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EXPERIMENTAL STUDIES ON THE RELATIONSHIP BETWEEN THE MAMMARY TUMORS AND THE ANTERIOR LOBE OF THE PITUITARY GLAND

by

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I. INTRODUCTION

A considerable number of the reports concerning the relation between mammary tumors and sexual hormones have been published. These reports consist of the following facts: 1) The development of tumors in mammary gland after experimental medication of estrogenic hormones 2) The observation of something unusual at measurement of sexual hormones in urine and blood of patients of mammary tumors 3) The acquisition of some effects after therapeutic extirpation of ovary, suprarenal or pituitary glands and after medication of sexual hormone preparations; and so on. Moreover, the recent statistics of our clinic show that the number of the patients of mammary tumors, especially mammary cancer and mastopathy has become more numerous, compared with that before World War II, and that the age of the patients has been also younger. The fact that the number of working women has been increased after the war and their sexual environment has been changed, seems to play an important rôle. ISEDA and NISHIYA in our institute, have already published it in detail in this journal, proving the imbalance of discharging values of sexual hormones in urine of the patients of mammary tumors.

From this point of view, KOSHI in our laboratory, giving mice various kinds of sexual hormones, observed changes of mammary glands, and furthermore practiced KOGURE's examination, in order to give variety to thier sexual environment. At each occasion of several times of their pregnancy and delivery, he forced them to wean in five days or immediately and made mammary tumors, which were compared with each other.

At my experiment, in these mice with mammary tumors, the changes of anterior lobe of the pituitary gland were investigated, the differences from the findings of pituitary gland at the cases of transplantation of transplantable mouse-cancer were studied.

II. CHANGES OF ANTERIOR LOBE OF PITUITARY GLAND IN MICE WITH MAMMARY TUMORS

Experimental Method

As the experimental animals, the hybrid mice of German strain were used. Each uterine couple cohabitted. After fertilisation, the males were isolated till delivery. And after the period of 30 days from delivery, they cohabitted again with the females. Thus, pregnancy and delivery were repeated. These experimental animals were classified into the following 3 groups, according to their lactating ways. 1) The group, which lactated till natural weaning after delivery. 2) The group, which did never lactate almost entirely after every delivery. 3) The group, which lactated only during 5 days after every delivery and afterwards did never lactate. The cases, in which mammary cancer and mastopathy-like changes developed, were the most numerous in the third group, and were somewhat less in the second group than in it, but further numerous, compared with those in the first group of normal lactation (according to KOSHI in our institute). About the anterior lobe of pituitary gland of the mice, in which mammary cancer or mastopathy-like changes developed during the experiment, the percentage of gland cells and their morphological findings were examined. As the control, the healthy female mice of same age to the ones with mammary cancer and mastopathy-like changes, were used.

The pituitary gland was fixed for 2 hours in BOUIN's solution, applying 0.5% trichloroacetic acid, instead of 0.5% acetic acid. For prevention of its destruction, the gland was fixed with the bone, as placed on Turkish saddle, and was embedded. From the tissues embedded with paraffine, 3～4μ continual sections were made on horizontal cut surface. The staining was ROMEIS's Cresazan method, GOMORI's Aldehydefuchsin method and Haematoxylin-eosin staining.

In each pituitary gland, the percentage of 3 kinds of gland cells, namely acidophile (α), basophile (β) and chromophobe (γ) cells, was calculated by INOUE's method, modified from RASSMUSSEN & HERRICK's method (Fig. 1). Among the continual sections the ones of almost middle position were selected and under oil immersion, in each visual field of them about 30 cells were calculated and differentiated, extending throughout whole fields. With this method, the visual fields were about 70 and all the calculated number of cells reached to some two thousand. The cells of circumferential regions were mostly altered in staining, due to the fixation, embedding and others, and were difficult to differentiate. Then they were excepted.

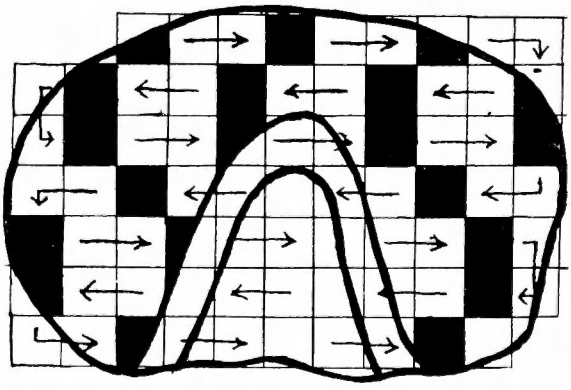


Fig. 1. The calculation method of the pituitary gland cells of mice by Inoue.
■ : Microscopic field to be counted
□ : Microscopic field not to be counted

Experimental Results

i) Weight of Pituitary Gland

The pituitary gland of normal mice is extremely small and is about 1mg in most cases. Sometimes its weighing errors are apt to be found, and its value is unreliable. In the present experiment, however, the measured value in 8 normal mice which were arbitrarily selected after about 400 days from their birth, was 0.9mg in average, and the average value pro 10g body weight was 0.35mg/10g (body weight).

The average weight of pituitary gland of the 8 mice with mammary cancer was 1.2mg, and the average weight pro 10g body weight was 0.38mg/10g (body weight). A slight hypertrophy was observable (Table 1).

Table 1. Weight of Pituitary Gland

	Normal Mice		Mice with Mammary Cancer	
No.	Body Weight	Weight of Pituitary	Body Weight	Weight of Pituitary
1.	27g.	1.0mg.	34g.	1.5mg.
2.	24	0.9	27	0.9
3.	25	0.8	28	0.9
4.	25	0.7	35	1.5
5.	27	1.0	28	1.0
6.	26	0.7	34	1.5
7.	26	0.9	32	1.2
8.	28	1.2	27	0.8
average	26.0g.	0.9mg.	30.7g.	1.2mg.

ii) Histological Findings of Anterior Lobe of Pituitary Gland

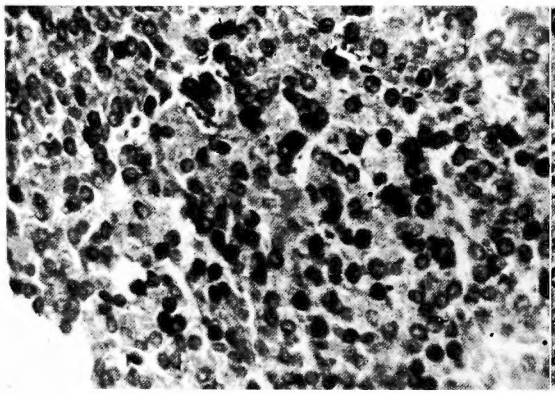
(1) Normal Case.

In anterior lobe of pituitary of mice, several gland cells are surrounded by interstitial tissues and form a cell fasciculus, and interfascicular spaces are the so called Sinusoid. According to GOMORI's staining method, the gland cells can be classified into 4 main kinds as follows: a) The α cells which contain azocarmin stainable granules in their protoplasm, b) the β cells which contain purple stained granules by aldehyde-fuchsin, c) the δ cells which are green stained by light green, d) the γ cells which are unstained by any coloring matter. The δ cell is considered a kind of the changed forms of β cell. And both the kinds cannot always be differentially colored. Then, both of them were calculated together as β cells in the present experiment.

The nucleus of these gland cells was generally large, compared with each cell body, and chromatin was scanty in it. Therefore, the protoplasm seemed to be narrow and most of nuclei were light seen. Among various kinds of gland cells, any remarkable difference of nucleus was not observable.

The α and γ cells were the greater part of all gland cells, and were found in every visual field, but the β cells were in a small number and were apt to gather together near the border region between the pars intermedia and the fore part of pituitary gland. But they also lay scattered in the other regions (Fig. 2, 3).

The average value of percentage of these 3 kinds of gland cells in 10 normal



Eig, 2. The anterior lobe of pituitary in a normal female mouse. Typical three kinds of cells are seen. ×400

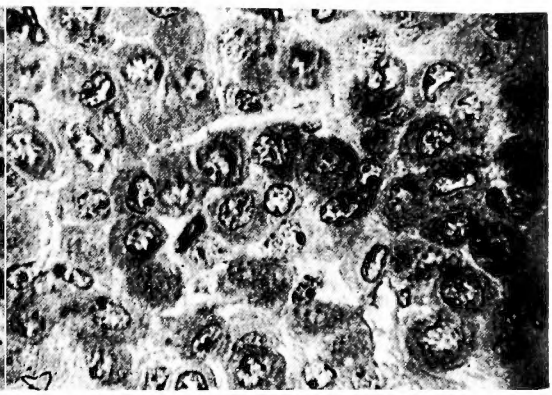


Fig. 3. The same above. ×1.000

Table 2. Percentage of Three Cell Types in Pituitary Glands of Normal Female Mice

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
1.	4.1	48.1	47.8
2.	4.3	52.8	42.9
3.	5.3	52.0	42.7
4.	6.0	54.8	39.2
5.	5.4	49.6	45.0
6.	4.3	55.1	40.6
7.	4.7	53.6	41.7
8.	4.1	58.3	37.6
9.	5.6	50.7	43.7
10.	5.8	49.1	45.1
x	5.0	52.4	42.6
s.	0.70	3.01	2.86
s. e.	0.23	0.95	0.91

x : Mean Value s : Standard Deviation s. e. : Standard Error

female mice was α cells 52.4% (48.1～58.3), β cells 5.0% (4.1～6.0) and γ cells 42.6% (37.6～47.8) (Table 2).

The α cells were almost roundish and in some cells the granules were filled in protoplasm and in some cells they were barely observed surrounding the nucleus. And in some cells a part of the granules were projected forth toward interstitial tissues, as though they showed the endocrine condition. In normal mice, the former were the most numerous.

The β cells had irregular polygonal forms, and somewhat larger than the other cells. About the condition of granules, various degrees were observed, like in the α cells.

The protoplasm of the γ cells was difficultly stainable, and the cell border was

indistinct and the state, in which only nuclei gathered together, was found.

(2) Group of Mastopathy-like Changes

The average percentage value of the 3 kinds of cells in the anterior lobe of pituitary gland of 10 female mice with mastopathy-like changes, was α cells 43.7% (38.6～46.7), β cells 4.9% (3.8～6.2) and γ cells 51.5% (48.6～57.6). Compared with normal female mice, the remarkable increase of γ cells and the decrease of α cells were observed. When the difference was statistically investigated, at the level of significance 5%, p: 0.05, and then it was significant (STUDENT's Test) (Table 3).

Table 3. Percentages of Three Cell Types in Pituitary

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
83	4.9	43.7	51.3
255	4.4	45.6	50.0
513	5.1	45.9	49.2
514	3.8	38.6	57.6
517	4.9	41.5	53.6
518	4.6	43.7	51.7
616	6.2	41.9	51.9
701	4.5	46.2	49.3
703	4.7	46.7	48.6
714	5.4	42.1	52.1
x	4.9	43.6	51.5
s.	0.61	2.45	2.51
s. e.	0.19	0.78	0.79

As to the histological findings of the anterior lobe in the group of mastopathy-like changes, in the majority of cases the stainability of α cells declined strikingly. The cases of granule-falling stadium were increased and some of their granules were observed in a small number, being omnipresent in one side of cell body. Also in some of the β cells, the basophile granules were decreased and they could be difficultly differentiated from the γ cells. But the grade of changes were slight, compared with that of the other cells. The γ cells were markedly increased, and the cell body were rather hypertrophied and the vacuolation was observed in some of them. These findings of cells were agreed with those at medication of a large dose of estrogen, and then they were corresponding to the so called "pregnancy cells". In the interstitial tissues, any other remarkable change was not seen than the dilatation of sinusoid (Fig. 4, 5).

(3) Group of Mammary Cancer

The mice, in which mammary cancer developed as large as a walnut, were employed. Their times of delivery were about 3～6, and their surviving period was 444 days in average.

The average percentage of the cells of pituitary gland in the 11 mice suffering from mammary cancer was α cells 45.9% (42.6～52.8), β cells 4.6% (3.3～5.4),

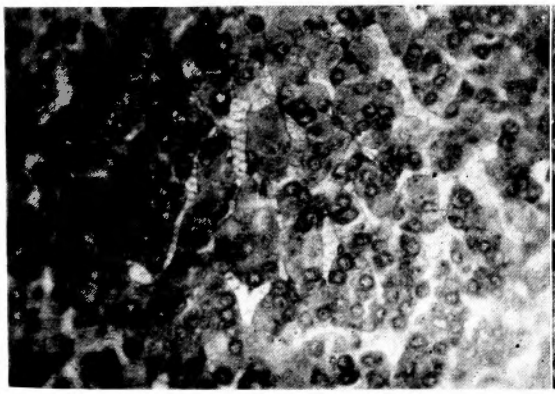


Fig. 4. A case of mastopathy-like changes. Chromophile granules are decreased and sinusoids are enlarged. ×400

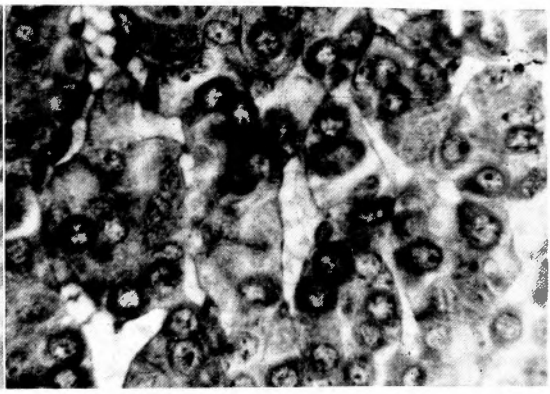


Fig. 5. The same above. ×1.000

Table 4. Percentages of Three Cell Types in Pituitary Gland of Mice with Mammary-Cancer

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
5.	5.6	42.6	51.8
19.	4.0	47.3	48.7
48.	4.6	47.0	48.4
59.	5.4	45.6	49.0
78.	4.7	43.9	51.4
92.	4.5	46.5	49.0
155.	3.5	49.6	46.5
175.	5.3	43.6	51.1
187.	4.1	44.6	51.5
235.	5.1	43.8	51.1
247.	3.3	52.8	43.9
x	4.6	45.9	49.5
s.	0.73	2.88	2.35
s. e.	0.23	0.89	0.73

which had the tendency of decrease, compared with those of the normal group without treatment, and γ cells 49.5% (43.9～51.8), which were strikingly increased on the contrary. When the difference was statistically analyzed, p 0.05 at the level of significance 0.5%, and it was significant (Table 4).

In the histological findings of anterior lobe of pituitary gland in these mice, like in those of the group of mastopathy-like changes, the stainability of α cells remarkably declined, and the cases of entgranuling stadium were increased and in some cases a small number of granules were omnipresent in one side of cytoplasm. The granules of β cells were decreased and the stainability declined. The number of the cells was also decreased and they were changed more intensively than the group of mastopathy-like changes. The number of γ cells was increased and each cell, as a whole, was small. Sometimes the mass of cells was formed. In the

interstitial tissues, the sinusoid was dilatated, the fasciculus was disordered and hyperemia was found. In other words, these findings showed that the damage of anterior lobe of pituitary was more intensive (Fig. 6, 7).

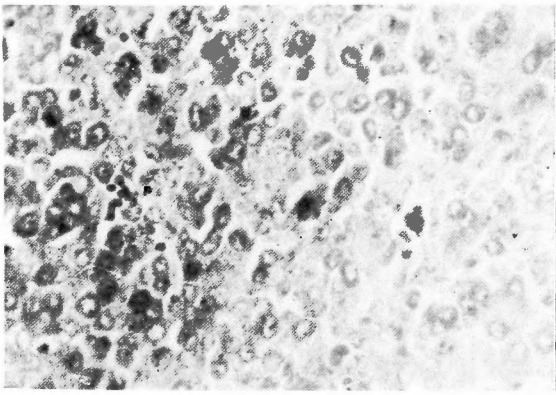


Fig. 6. A case of breast-cancer. The mass of cells are formed, the fasciculus is disordered, and hyperaemia is seen. ×400

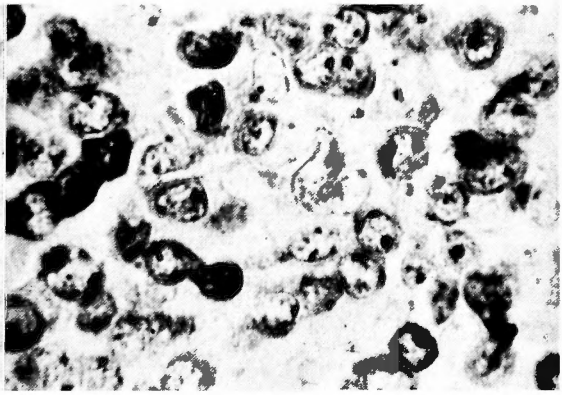


Fig. 7. The same above. ×1.000

III. CHANGES OF ANTERIOR LOBE OF PITUITARY DUE TO TRANSPLANTED CANCER

Are the above mentioned changes in anterior lobe of pituitary in the group of naturally developed mammary tumors really the cause of tumor generation or the result? For the purpose of investigating it, the author practiced the transplantation of EHRLICH's tumor, which had an extreme malignancy, and of BASHFORD's cancer, which was comparatively mitigatory, and the injection of tissue emulsion of spontaneously developed mammary cancer in mice. And the changes of anterior lobe of pituitary gland were respectively examined.

Experimental Method.

The employed animals were all the adult female and hybrid mice, of about 20 g body weight.

1) EHRLICH's ascites cancer 0.1cc, cultivated successively in vivo, was injected subcutaneously in back skin of each mouse, to make a subcutaneous tumor. In 2～3 weeks after the injection when the tumor developed as large as walnut, the animal was suffocated and killed, for the experiment.

2) As BASHFORD's cancer, the one of the Institute of Pathology of Osaka University was selected, and it was cultivated, for 18 or 19 generations, successively in the subcutaneous tissue of back skin. The transplanted cancer tissue, except the necrotic part of central region, was sectioned into about $1mm^3$, was immersed in penicilline saline solution, and was embedded into subcutaneous tissue of back skin of each mouse. After 2 months from the transplantation, the experiment was carried out in the animals, in which the tumor developed as large as walnut.

3) The cancer tissue resected from the mice with spontaneously developed

cancer, was crushed enough and the physiologic saline solution, of ten times as much as it, was poured to it. After shaking, it was centrifuged, the sediments were removed, and the tissue emulsion of mammary cancer was made. Immediately 0.1cc of it was injected into the subcutaneous tissue of each mouse. In one week or one month, the animals were suffocated and killed, for the experiment.

Thus, in the 3 groups, as previously mentioned, the pituitary gland was fixed for 2 hours in BOUIN's solution, and after embedding and staining, the percentage of cells of anterior lobe was calculated, by means of INOUE's method modified from RASSMUSSEN's method.

Experimental Results

1) Group of Subcutaneous Transplantation of EHRLICH's Cancer

When, for the purpose of forming a solid tumor in subcutaneous tissue, the author injected EHRLICH's ascites cancer in back skin, it developed at the injected region without latent time. Afterward, the tumor developed rapidly and successively, and after 2 weeks the size became as large as a thumb or a walnut. Sometimes it reached at the retroperitoneum. Moreover, the successful rate of transplantation was 100%. When the findings of anterior lobe of pituitary of the 14 mice in this stadium were investigate, the average percentage value of the 3 kinds of cells was α cells 50.7% (46.6～53.7), β cells 4.7% (3.1～6.7) and γ cells 44.6% (39.7～50.3), and these values had no significant difference from those of normal group without treatment (p. 0.05) (Table 5).

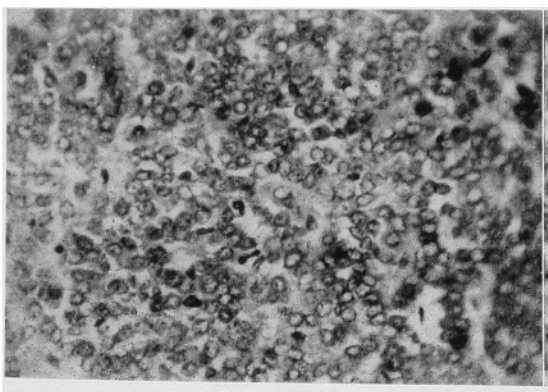


Fig. 8. A case of transplantation of EHRLICH's cancer.
Vacuolation and mitosis of cells are seen. ×400

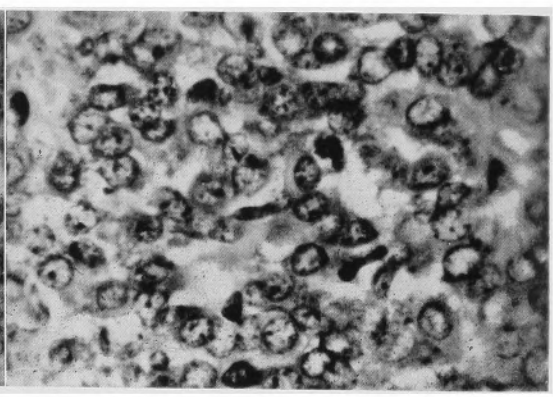


Fig. 9. The same above. ×1.000

On the histological standpoint, however, the α cells had the findings with large vacuolation, and in 5 of 13 cases 1～2 cells, which were acting mitosis, were observed in all the visual fields. Till now, it has been said that the pituitary gland cells, in which mitosis was found, were very rare. Then, what caused it, is the problem to be dissolved in future (Fig. 8, 9).

2) Group of Transplantation of BASHFORD's Cancer

When the tissue specimen of BASHFORD's cancer was transplanted in subcutis

Table 5. Percentages of Three Cell Types in Pituitary Glands of Mice transplanted Ehrlich's Cancer

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
1.	5.8	51.0	42.2
2.	5.2	50.4	44.4
3.	2.5	51.9	45.6
4.	5.2	52.2	42.6
5.	4.6	51.8	43.6
6.	3.1	46.6	50.3
7.	3.6	48.8	47.6
8.	4.2	53.7	42.1
9.	4.1	48.8	47.1
10.	6.2	48.8	45.0
11.	6.7	53.6	39.7
12.	5.4	49.8	44.8
13.	4.7	51.2	44.1
x	4.7	50.7	44.6
s.	1.17	1.98	2.64
s. e.	0.32	0.55	0.73

Table 6. Percentages of Three Cell Types in Pituitary Glands of Mice tansplantated Bashford's Cancer

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
1	6.8	48.6	44.6
2	4.6	54.7	40.7
3	6.4	50.4	43.2
4	7.0	54.8	38.2
5	4.2	50.0	45.7
6	5.4	47.4	47.2
x	5.7	51.0	43.3
s.	1.16	2.38	3.03
s. e.	0.47	0.93	1.24

of mice, induration was made after the latent time of about 7 days, and it gradually developed. After about 2 months a tumor as large as a walnut was formed. The successful rate of transplantation was about one half. In the 6 successful cases of tranplantation, the percentage of anterior lobe cells of pituitary gland, as showed in Table 6, had no significant difference, compared with that of the normal group without treatment, and no increase of γ cells, which was oberved in cases of mammary cancer and mastopathy-like changes, were seen.

3) Group of Injection of Tissue Emulsion of Mammary Cancer

The injected dose was considerably large. After more than one week, however, no change was risen in the injected region and the body weight of animals had no great difference compared with that before the injection. The percentage of

Table 7. Percentages of Three Cell Types in Pituitary Glands of Mice injected with Emulsion of Breast Cancer Tissue

No.	Basophil (%)	Acidophil (%)	Chromophobe (%)
1.	5.2	48.1	46.7
2.	4.2	52.4	43.4
3.	4.6	49.3	46.1
4.	4.7	51.4	43.9
5.	3.8	47.0	49.2
6.	4.3	44.8	50.9
7.	5.3	54.2	40.5
x	4.7	49.6	45.7
s.	0.55	3.04	3.29
s. e.	0.21	1.15	1.24

anterior lobe cells of pituitary gland in these 7 cases, as showed in Table 7, had no significant difference compared with that of the normal group without treatment and was distinctly different from the findings of group of mammary cancer. In the histological findings, no special change was observed.

IV. DISCUSSION

As to the rôle of sexual hormones at development of mammary tumors, since the pioneer investigation of LACASSAGNE, a plenty of researches have been reported and nowadays it has been made clear that estrogen causes the enlargement and hypertrophy of mammary gland and furthermore it is closely connected to the development of mammary tumors. But, whether or not the changes of mammary gland caused by these sexual hormones can develop only under the existence of anterior lobe of pituitary, is not yet settled. TURNER and others supposed that estrogen acted first on the anterior lobe of pituitary and mammogenic hormone was produced and after that the development of mammary gland was promoted by it. On the contrary, GARDNER and his collaborators pointed out that even in the animals, of which pituitary gland was removed, estrogen developed the mammary gland, but they found that the simultaneous medication of lactogen promoted much more the development of mammary gland and concluded that estrogen raised the sensitivity to lactogen.

Anyhow, from the fact that the anterior lobe of pituitary, as the higher centrum of endocrine organs of sexual gland system, is related to the change of estrogenic hormones, it is not difficult to suppose that it has influences on the development of mammary gland and on the generation of mammary tumors.

But, till now the opinions on the changes of anterior lobe of pituitary in animals or patients with tumors, are conflicting, and about the mechanism of cells of pituitary gland there are a good deal of unknown points. Any definite theory was not yet settled.

As the parenchyma cells of anterior lobe of pituitary, the α, β and γ cells

have been found. ROMEIS classified, according to his Cresazan staining, the α cells into α and ε cells, the β cells into β and δ cells, and the γ cells into γ and undifferentiated cells, namely into 6 kinds in amount, but in general, they were classified into the above mentioned 3 kinds. On the relation of these 3 kinds of cells it was formerly considered that the γ cells were matured to the α cells, which were changed into the β cells, finishing secretion, and they returned again to the original form of γ cells. But nowadays, according to the studies on mitochondria and GOLGI's apparatus, the change between α and β cells is not recognized. Some of the γ cells develope only to α cells and some only to β cells. It is considered that after both kinds of cells finished secretion, and were deprived of granules, they returned to the former form of γ cells.

According to my experiment, in the hybrid normal female mice, after about 400 days from their birth, the weight of pituitary gland was 0.9mg in the average of 8 cases and the average weight pro 10g body weight was 0.35mg/10g (body weight). They were agreed with those reported by the other several authors. The percentage of 3 kinds of cells was α cells 52.4% the most numerous, γ cells 4 2.6% the next and β cells 5.0% the least number. These values are considerably different, according to the authors. Some reported that the α cells were the most numerous, the γ cells were the next and the β cells were the least. It might be because the differentiation of β and γ cells is sometimes difficult and some measuring errors might be easy to occur. Simultaneously, it can be considered that the kinds, sex and age of animals, and diet, climate and the other conditions have influences on the findings.

Now, in the mice, which were forced into weaning after 5 days or immediatly at each occasion, repeating several times of pregnancy and delivery, the average weight of pituitary gland in 8 cases was 1.2mg and its average weight pro 10g body weight was 0.38mg/10g (body weight). These values had the tendency of slight increase, compared with those of normal female mice. About the weight increase of pituitary gland in the animals with mammary cancer, there are the reports of BIELSCHOWSKY, CRAMER & HORNING and others, and especially BIELSCHOWSKY, being able to perform an exact measurement in a new strain of mice (NZY), reported that the pituitary gland was hypertrophied two times as large in the group of mammary cancer.

In the percentage of cells of anterior lobe of pituitary gland in the mice with mammary cancer and mastopathy-like changes, the increase of γ cells and the decrease of α cells were remarkable, but in the grade no difference was found. About the β cells, the percentage had a slight decrease in the group of mammary cancer, the stainability of granules declined and some fallen granules were observed, while in the group of mastopathy-like changes, such findings were never seen.

These findings were considered to be the process when the α cells discharged hormones and changed into the γ cells which had no secretion function, and when the hormone activity of α cells declined, as the result. And the findings at the time of medication of a large dose of estrogen were also similar, and about such

experiments a plenty of reports have been published. They are almost same in the results and the increase of γ cells is recognized in most of them. Especially, CRAMER & HORNING observed the enlargement of anterior lobe of pituitary gland, the tubercular proliferation of γ cells and sometimes the adenoma, in the rats with mammary cancer due to estrogen.

On the other hand, the 2 kinds of hormones of gonadotropin are lutenizing hormone (LH) and follicle stimulating hormone (FSH). From what cells are they secreted? It is yet unknown, but TAKEWAKI, judging from the experiments of WOLF, FINERTY and PURVES about sexual cycle and extirpation of sexual glands, showed some suggestion that FSH was originated from the β cells and LH was from the α cells.

From these standpoints, it is also too early to discuss immediately the secretion quantity of LH and FSH, but it is undoubted that the hormone imbalance, which brings at least the relative surplus of estrogen, exists in the anterior lobe of pititary gland in the animal with mammary cancer. Furthermore, in the cases of mammary cancer, compared with the group of mastopathy-like changes, the changes of interstitial tissue are remarkable and the β cells had the tendency to decrease. And it might be said that the disfunction of anterier lobe of pituitary gland is more intensive. In a word, these findings can be considered as the proof that mastopahy-like changes are altering to mammary cancer.

From the above mentioned results, the existence of sexual hormone imbalance in mice with mammary tumors is almost certain. However, whether these organic changes are the caused of development of mammary tumors or the secondary changes due to the tumor, is not yet decided. For the purpose of investigating this question, the author, performing the transplantation of EHRLICH's tumor and BASHFORD's cancer and the injection of tissue emulsion of mammary cancer in mice, studied on the respective changes of anterier lobe of pituitary gland.

GUYER and others, transplanting the mouse cancer of FLEXNER's strain into white mice, found the hypertrophy of pituitary gland and the increase of α cells, and furthermore observed the similar findings even in the white mice in which the mouse cancer of WALKER's system was transplanted. These findings were not the specific changes of cancer, but in the case of transplantation of kindney tissue the same findings could be found. Therefore, Mc.EUEN and others considered that a part of such findings was merely caused by the resorption of necrotic tissue which was produced from the destroyed tissue at the central part of tumor. Recently, MORI, carrying out the transplantation of mammary cancer in mice, found a slight decrease of the α cells but he reported that the γ cells had no change. And he presumed that these findings would be the changes of pituitary gland caused by toxin of mammary cancer.

In my experiment, the percentage of 3 kinds of cells had no great difference from that of the normal group without treatment, and only in 5 of the cases of transplantation of EHRLICH's cancer, which had an extreme malignancy, mitosis was observed, which was generally considered difficult to occur in pituitary gland. It is entirely different from the findings of mammary cancer, and it can be said

hat it is caused by malignant tumour itself. The fact that the findings at the ime, when from the tissue of mammary cancer itself of mouse the emulsion was nade and was injected, agreed with these findings, is presumably support the ıbove mentioned idea.

The matter that the findings in a series of these experiments are almost imilar, reminds us of a lot of unspecific changes originated from something in ancer tissue.

As above mentioned, the changes of anterior lobe of pituitary gland in the nice, in which mammary tumors naturally developed, are distinctly different from hose at the transplantation of cancer, and it is distinct at least that they are not enerated as the result of development of mammary cancer, but the matter that hey play a causal part, is presumably suggestible.

V. CONCLUSION

On each occasion of repeated several times of pregnancy and delivery, the mice were forced into weaning after 5 days or immediately, and mammary cancer and nastopathy-like changes were developed. The anterior lobe of pituitary gland in hese mice was microscopically examined under Cresazan stainining. The quantitıtive measurement of cells was based on INOUE'S method, modified from RASSMUSEN'S method. The findings at the transplantation of EHRLICH'S cancer and BASHFORD'S nammary cancer of mouse were compared with those at development of the nammary cancer and mastopathy-like changes. The results were as follows.

(1) In the anterior lobe of pituitary gland in the cases of mammary cancer nd mastopathy-like changes, the increase of chromophobe cells and the decrease of cidophile cells were remarkable, and in most of the cases, the stainability of ranules declined and a lot of entgranulating were seen. Particularly, in the cases f mammary cancer, even the basophile cells were had the tendency of decrease nd changes of granules were observed. The changes of interstitial tissue were oticeable in the case of cancer, and the damage of anterior lobe of pituitary gland vas remarkable, compared with those in the cases of mastopathy-like changes. 'hese findings were similar to those at a long term medication of large dose of strogen pellet.

(2) In the mice of subcutaneous injection of EHRLICH'S ascites cancer, the ıitosis was distinctly observed in 5 cases, and except it, any quantitative change f cells was not found. In the mice of transplantation of BASHFORD'S cancer, the indings were also similar. These findings are considered unspecific changes caused y something in cancer tissue and the fact that the changes of anterior lobe cells f pituitary gland at the injection of tissue emulsion of mammary cancer were lso similar, might support this idea.

(3) Judged from these matters, it can be considered that the findings of

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anterior lobe of pituitary gland in the mice with mammary cancer and mastopathy-like changes, show the hormone imbalance of pituitary sexual glands system, and furthermore that they presumably play a causal part in development of tumors.

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乳腺腫瘍の発生と脳下垂体前葉の関係

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さきに，教室の越は岐阜産雑種マウスについて，妊娠出産を繰返し，その都度五日間で，或いは直ちに授乳を中止せしめることによつて，高率に乳癌及びマストパチー様変化を惹起せしめた．そこで自分は斯く越の作成した乳腺腫瘍発生マウス脳下垂体前葉を組織化学的に検索して，これとEhrlich癌，Bashford癌移植時及び自然発生マウス乳癌抽出液注射時に於ける脳下垂体前葉の変化とを比較検討し，乳腺腫瘍の発生と脳下垂体前葉の関係を検討したのであるが，その知見を総括して次の結果をえた．

1) マストパチー様変化を来したマウスの脳下垂体前葉に於ては，好色素顆粒の染色性が著しく低下し，三種細胞の百分率ではα細胞の減少と，γ細胞の増加が認められ，β細胞の比率は正常と差はなかつた．この組織像は，恐らくα細胞がホルモン放出を行つている時期に一致するものと思われる．

2) 乳癌発生マウスの脳下垂体前葉では，マストパチー様変化の場合に於けると同様にγ細胞の増加が著明であつて，組織学的にも，腺細胞の萎縮，空泡形成が観られ，細胞顆粒は顕著に減少し，細胞裂間隙が拡大している等々の所見が認められた．

これ等乳腺腫瘍発生マウス脳下垂体前葉の所見は，Estradiolの長期投与時の脳下垂体前葉の変化とよく一致しているのである．

3) Ehrlich腹水癌をマウスの皮下に注射し固形腫瘤を形成せしめ，，又Bashford癌を背部に移植し，腫瘤がクルミ大になつたものの脳下垂体前葉細胞の百分率は，何れも正常と差はなく，ただ組織学的にEhrlich腹水癌移植例に，空泡形成が観られ，13例中5例に有絲核分裂像が認められた．

4) 乳癌組織の壊死崩壊物質の吸収による脳下垂体前葉の変化を観る目的で，自然発生マウス乳癌組織の抽出液を，健常マウスの皮下に注射したものの脳下垂体前葉は，組織学的にも，また三種細胞百分率にも変化を認めなかつた．

5) 以上の所見から，乳腺腫瘍自然発生マウスの脳下垂体前葉の変化は，腫瘍による二次的変化ではなく多分に原因的役割を演じているものと考えられ，しかもそれが性ホルモン＝アンバランスの出現と深い関係にある事を示唆しているものと考える．