basophilic granules, which were easily stainable with anilin blue or light green, gross orangophil granules with positive PAS reaction became evident within the cytoplasm of the basophil cells which many investigators called thyroidectomy cells (Figs. 11 and 12). However the orangophil gross granules could not be found in true thyroidectomy cells of the thyroidectomized mice which were not treated with methylthiouracil (group III). GRIESBACH²³⁾ stated that the peculiar orangophil (therefore acidophil-like) cells observed in the thiouracil groups were not acidophil in nature. Fine proper basophil granules in the cytoplasm illustrated in Figs. 11 and 12 should be noticed! About one year after the onset of the experiment the cells became more and more extraordinary in size. The basophilic granules disappeared completely, and the cytoplasm was filled with the gross orangophil granules. The cells in this period simulated acidophil cells.

II stage (stage of disintegration and reconstruction).

The anterior pituitary gained macroscopically in size, and the cytoplasm of the basophil cells began to swell and disintegrate histologically. Then the orangophil gross granules became scattered between the cells (Fig. 13). On the other hand a number of regenerated chromophobe cells appeared, which also took the same course sooner or later, namely from proliferation and hypertrophy to disintegration. Such circles took place repeatedly and the regenerative process became more and more intense. In addition to the peculiar basophil cells in the cycle, basophil cells of another type could be observed, whose granules were PAS reaction positive, and stainable with aldehyde fuchsin (Fig. 14). The shape and size of the cells were not altered, however they gained somewhat in number during this stage. Proper eosinophilic cells of the anterior pituitary and those of the intermediate lobe did not change.

III stage (stage of adenoma formation).

About two years after the beginning of the experiment, a number of chromophobe cells began to proliferate in the lateral part of the anterior pituitary, forming a nodule, which was distinguishable even macroscopically from the neighboring relatively normal area. This nodule gained in size progressively and finally developed into a histological adenoma (microadenoma or microtumor), the definition of which will be given later in the chapter of discussion. The adenoma was histologically composed of two types of cells. One was a round or oval cell similar to a chromophobe cell, although the cytoplasm of the cell was larger and the boundary was less distinct (Figs. 15 and 16). It was remarkable that PAS positive fine granules scarcely stainable with aldehyde fuchsin were discovered in the chromophobe-like cytoplasm (Fig. 17). The other cell was basophilic, polyhedral shaped, and connected with each other forming a network (Fig. 18). These cells were smaller in size and less in number than the former cells. The granules of the cytoplasm were PAS positive and with aldehyde fuchsin easily stainable.

The microtumors in this group are given in Figs. 5 and 8.

(iii) The anterior pituitary cell distribution of the mice of the IV group: Influences of the long term feeding of methylthiouracil are shown in Table 12. Comparing it with Tables 9, 10 and 11 (group I, II and III), increased percentage of the basophil cells is notable.

Table 10

II. GROUP (The mice with castration and without methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 24	26 days	48.4 %	6.7%	44.9 %
No. 28	96 days	48.4 %	8.6%	43.0 %
No. 35	538 days	55.8 %	1.2%	43.0 %

Table 11

III GROUP (The mice with thyroidectomy and without methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 38	29 days	48.6 %	5.2 %	46.2 %
No. 41	93 days	56.5 %	7.4 %	36.1 %
No. 43	451 days	26.3 %	15.0 %	58.7 %
No. 46	660 days	37.8 %	3.9 %	58.3 %

Table 12

IV GROUP (The mice without surgical procedures and with methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 48	30 days	42.9 %	5.2 %	51.9 %
No. 53	126 days	25.8 %	14.0 %	60.2 %
No. 70	546 days	52.0 %	16.7 %	31.3 %
No. 71	563 days	39.6 %	16.5 %	43.9 %
No. 72	587 days	29.1 %	55.4 %	15.5 %
No. 74	638 days	Histological adenoma		

(4) Changes of the anterior pituitary in the mice castrated and fed the methylthiouracil diet (group V)

(i) Change in size: Macroscopic hypertrophy of the pituitary in this group was first recognized on the 292nd day, while in the group IV on the 320th day. In the mice castrated and fed the methylthiouracil diet, macroscopic hypertrophies

Table 13

	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were observed	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were not observed
IV GROUP, the mice without surgical procedures and with methylthiouracil administration	5	11
V GROUP, the mice with castration and with methylthiouracil administration	12	9

$$\chi^2=1.52 \quad t=1.24 \quad P=\frac{1}{2}-F(t)=0.11$$

of the pituitaries were observed in 57 per cent (12 out of 21), and in the mice fed the diet and treated not surgically, in 31 per cent (5 out of 16) of the animals sacrificed after the 292nd day. The rate of the former seems to be greater than that of the latter, however by χ^2 test it is not significant that the castration promotes the development of pituitary hypertrophy (Table 13).

The macroscopic adenoma (gross tumor) in the group V was first recognized on the 497th day, while in the group IV on the 638th day. In the mice of the group V the gross tumors were disclosed in 15 per cent (2 out of 13) and in the mice of the group IV in 20 per cent (1 out of 5) of the animals autopsied after the 497th day. By χ^2 test the facilitating effect of castration on the tumorigenesis proved to be not significant (Table 14).

Table 14

	Number of the mice in which gross tumors of the anterior pituitaries were observed	Number of the mice in which gross tumors of the anterior pituitaries were not observed
IV GROUP, the mice without surgical pocedures and with methylthiouracil administration	1	4
V GROUP, the mice with castration and with methylthiouracil administration	2	11

$\chi^2=0.22$ $t=0.468$ $P=\frac{1}{2}-F(t)=0.32$

(ii) Histological findings: Histological changes of the anterior lobe in the mice castrated and fed the methylthiouracil diet were essentially identical to those in the mice of the group IV, however the changes in the former appeared to develop more frequently and in the earlier stage than in the latter. The micro-tumor in the group V was first observed on the 497th day, whereas in the group IV on the 638th day. In the mice of the former group the microtumors were discovered in 31 per cent (4 out of 13), and in the latter group in 20 per cent (1 out of 5) of the animals sacrificed after the 497th day. By χ^2 test it is proved to be not significant that the rate is greater in the former group than in the latter (Table 15).

The microtumors in this group are given in Figs. 6 and 9.

Table 15

	Number of the mice in which microtumors of the anterior pituitaries were observed	Number of the mice in which microtumors of the anterior pituitaries were not observed
IV GROUP, the mice without surgical procedures and with methylthiouracil administration	1	4
V GROUP, the mice with castration and with methylthiouracil administration	4	9

$\chi^2=0.017$ $t=0.131$ $P=\frac{1}{2}-F(t)=0.45$

What is called castration cell was similar to the peculiar pituitary cell developed by feeding of the methylthiouracil diet, and nearly indistinguishable from each other in the early stage. However in the late stage the orangophil gross granules could be observed in the methylthiouracil cell, but not in the castration cell. In the mice castrated and fed the diet without the drug, macroscopic hypertrophy and gross adenoma as well as microadenoma could not be found, although small localized proliferation of the chromophobe cells, which was not yet to be named microtumor, was observed in the lateral area of the anterior pituitary in some senile mice.

(iii) The anterior pituitary cell distribution of the mice of the V group: Comparing Table 16 with Tables 9, 10 and 12, basophil cell ratio tends to increase with age in the IV and V group, and more remarkably in the latter than in the former, while eosinophil cell ratio decreases more evidently in the former.

Table 16
V GROUP (The mice with castration and with methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 75	30 days	33.4 %	12.4 %	54.2 %
No. 78	96 days	68.0 %	29.7 %	12.3 %
No. 93	497 days	Histological adenoma		
No. 94	521 days	39.0 %	18.1 %	42.9 %
No. 95	521 days	32.3 %	19.0 %	48.7 %
No. 96	558 days	Histological adenoma		
No. 97	558 days	21.4 %	17.6 %	61.0 %
No. 99	567 days	52.2 %	26.9 %	20.9 %
No. 102	573 days	Histological adenoma		
No. 103	573 days	Histological adenoma		

(5) Changes of the anterior pituitary in the mice thyroidectomized and fed the methylthiouracil diet (group VI)

(i) Change in size: The macroscopic hypertrophy in this group was first recognized on the 180th day, earliest among the six groups, while in the IV group on the 320th day. In the mice of the VI group the macroscopic hypertrophies of

Table 17

	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were observed	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were not observed
IV GROUP, the mice without surgical procedures and with methylthiouracil administration	5	15
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	10	2

$$\chi^2 = 9.6 \quad t = 3.10 \quad P = \frac{1}{2} - F(t) = 0.00097$$

the anterior pituitaries were observed in 83 per cent (10 out of 12) and in the mice of the IV group (thiouracil animals with no operation) in 25 per cent (5 out of 20) of the animals sacrificed after the 180th day. The rate of the former seems to be greater than that of the latter, and in χ^2 test it is significant that the thyroidectomy facilitates the development of pituitary hypertrophy (Table 17).

The gross tumor in the group VI was first recognized on the 276th day, while in the IV group on the 638th day. In the mice of the former group the gross tumor were disclosed in 22 per cent (2 out of 9) and in the mice of the latter group in 6 per cent (1 out of 16) of the animals autopsied after the 267th day. χ^2 test discloses that the promoting effect of thyroidectomy on the gross adenoma development is not significant (Table 18).

Table 18

	Number of the mice in which gross tumors of the anterior pituitaries were observed	Number of the mice in which gross tumors of the anterior pituitaries were not observed
IV GROUP, the mice without surgical procedures and with methylthiouracil administration	1	15
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	2	7

$$\chi^2 = 0.29 \quad t = 0.539 \quad P = \frac{1}{2} - F(t) = 0.30$$

(ii) Histological findings: Histological changes of the anterior pituitary in the mice thyroidectomized and fed the methylthiouracil were essentially identical with those in the mice fed the diet and treated not surgically, however the changes in the former appeared to develop more frequently and in the earlier stage than in the latter.

The microtumors in this group are given in Figs. 7 and 10. They were disclosed in 67 per cent (2 out of 3), and in the group IV in 20 per cent (1 out of 5) of the animals sacrificed after the 481st day. By χ^2 test it is proved to be not significant that the ratio is higher in the former group than in the latter (Table 19).

Table 19

	Number of the mice in which microtumors of the anterior pituitaries were observed	Number of the mice in which microtumors of the anterior pituitaries were not observed
IV GROUP, the mice without surgical procedures and with methylthiouracil administration	1	4
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	2	1

$$\chi^2 = 0.32 \quad t = 0.565 \quad P = \frac{1}{2} - F(t) = 0.39$$

What is called thyroidectomy cell was similar to the peculiar pituitary cell developed by feeding of the methylthiouracil diet, and nearly indistinguishable from

each other in the early stage, however in the late stage the orangophil gross granules could be observed in the methylthiouracil cell, but not in the thyroidectomy cell. In the mice thyroidectomized and fed the diet without the drug, macroscopic hypertrophy and gross adenoma as well as microtumor could not be found, although localized proliferation of the chromophobe cells, which could not yet be named microtumor, was observed in the lateral area of the anterior pituitary in some aged mice.

(iii) The anterior pituitary cell distribution of the mice of the group VI: Comparing Table 20 with Tables 9, 10, 11 and 12, the cell ratios of the group VI are nearly the same as those of the group IV and V (Table 20).

Table 20

VI GROUP (the mice with thyroidectomy and with methylthiouracil administration)

	Age when autopsied	Eosinophilic cell	Basophilic cell	Chromophobe cell
No. 106	31 days	34.4 %	8.4 %	57.2 %
No. 111	122 days	58.0 %	12.2 %	29.8 %
No. 124	481 days	Histological adenoma		
No. 126	503 days	Histological adenoma		

(6) Comparison between the group V and VI (the methylthiouracil mice castrated and those thyroidectomized)

Both castration and thyroidectomy seemed somewhat to enhance the tumorigenic action of the drug. Which is more facilitative, castration or thyroidectomy? The macroscopic hypertrophy in the group V was first recognized on the 292nd day, while in the VI group on the 180th day. In the former group the hypertrophies were observed in 48 per cent (12 out of 25) and in the latter group 83 per cent (10 out of 12) of the mice killed after the 180th day. By χ^2 test it is significant at the probability of risk below 10 per cent that thyroidectomy is more accelerative than castration (Table 21).

Table 21

	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were observed	Number of the mice in which macroscopic hypertrophies of the anterior pituitaries were not observed
V GROUP, the mice with castration and with methylthiouracil administration	12	23
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	10	2

$$\chi^2 = 2.86 \quad t = 1.69 \quad P = \frac{1}{2} - F(t) = 0.051$$

The gross tumor in the group V was first disclosed on the 497th day, while in the group VI on the 276th day. In the former group the gross tumor were observed in 10 per cent (2 out of 21), and in the latter 22 per cent (2 out of 9) of the mice autopsied after the 276th day. The ratio in the latter group seems

to be greater than that of the former. By χ^2 test, however, it is not significant that thyroidectomy promotes more strikingly the gross adenoma development than castration (Table 22).

Table 22

	Number of the mice in which gross tumors of the anterior pituitaries were observed	Number of the mice in which gross tumors of the anterior pituitaries were not observed
V GROUP, the mice with castration and with methylthiouracil administration	2	19
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	2	7

$$\chi^2 = 0.12 \quad t = 0.346 \quad P = 1/2 - F(t) = 0.37$$

In the group V the microtumor was first revealed on the 497th day whereas in the group VI on the 481st day. These microadenomas developed in 31 per cent (4 out of 13) of the mice in the former group, and 67 per cent (2 out of 3) of the mice in the latter, which were autopsied after the 481st day. By χ^2 test it is not significant that thyroidectomy has a more facilitating action on the development of the gross tumor than castration (Table 23).

In the pituitary cell distribution there were no distinct differences between both groups (Tables 16 and 20).

Table 23

	Number of the mice in which microtumors of the anterior pituitaries were observed	Number of the mice in which microtumors of the anterior pituitaries were not observed
V GROUP, the mice with castration and with methylthiouracil administration	4	9
VI GROUP, the mice with thyroidectomy and with methylthiouracil administration	2	1

$$\chi^2 = 0.256 \quad t = 0.495 \quad P = 1/2 - F(t) = 0.31$$

DISCUSSION

(1) Definition of the adenoma

Criteria to define the experimental pituitary adenoma are still obscure. Some investigators⁸⁾³⁴⁾⁵⁷⁾ define as a tumor every pituitary enlargement, but some others¹⁶⁾¹⁷⁾¹⁸⁾⁵¹⁾ only the enlargement more than several times the normal weight. Among the tumors thus defined, simple proliferation of pituitary cells may be included. Though a nodule composed of the cells of a single type is called adenoma, the localized cell proliferations observed in the lateral parts of the anterior lobes in normal old mice (as illustrated in Fig. 4) may not be considered as adenomas, because it is

necessary to be called adenomas that the noduli of proliferated cells have the size over a certain degree, even in case they are microscopic noduli of the cells of a single type. In this study the microtumor is defined as a localized cell proliferation forming a group or nodulus over one millimeter in diameter composed of pituitary cells of a single type, which is distinguished sharply from the surrounding tissue.

(2) Histogenesis of the adenoma cells

Since the experimental adenoma developed in the lateral part of the anterior pituitary, where the chromophobe cell proliferation was observed also in normal senile mice, the chromophobe cell is regarded as the origin of the experimental adenoma. However precise histological observation of the tumor cells revealed that the adenoma was composed of two types of cells. The cells of the first type were chromophobe-like ones, which however had delicate granules with positive PAS reaction. These cells seem to be developing from stock cells (die Stammzellen) into basophil cells, and the granules were extremely similar to those of the δ cells²³⁾²⁴⁾²⁵⁾ (HALMI), which have been thought by many workers⁴¹⁾⁴²⁾ to be FSH secreting ones. The cells of the second type were smaller in number and contained basophilic granules in the cytoplasm which were PAS reaction positive and with aldehyde fuchsin stainable. These cells would be identical with the β cells²³⁾²⁴⁾²⁵⁾ (HALMI and GRIESBACH), which many investigators⁴¹⁾⁴²⁾ have considered as the source of TSH. From these histological findings all the cells forming the experimental adenoma are thought to be basophilic in origin.

Table 24

		IV GROUP	V GROUP	VI GROUP	INFERENCES
Surgical procedure		None	Castration	Thyroid-ectomy	By χ^2 test
Administration of methylthiouracil		With methylthiouracil	With methylthiouracil	With methylthiouracil	
Shortest intervening period of time from the onset of the experiment to the development of macroscopic hypertrophy		320 days	292 days	180 days	
Shortest intervening period of time from the onset of the experiment to the development of gross tumor		638 days	497 days	276 days	
Shortest intervening period of time from the onset of the experiment to the development of microtumor		638 days	497 days	481 days	
Frequency of the macroscopic hypertrophies	among the mice examined after the 180th day	25%	48%	83%	$IV < VI$ * $V < VI$ * $IV < V$ **
	among the mice examined after the 292nd day	31%	57%		
Frequency of the gross tumors	among the mice examined after the 276th day	6%	10%	22%	$IV < VI$ ** $V < VI$ ** $IV > V$ **
	among the mice examined after the 497th day	20%	15%		
Frequency of the microtumors	among the mice examined after the 481st day	20%	31%	67%	$IV < VI$ ** $V < VI$ ** $IV < V$ **

* Significant, because the probability of risk (P) is below 10 per cent.

** Not significant, because P is not below 10 per cent.

(3) Goiter and exophthalmos

Goiter and exophthalmos produced by the long term administration of methylthiouracil seemed to result from hypersecretion of thyroid stimulating hormone (TSH) in the anterior pituitary. Thiouracil derivatives are said to bind easily iodine and produce inactive iodine compounds in vivo, so that the experimental animals will fall in the condition of relative lack of iodine, and the thyroxine synthesis may be derailed⁽⁷⁾⁽³⁹⁾. As a biological response to it, the hypersecretion of TSH in the anterior pituitary may result, and then the thyroid gland begins to enlarge. The second action of TSH is the development of exophthalmos, which was discovered by LOED and ARON.⁽¹²⁾⁽¹³⁾⁽³²⁾⁽⁵³⁾ In this study hypothyroid exophthalmic goiter resulted from the feeding of methylthiouracil diet. It is assumed from the experimental data that TSH is secreted from the aforesaid basophil cells composing the adenoma.

(4) The facilitating effect of castration and thyroidectomy on the tumorigenesis due to methylthiouracil.

The adenoma production in the thyroidectomized thiouracil-animal group, are compared with that in the castrated group (Table 24). Notwithstanding the minority of the number of animals used in the present study, it may be concluded that both thyroidectomy and castration accelerate the tumor development, and the former is more promoting than the latter. It is not surprising that the thyroidectomy promotes the adenoma formation, because the secretion of TSH may be raised by the procedure. If gonadotrophin (FSH) and TSH secreting pituitary cells are different from each other (the δ cell and β cell), as some investigators⁽²³⁾⁽⁴¹⁾⁽⁴²⁾ suggest, it might be difficult to explain that castration also facilitates the adenoma production. In this connection the following data of FURTH⁽¹⁴⁾ is of much interest. In 1951 he proved that a pituitary adenoma experimentally produced in a mouse by the destruction of thyroid gland tissue with I^{131} secreted TSH as well as FSH.

SUMMARY AND CONCLUSIONS

(1) Localized chromophobe cell proliferations are frequently met with in the lateral portions of the anterior pituitary lobes of old mice, though they could not be considered as hypophyseal adenomas.

(2) A number of male dd strain mice, 4 to 6 weeks old, were fed 0.5 per cent methylthiouracil diet and sacrificed at various intervals of time. In the anterior lobe, proliferation and enlargement of basophil cells developed after several months. The cells began to be disintegrated gradually, at the same time reconstructive process took place. After two years treatment chromophobe-like adenomas could be found macroscopically and histologically in the anterior pituitaries in some mice.

(3) Many workers have regarded the experimental adenoma as chromophobe, however by precise observation two types of cells were discriminated in the tumor. One was round or oval and similar to chromophobe cell, except that the cytoplasm was larger and the cell boundary was less distinct. It is remarkable that fine

granules with positive PAS reaction and scarcely stainable with aldehyde fuchsin were discovered in the chromophobe-like cytoplasm. The cell seems to be δ cell (HALMI) developed from the stock cell. The other was basophilic, shaped polyhedral and connected with each other, forming a network. The latter was smaller in size and less in number than the former. The granules in the cytoplasm were of positive PAS reaction and easily stainable with aldehyde fuchsin. This cell seems to be identical with β cell (HALMI). It is likely that these two types of cells forming the experimental adenoma are both basophilic in origin.

(4) Castration as well as thyroidectomy promotes the development of the thiouracil-adenoma, shortens the intervening period from the beginning of experiment to the adenoma formation and increases the frequency of the tumor production, however thyroidectomy is more facilitative than castration.

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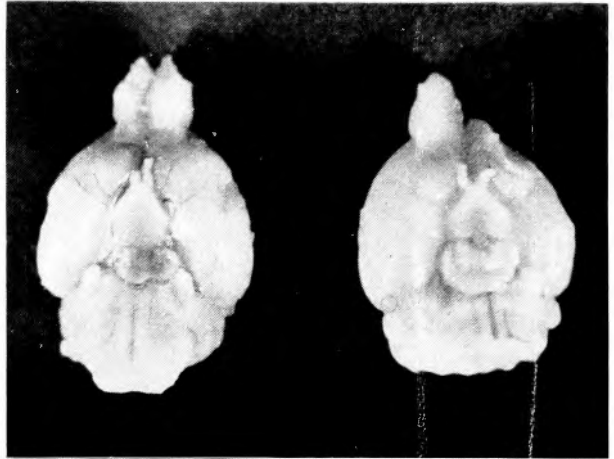
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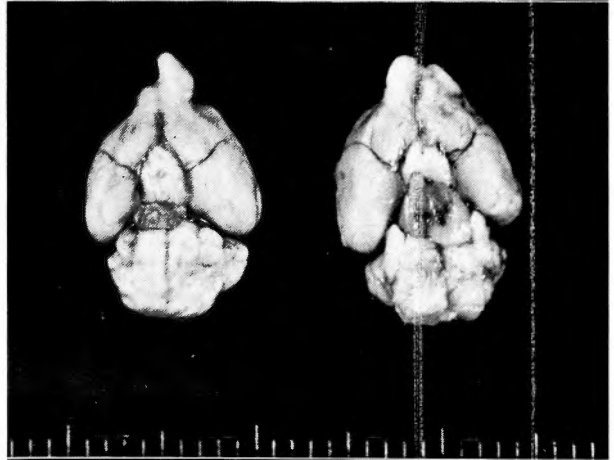
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Fig. 5

Right : No. 74 (IV Group)
Adenoma.
Left : No. 22 (I Group)
Control.

**Fig. 6**

Right : No. 102 (V Group)
Adenoma.
Left : No. 20 (I Group)
Control.

**Fig. 7**

Right : No. 124 (VI Group)
Adenoma.
Left : No. 43 (III Group)
Control.



Macroscopic pituitary adenomas, as seen from the base of brain, (after fixed in Halmi-Bouin solution and rinsed in water). The minimal gradation of scale indicates one millimeter.

IV GROUP

The mice without surgical procedures and with methylthiouracil administration.

No. 23

Control.



No. 74



Fig. 8 Histological adenoma in a mouse of the group IV.

V GROUP

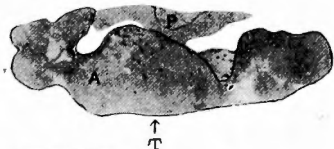
The mice with castration and with methylthiouracil administration.

No. 35

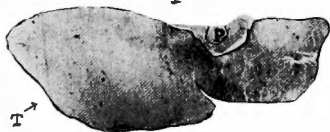
Control, with castration only.



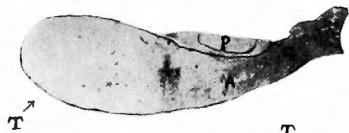
No. 93



No. 96



No. 102



No. 103



Fig. 9 Histological adenomas in mice of the group V.

VI GROUP

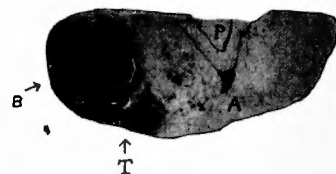
The mice with thyroidectomy and with methylthiouracil administration.

No. 43

Control, with thyroidectomy only.



No. 124



No. 126

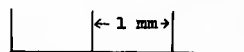


Fig. 10 Histological adenomas in mice of the group VI.

Microphotograph showing the widest frontal section of the adenomatous pituitary, (paraffin section).

A : Anterior Lobe.

P : Posterior Lobe.

T : Histological Adenoma.

B : Bleeding within the adenoma.

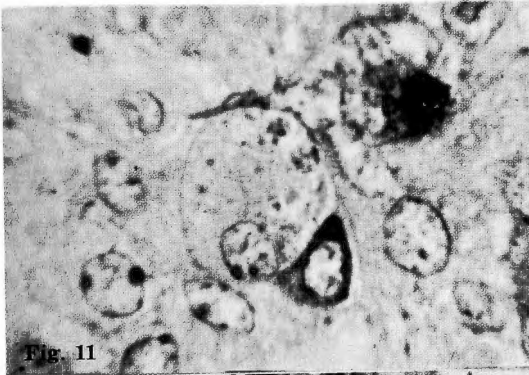


Fig. 11



Fig. 12

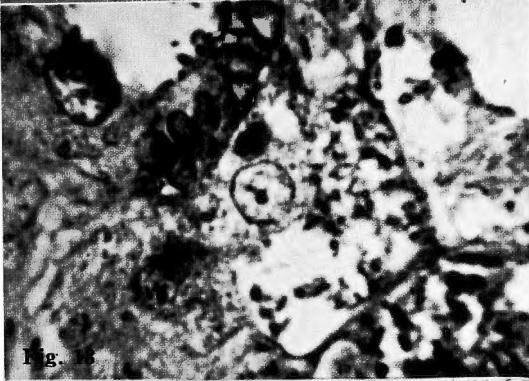


Fig. 13

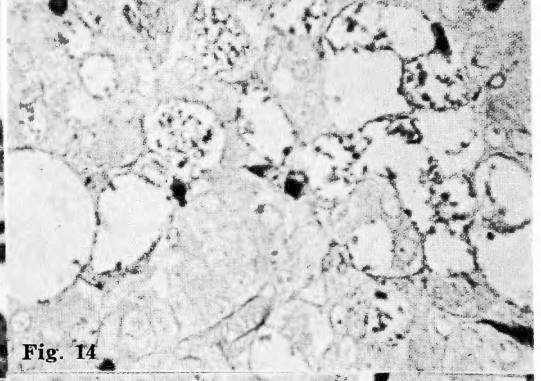


Fig. 14

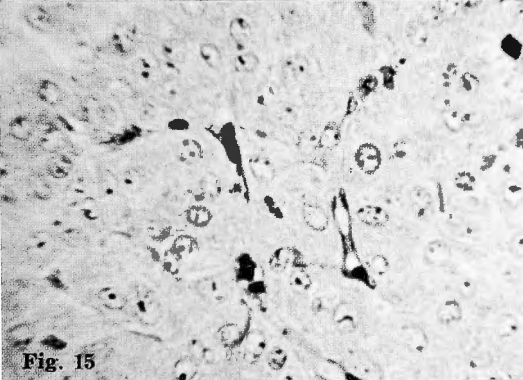


Fig. 15

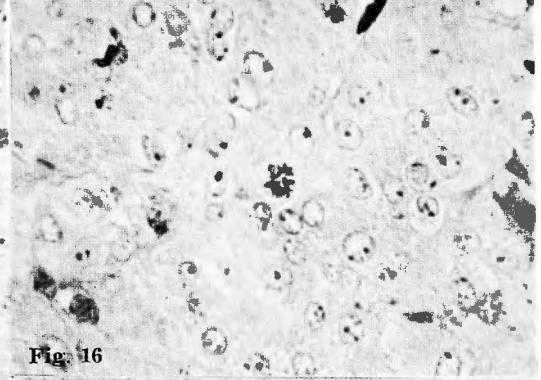


Fig. 16

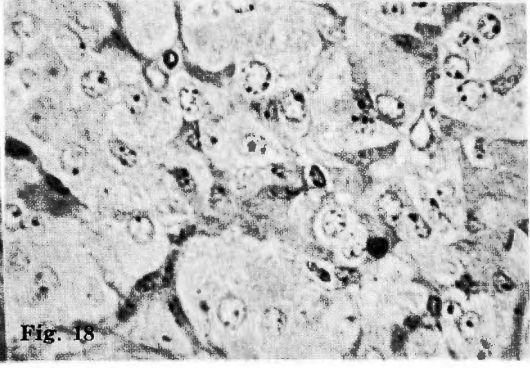
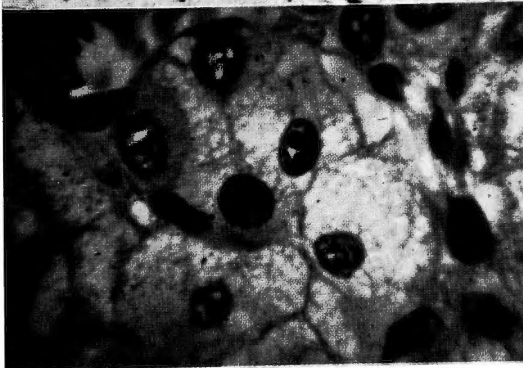


Fig. 18

- Fig. 11** The basophil cell in the early stage of hypertrophy and proliferation, azan stain, $\times 100 \times 15$.
- Fig. 12** The basophil cell in the late stage of hypertrophy and proliferation, azan stain, $\times 100 \times 15$.
- Fig. 13** Orangophil granules scattered in the cellular interspace, azan stain, $\times 100 \times 15$.
- Fig. 14** The orangophil granules in low power magnification, aldehyde fuchsin azan stain, $\times 40 \times 15$.
- Fig. 15** The adenoma cells of the first type, azan stain, $\times 40 \times 15$.
- Fig. 16** The adenoma cells of the first type, aldehyde fuchsin azan stain, $\times 40 \times 15$.
- Fig. 17** Fine PAS-reaction-positive granules (fine red granules) observed in the adenoma cells of the first type, PAS reaction, $\times 100 \times 10$.
- Fig. 18** The adenoma cells of the second type, aldehyde fuchsin azan stain, $\times 40 \times 15$.

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(*Written in Japanese.)

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メチールサイオユラシール長期投与による 実験的脳下垂体腺腫

特に去勢或は甲状腺切除の影響に関して

三重県立大学第1外科教室 (主任: 山本俊介教授)

鏡 友 雄

メチールサイオユラシールを飼料の中に0.5%の割合に混入して雄 dd 系マウスを飼育することにより、約2ケ年の後脳下垂体前葉の側方部に腺腫様変化が見られた。此は肉眼的にも、組織学的にも腺腫といえるものであつた。

老令のマウスに於ても脳下垂体前葉の側方部にクロモフオーブ細胞の限局的増加が高率に見られたが、此の様な変化は未だ腺腫といえる程度ではなかつた。

サイオユラシール誘導体投与により発生する腺腫は従来の報告によれば何れもクロモフオーブ腺腫と記載されているが、精く観察することにより此の腺腫は2種類の構成要素から成立つていた。主たる構成要素の一見クロモフオーブ様の細胞は細胞質内に微細なPAS反応陽性でアルデヒドフクシン染色性陰性の顆粒を有することより、基本細胞よりデルタ細胞へ発展する

過程の一段階にあるものと考えられる。他の構成要素たる細胞は前者に比して小さく数も少く互に融合接続して網状構造をなす多角形の細胞で、細胞質内にPAS反応陽性アルデヒドフクシン染色性陽性の物質を有することより、ベータ細胞に属すべきものと考えられる。以上の如く此の腺腫を構成する細胞要素は何れもパゾフィール細胞の系統に属すべきものと考えられる。

メチールサイオユラシール投与と去勢或はメチールサイオユラシール投与と甲状腺切除を併せ行うことにより、メチールサイオユラシール投与のみの場合よりも脳下垂体腺腫発生迄に要する日数を短縮し且つ発生率を上昇させることが出来た。此の促進作用は去勢よりも甲状腺切除の方が大であつた。