HISTOLOGICAL CHANGES OF THE HYPOPHYSIS, OTHER ENDOCRINE ORGANS AND BONES OF MICE CAUSED BY ORAL ADMINISTRATION OF A LARGE QUANTITY OF LEUCIN.

by

TERUKAZU IMAI

The First Surgical Division, Kyoto University Medical School
(Chief, Prof. Dr. CHISATO ARAKI)
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INTRODUCTION

Engel and several other authors reported that ACTH and adrenocortical hormone have an intimate relation with the metabolism of amino acid, while, on the other hand, there are very few reports that some kinds of amino acid have some influence on the secretion of a certain hormone. HARADA and his coworkers found that the administration of a large amount of histidin to rats brought about an increase in body weight, and also an increase in α-cells, hypertrophy of δ-cells and atrophy of β-cells in the anterior lobe of the pituitary gland. In addition, they observed that the administration of histidin restored not only the decrease in body weight but also the decrease in α-cells and the hypertrophy and hyperplasia of β-cells of the adenohypophysis occurring in thyroidectomized rats. Also, they reported that in rats having been administered lucin for four weeks, an increase in body weight together with an increase in the α-cells in the pituitary took place.

In the hope of experimental production of acidophilic adenoma of the hypophysis I administered a large amount of leucin to mice for long periods and examined the hypophysis, other endocrine organs and bones histologically.

EXPERIMENT I

ANIMALS AND METHOD.

Animals used for this experiment were mice, both male and female, one month after birth. They were put into separate wire-mesh boxes and brought up. An amount of 150 mg leucin was administered orally each day and their body weights were weighed every four days early in the morning before feeding. After 10 weeks and 20 weeks respectively, they were killed, and the hypophyses, thyroids, adrenal glands, sexual organs and bones were histologically examined.

The number of experimental animals used were 10 males and 10 females for both the leucin administered group and the control group respectively. Five of each group were killed after 10 weeks and the remaining 5 after 20 weeks.

Leucin was dissolved in lukewarm water and then hardened with casein into

a solid food in form of a cube of approximately 0.7 cm^3, and administered orally with an addition of a little amount of vitamin B_1.

The hypophysis was fixed in Bouin's solution and imbedded in paraffin and stained by the GOMORI's aldehyde-fuchsin method and the Halmi's method. The thyroid, gonad and adrenal cortex were fixed in formalin and stained by hematoxylin-eosin. For the adrenal cortex, the Sudan III stain was also used. The bones, after having been decalcified by 10% trichloroacetic acid, were imbedded in celloidin and stained by hematoxylin-eosin.

RESULT OF EXPERIMENT

I. BODY WEIGHT

From the beginning of the experiment, the body weights of the mice were continuously weighed in every fourth morning before feeding. No prominent

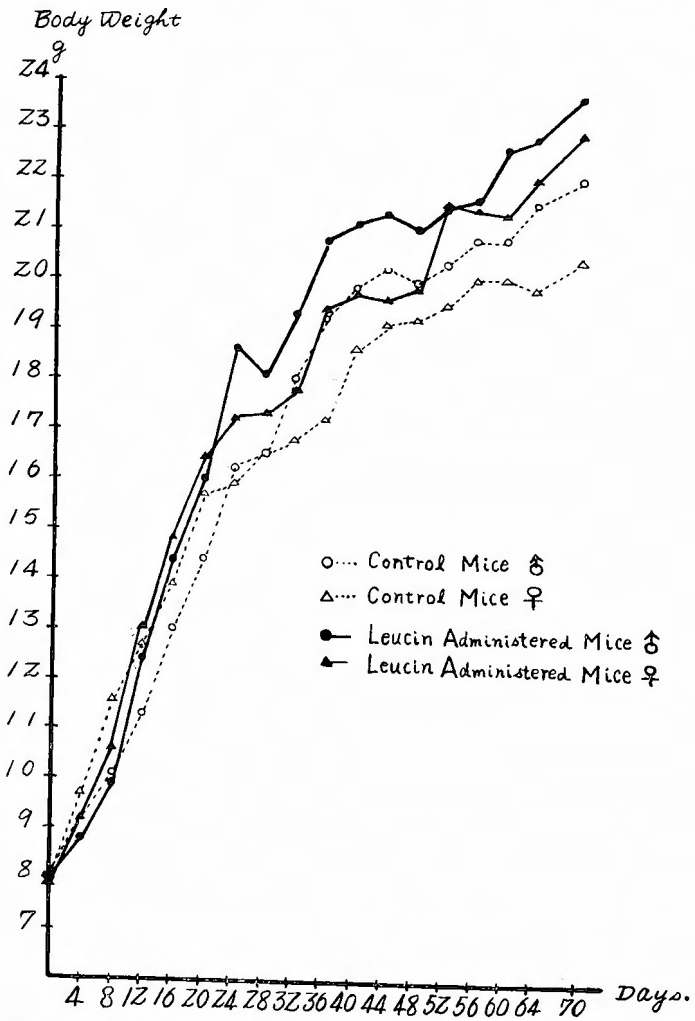

Fig. 1 Body Weight Increase Curve in Both Leucin Administered and Control Mice.

difference in the body weight was noticed during the period of 10 weeks between the control and the leucin group. After 10 weeks, the average weight of the control males was 22.4 g and that of the leucin administered males 22.4 g, whereas that of the control females was 20.3 g and that of the leucin administered females 23.7 g. In other words, a slightly more increase in the body weight was noticed in the leucin administered group than in the control group. But after a period of 20 weeks, considerable differences in the body weight were noticed between the control group and the leucin group, roughly in accord with the morphological change of adenohypophysis to be mentioned later. Namely, the average body weight

Table 1 Body Weight in the Leucin Administered and Control Groups.

	Days	Before	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	70	140
Control Group ♂	No. 1	8.0	8.0	10.0	12.0	14.0	15.0	18.0	17.0	19.0	20.0	18.0	18.0	19.0	18.5	21.0	22.0	24.0	26.0	
	No. 2	7.0	8.0	10.5	11.0	13.0	15.0	16.0	17.0	17.0	16.0	17.0	18.0	16.0	18.0	19.0	19.0	20.0	22.0	
	No. 3	8.0	10.0	10.0	11.0	14.0	14.0	15.5	16.0	18.0	18.0	20.0	20.0	18.5	19.0	19.0	18.0	18.0	20.0	
	No. 4	9.0	10.0	11.0	12.0	12.0	12.0	15.0	16.5	18.0	18.0	21.0	21.5	20.0	20.0	19.0	20.0	20.0	21.5	
	No. 5	7.0	9.5	11.5	12.0	14.0	15.0	16.0	17.0	18.5	19.0	20.0	22.5	20.0	21.0	21.0	20.0	22.5	22.5	
	No. 6	8.5	9.0	9.0	10.5	14.0	16.0	18.0	15.0	20.0	22.0	22.0	23.0	22.0	23.0	22.5	23.0	24.0	24.0	28.0
	No. 7	9.0	10.0	10.0	11.5	13.5	15.5	18.0	18.5	18.5	21.0	22.0	21.0	22.0	21.0	23.0	23.0	23.0	22.0	26.0
	No. 8	8.0	8.0	10.0	11.5	11.0	12.0	14.0	16.0	16.0	17.0	18.0	19.0	20.0	19.0	18.0	18.0	19.0	20.0	21.0
	No. 9	8.0	9.0	10.0	11.0	12.0	14.0	16.0	18.0	19.0	21.5	20.0	20.0	21.0	21.0	22.0	22.0	23.0	22.0	24.0
	No.10	8.0	10.0	9.0	10.5	12.5	13.0	15.5	14.0	16.0	19.0	20.0	19.0	20.5	22.0	23.0	22.5	21.0	20.0	25.0
Leucin Administered Group ♂	No. 1	8.0	7.0	7.0	10.5	12.0	14.0	14.0	15.0	18.0	18.0	19.0	20.0	19.0	21.0	21.5	20.5	20.5	21.0	
	No. 2	8.5	10.0	11.0	12.0	13.0	17.0	20.0	20.0	20.0	21.0	23.0	22.0	23.0	22.5	21.0	22.0	22.0	23.0	
	No. 3	7.0	8.0	7.0	10.0	11.5	14.0	16.5	18.0	19.0	19.0	20.0	20.0	20.0	21.0	22.0	22.0	22.5	24.0	
	No. 4	7.0	8.0	8.0	9.0	13.0	15.0	17.0	18.0	20.0	22.0	22.0	21.0	20.0	21.0	20.0	20.0	21.0	22.0	
	No. 5	8.0	8.0	10.0	11.0	15.0	15.0	16.0	17.5	18.0	18.5	18.0	18.5	17.5	19.0	20.0	22.0	21.0	22.0	
	No. 6	8.5	10.0	12.5	15.0	15.5	16.0	16.0	17.0	20.0	19.0	18.0	20.0	20.0	21.0	22.0	22.0	24.0	25.5	28.0
	No. 7	9.0	10.0	10.0	14.0	14.0	18.0	19.5	18.0	20.0	21.0	22.0	23.0	22.0	20.0	22.0	23.0	23.0	24.0	32.0
	No. 8	8.5	9.0	10.0	11.5	16.0	16.0	20.0	20.0	21.0	21.0	23.0	24.0	24.0	23.5	26.0	25.0	26.0	26.0	27.0
	No. 9	8.0	10.0	11.5	14.0	17.5	16.5	18.5	19.0	19.0	21.0	23.0	22.0	23.0	23.0	24.0	25.0	24.0	24.0	30.0
	No.10	7.0	8.0	12.0	13.5	16.0	18.5	18.0	18.0	18.0	21.0	22.0	22.0	21.0	22.0	22.0	24.0	24.0	24.0	24.0

Table 2 Body Weight in the Leucin Administered and Control Groups.

	Days	Before	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	70	140
Control Group ♀	No. 1	8.0	10.0	11.5	13.0	13.5	16.0	17.0	16.0	16.0	17.5	18.0	18.0	18.0	19.5	20.0	20.5	20.0	20.5	
	No. 2	8.0	9.5	12.0	13.5	14.0	16.0	15.0	16.0	18.0	16.5	17.5	18.0	17.5	18.0	18.5	18.5	18.0	18.0	
	No. 3	9.0	10.0	13.0	14.0	16.0	16.0	15.0	18.0	17.0	18.0	19.0	19.5	20.0	20.0	19.5	19.5	20.0	20.0	
	No. 4	8.0	9.5	12.0	14.0	13.0	14.0	17.0	16.5	18.0	18.0	18.5	20.0	20.0	20.5	20.5	20.0	20.0	20.0	
	No. 5	8.0	10.0	12.0	12.0	14.0	19.0	19.0	20.0	20.0	18.0	21.0	23.0	22.0	21.0	22.0	22.0	21.0	23.0	
	No. 6	7.0	10.0	11.5	13.5	14.5	15.5	15.0	18.0	16.0	18.0	18.0	18.0	19.0	20.0	22.0	20.0	20.0	21.0	26.0
	No. 7	9.0	10.0	11.5	12.0	15.0	14.0	15.0	15.0	17.0	18.0	17.0	18.0	18.0	19.0	20.0	19.0	19.0	19.0	21.0
	No. 8	8.0	10.0	11.0	12.0	14.0	14.5	14.0	15.0	16.0	16.0	18.0	18.0	18.0	18.5	19.0	20.0	20.0	20.0	22.0
	No. 9	9.0	10.0	11.5	12.5	14.0	16.0	18.0	16.0	17.0	18.0	20.0	20.0	19.0	18.0	18.5	19.0	19.5	21.0	23.0
	No.10	8.0	9.0	11.0	10.5	13.0	14.5	15.0	14.5	14.5	15.0	17.5	19.0	20.0	21.0	21.0	20.0	20.0	21.0	23.0
Leucin Administered Group ♀	No. 1	8.0	8.5	8.0	11.5	12.0	11.5	16.0	14.5	16.0	17.0	17.5	17.0	16.0	17.5	17.5	17.5	18.0	18.5	
	No. 2	7.0	8.5	10.0	14.0	17.5	17.0	17.5	18.5	19.0	18.5	18.0	18.0	21.0	22.0	23.0	24.0	23.5	26.0	
	No. 3	9.0	9.0	8.5	12.0	13.0	18.0	18.0	18.0	18.5	19.5	19.5	20.0	20.0	20.5	20.5	19.0	21.0	22.0	
	No. 4	8.0	10.0	13.0	14.0	16.0	17.0	18.0	18.5	18.5	19.0	20.0	20.0	20.0	22.0	23.0	22.0	24.0	26.0	
	No. 5	8.5	11.5	12.0	12.5	16.0	18.0	18.5	18.0	18.0	20.0	22.0	20.0	20.0	20.0	22.5	23.0	21.5	26.0	
	No. 6	7.0	8.0	10.0	13.5	16.0	18.0	18.0	18.0	18.0	17.0	20.0	21.0	21.0	21.0	22.0	22.0	21.0	23.0	30.0
	No. 7	8.0	10.0	11.5	12.0	12.5	11.5	16.0	17.0	17.0	21.0	20.0	20.0	20.0	19.0	20.0	20.0	21.0	20.0	23.0
	No. 8	8.0	7.0	10.0	12.5	13.0	16.0	18.0	19.0	20.0	23.0	21.0	21.0	23.0	24.5	26.0	25.0	27.0	27.0	28.0
	No. 9	7.0	9.0	12.0	16.0	17.5	16.5	17.0	16.0	17.0	19.0	19.0	18.0	18.0	18.0	19.0	20.0	19.0	20.0	22.0
	No.10	8.5	10.0	10.5	12.0	11.0	14.0	11.5	15.0	16.0	20.0	20.0	21.0	19.0	20.0	20.0	20.0	21.0	20.0	23.0

of the male mice of the control group weighed 24.4 g with the maximum weight of 28.0 g and the minimum weight of 21.0 g, whereas the average weight of the leucin administered group was 28.2 g with the maximum weight of 32.0 g and the minimum weight of 24.0 g. The control females showed an average of 23.0 g with the maximum weight of 26.0 g and the minimum weight of 21.0 g, whereas the leucin administered females weighed in average 25.2 g, with the maximum weight of 30.0 g and the minimum weight of 22.0 g. In other words, the males and females of the leucin administered groups showed a prominent increase over those of the control groups (Fig. 1, Tables 1 and 2).

‖. HYPOPHYSIS

All hypophyses were fixed in Bouin's solution, imbedded in paraffin and then cut into serial sections of approximately 3 microns in the horizontal plane. Sections near the middle of all section series were stained by the GOMORI's aldehyde-fuchsin

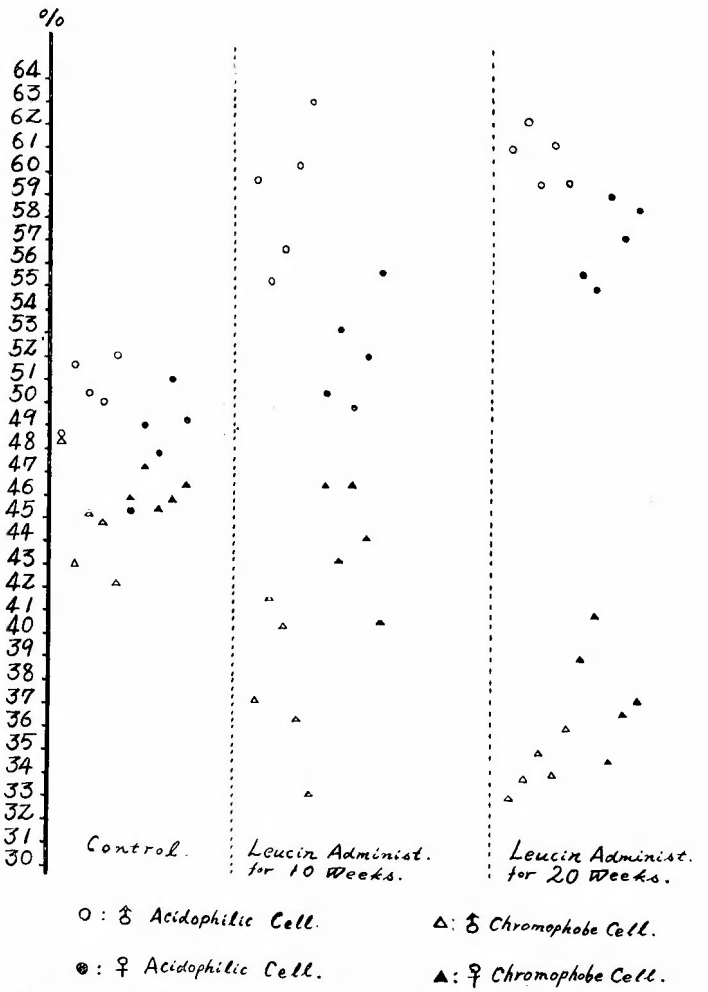

Fig. 2 Percentages of Acidophilic and Chromophobe Cells in Adenohypophysis of Leucin Administered Mice.

method and the HALMI's method. Then, by the Rasmussen's method, the number of acidophilic cells (α-cells), chromophobe cells (γ-cells) and basophilic cells (β-cells) of the anterior lobe were counted and their percentages were taken.

In all the leucin administered animals, there was an increase in α-cells and a decrease in γ-cells in both the 10 week administered group and the 20 week administered group. The changes were more prominent in the 20 week administered group. There was no change in β-cells.

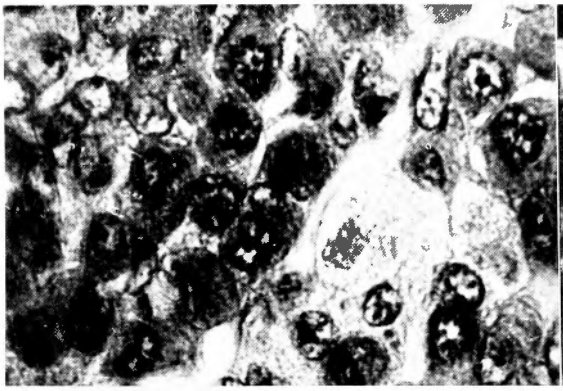

Fig. 3 Hypohysis in Control Mice. Halmi's Stain × 1000

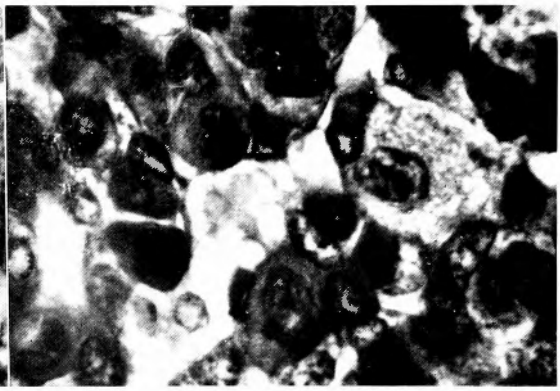

Fig. 4 Hypophysis in Mice Administered Leucin for 10 Weeks. Halmi's Stain × 1000

However, the change in the form of the anterior lobe cells, namely hypertrophy, atrophy of the cells, or the appearance of any abnormal cells could not be found (Fig. 2 and Table 3) (Figs. 3, 4 and 5).

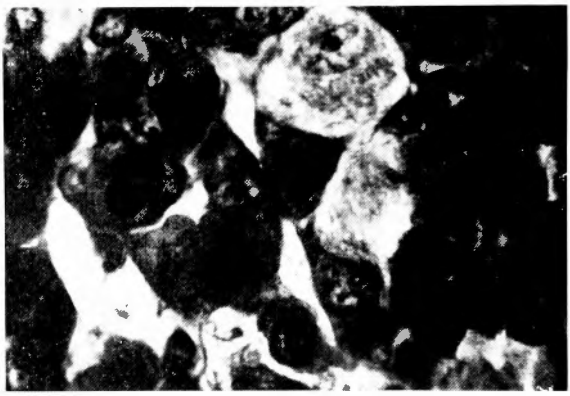

Fig. 5 Hypophysis in Mice Administered Leucin for 20 Weeks. Halmi's Stain × 1000

Table 3 Percentages of Three Kinds of Cells in Adenohypophysis of Leucin Administered Mice.

		Acidophilic Cell	Chromophobe Cell	Basophlilc cell
Control	숫	50.2	45.4	4.4
Leucin Administered for 10 Weeks.	숫	58.9	37.7	3.4
Leucin Administered for 20 Weeks.	숫	60.7	34.6	4.7
Control	우	49.0	45.5	5.5
Leucin Administered for 10 Weeks.	우	52.2	41.1	3.7
Leucin Administered for 20 Weeks.	우	57.2	37.9	4.9

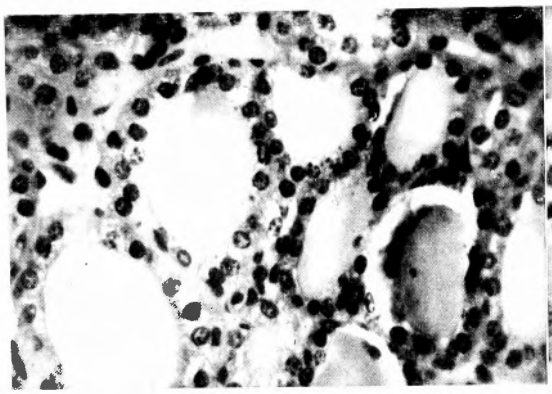

Fig. 6 Thyroid Gland in Control Mice. H. E. Stain × 400

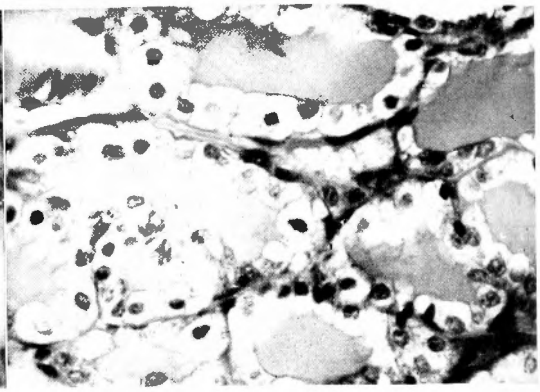

Fig. 7 Thyroid Gland in Mice administered Leucin for 10 Weeks. H. E. Stain × 400

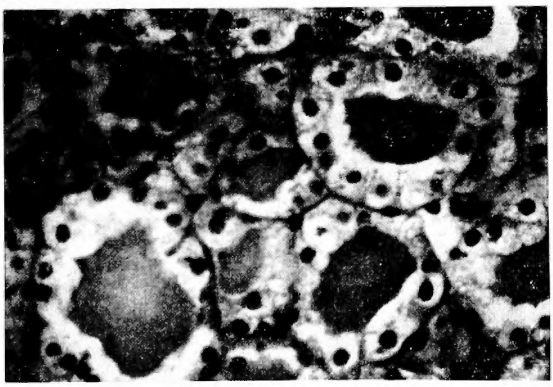

Fig. 8 Thyroid Gland in Mice Administered Leucin for 20 Weeks. H. E. Stain × 400

Ⅲ. THYROID GLAND

Thyroid glands were fixed in 10% formalin, imbedded in paraffin and stained by hematoxylin-eosin.

In the group to which leucin was administered for 10 weeks, five out of ten, and in that for 20 weeks, four out of ten were noticed to have tall epithelial cells with elongated nuclei, some of which were poor in chromatin. However, there was almost no remarkable change in the blood vessels and in the amount of colloid (Figs. 6, 7 and 8.).

IV. ADRENAL CORTEX

Adrenal glands were stained by hematoxylin-eosin and by the Sudan III method.

Generally, in the case of hyperfunction of the adrenal cortex, there are usually the increase in the weight of adrenal glands, the hypertrophy of the cells in the fascicular zone and the decrease in the lipoids contained in them, but in the leucin administered mice there was no prominent increase in the weight of the adrenal gland (Table4). However, the histological examination of the adrenal glands of both the 10 week leucin administered mice and the 20 week leucin administered mice revealed a remarkable hypertrophy of fascicular zone cells, and a decrease in lipoid granules in their protoplasms. This is in accord with the morphological change of adrenal cortex seen in the case where ACTH is administered for a long period. Furthermore, worthy of note were prominent hypertrophy and hyperplasia

Table 4 Mean Value of Weights of Suprarenals in Both Leucin Administered and Control Groups.

		10 Weeks			20 Weeks		
			Suprarenals			Suprarenals	
		Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	♂	22.4	6.6	0.29	24.4	5.0	0.20
Leucin Administration	♂	22.4	8.5	0.38	28.2	6.4	0.23
Control	♀	20.3	13.1	0.65	23.0	12.0	0.50
Leucin Administration	♀	23.7	11.0	0.46	25.2	14.2	0.59

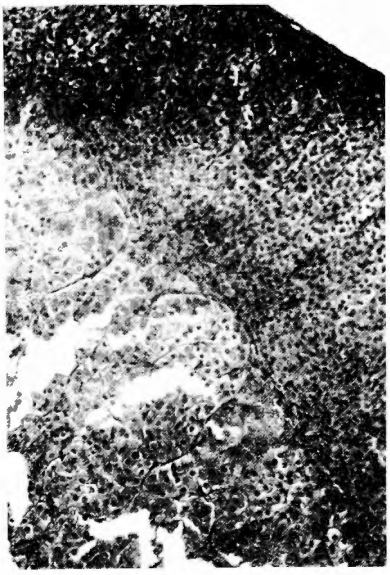

Fig. 9 Adrenal Gland in Control Mice. H. E. Stain × 100

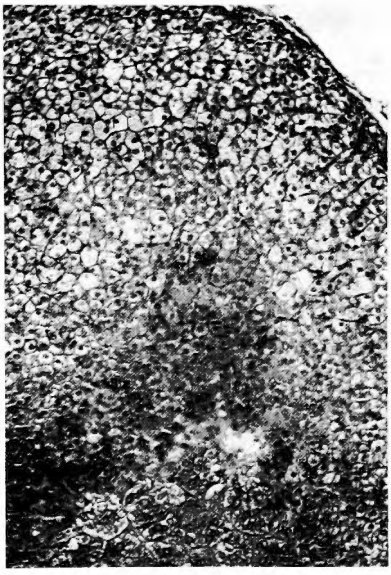

Fig. 11 Adrenal Gland in Male Mice Administered Leucin for 10 Weeks. H. E. Stain × 100

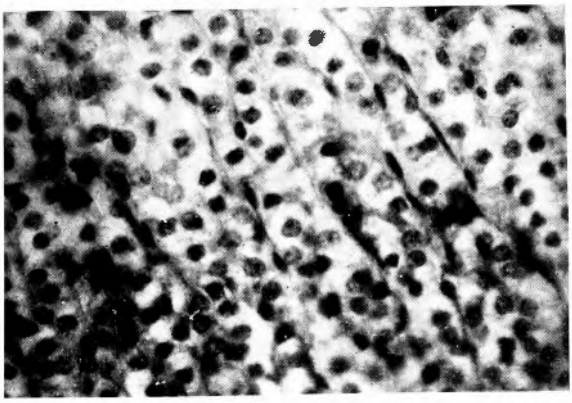

Fig. 10 Adrenal Gland in Control Mice. H. E. Stain × 400

of the X-zone in female mice with many vacuoles in the same zone. These changes were especially marked in the 20 week administered group.

At a glance, this is similar to the "fatty metaplasia" as advocated by Ohi, seen in the case where progesterone or testosterone have been administered, but no lipoid drop could be found in the vacuoles of the X-zone by fat stain. Therefore, this seems to be none other than a simple vacuole formation (Figs.

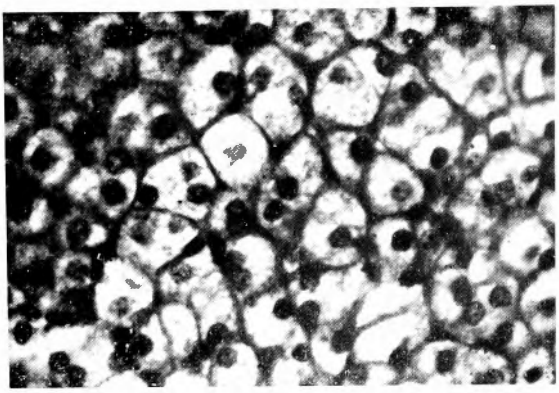

Fig. 12 Adrenal Gland in Male Mice Administered Leucin for 10 Weeks. H. E. Stain × 400

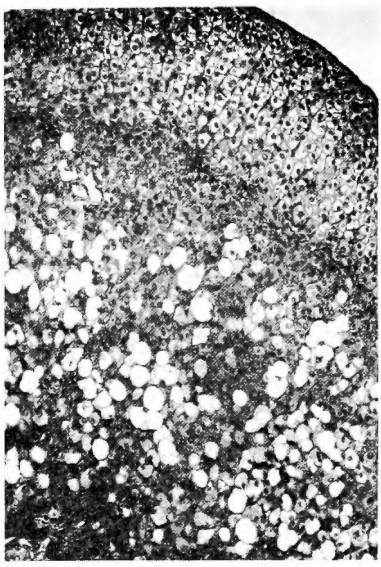

Fig. 13 Adrenal Gland in Female Mice Administered Leucin for 20 Weeks. H. E. Stain × 100

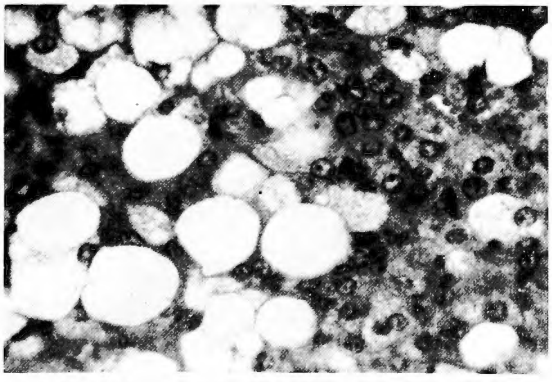

Fig. 14 Adrenal Gland in Female Mice Administered Leucin for 20 Weeks. H. E. Stain × 400

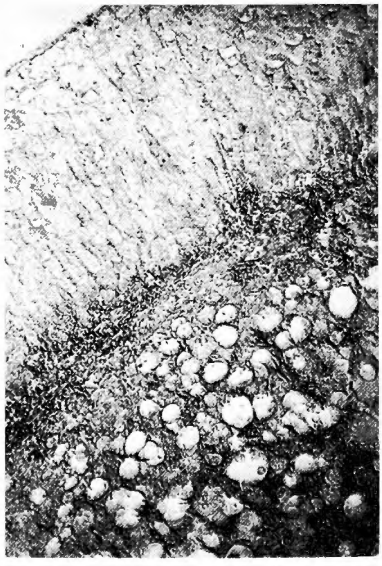

Fig. 15 Adrenal Gland in Female Mice Administered Leucin for 20 Weeks. Sudan Ⅲ Stain × 100

9, 10, 11, 12, 13, 14 and 15).

Ⅳ. GONAD

No prominent increase or decrease was noticed in the weight and in the ratio to body weight of the gonad in both of the groups to which leucin had been administered for 10 weeks and 20 weeks respectively (Tables 5 and 6).

Table 5 Mean Value of Weights of Testes in Both Leucin Administered and Control Groups.

	10 Weeks			20 Weeks		
		Testes			Testes	
	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	22.4	179	8.00	24.4	201	8.24
Leucin Administration	22.4	250	11.16	28.2	202	7.23

Table 6 Mean Value of Weights of Ovaria in Both Leucin Administered and Control Groups.

	10 Weeks			20 Weeks		
		Ovarium			Ovarium	
	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	20.3	22.4	1.10	23.0	19.0	0.83
Leucin Administration	23.7	25.8	1.09	25.2	14.2	0.59

Microscopically, the testes of both groups showed a certain increase in number of the interstitial Leydig cells, but there was no noticeable change in spermatogenesis in the seminiferous tubles.

The ovaria of both of the leucin administered groups showed an exceeding increase in corpora lutea, i. e. in the sections made from the middle part of the ovarium, 15 to 23 corpora lutea were counted in the leucin administered groups as compared with 7 to 10 corpora lutea in the control groups. It goes without saying that the increase in corpora lutea in the leucin administered groups was due to the acceleration of luteinization. No abnormality was found in the development of follicles (Figs. 16, 17, 18, 19, 20 and 21).

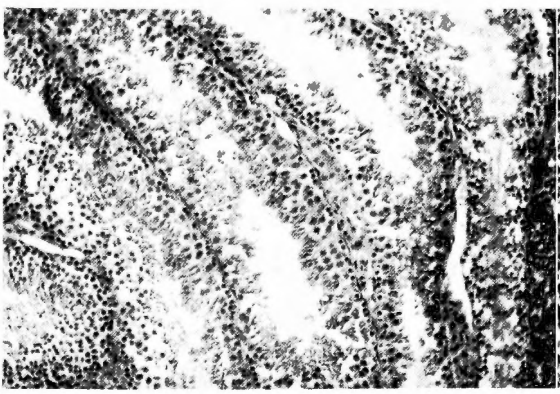

Fig. 16 Testis in Control Mice. H. E. Stain × 100

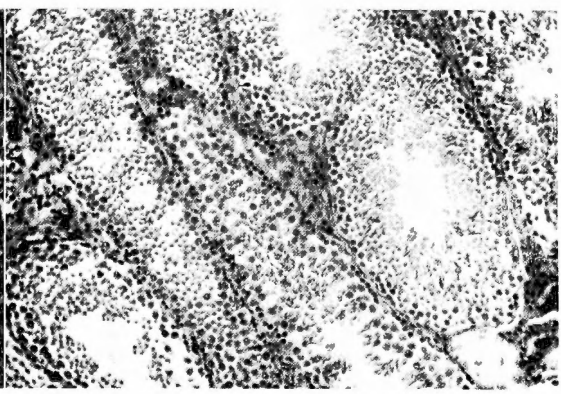

Fig. 17 Testis in Mice Administered Leucin for 10 Weeks. H. E. Stain × 100

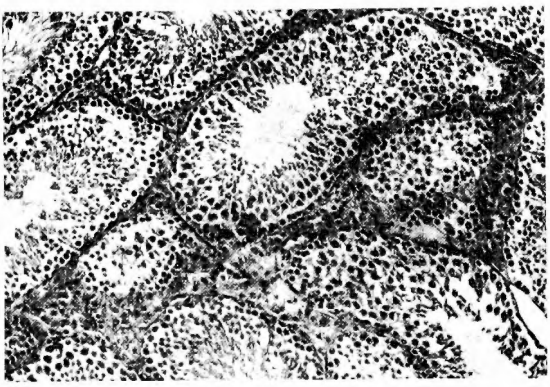

Fig. 18 Testis in Mice Administered Leucin for 20 Weeks. H. E. Stain × 100

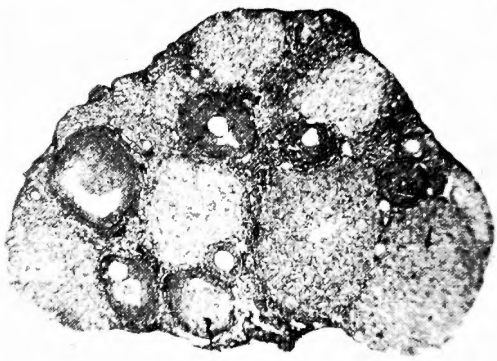

Fig. 19 Ovarium in Control Mice. H. E. Stain × 20

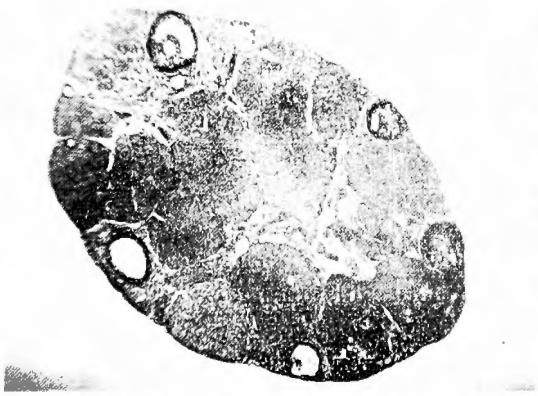

Fig. 20 Ovarium in Mice Administered Leucin for 10 Weeks. H. E. Stain × 20

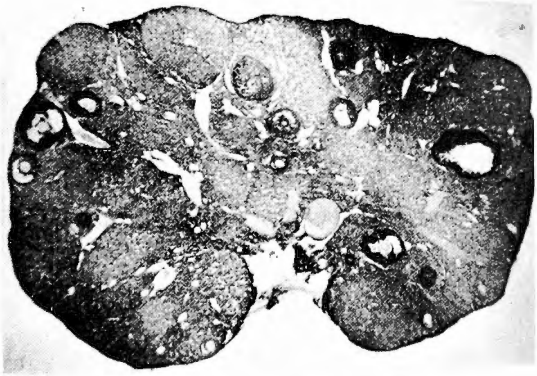

Fig. 21 Ovarium in Mice Administered Leucin for 20 Weeks. H. E. Stain × 20

For the purpose of observing the effect on body weight by the administration of leucin to mice from the intrauterine life, the following experiment was made.

Leucin administered mice were crossed. Also during the period of pregnancy and of lactation, leucin was continuously administered. The offsprings of these mice were put into separate mesh-boxes one month after birth. Thereafter, 150 mg of leucin was administered orally each day for 10 weeks and 20 weeks respectively as previously mentioned, after which the mice were sacrificed and the endocrine organs were histologically examined.

RESULT OF EXPERIMENT

I- BODY WEIGHT

In making a comparison of the body weights of mice one month after birth, the litters each of which consisted of the same number of babies, were used. Namely, 3 groups each consisting of 6 babies of the same litter were used each for leucin administered and control groups, respectively.

In the groups which had been administered leucin since the intrauterine life

an increase in body weight was more marked in comparison to the control groups. Males of the control groups had the maximum body weight of 9.0 g and the minimum weight of 7.0 g, whereas those of the leucin administered groups showed the maximum body weight of 14.0 g and the minimum of 6.0 g. Of the female groups, the control groups showed the maximum body weight of 9.0 g and the minimum of 8.0 g, whereas the leucin administered females showed the maximum of 14.0 g and the minimum of 7.0 g. Namely, Leucin administered groups, the body weight of 12.0 g to 14.0 g were seen in 8 out of 18 mice.

The increase in body weight during the following 10 weeks was practically the same in grade as in the previous experiment and the animals of both sexes maintained the difference in the body weights which had been present from the

Table 7 Body Weight in the Leucin Administered and Control Groups.

	Days	Before	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	70	140
Control Group ♂	No. 1	8.0	8.0	10.0	12.0	11.0	15.0	18.0	17.0	19.0	20.0	18.0	18.0	19.0	18.5	21.0	22.0	24.0	26.0	
	No. 2	7.0	8.0	10.5	11.0	13.0	15.0	16.0	17.0	17.0	16.0	17.0	18.0	16.0	18.0	19.0	19.0	20.0	22.0	
	No. 3	8.0	10.0	10.0	11.0	14.0	14.0	15.5	16.0	18.0	18.0	20.0	20.0	18.5	19.0	19.0	18.0	18.0	20.0	
	No. 4	9.0	10.0	11.0	12.0	12.0	12.0	15.0	16.5	18.0	18.0	21.0	21.5	20.0	20.0	19.0	20.0	20.0	21.5	
	No. 5	7.0	9.5	11.5	12.0	14.0	15.0	16.0	17.0	18.5	19.0	20.0	22.5	20.0	21.0	21.0	20.0	22.5	22.5	
	No. 6	8.5	9.0	9.0	10.5	14.0	16.0	18.0	15.0	20.0	22.0	22.0	23.0	22.0	23.0	22.5	23.0	24.0	24.0	28.0
	No. 7	9.0	10.0	10.0	11.5	13.5	15.5	18.0	18.5	18.5	21.0	22.0	21.0	22.0	21.0	23.0	23.0	23.0	22.0	26.0
	No. 8	8.0	8.0	10.0	11.5	11.0	12.0	14.0	16.0	16.0	17.0	18.0	19.0	20.0	19.0	18.0	18.0	19.0	20.0	21.0
	No. 9	8.0	9.0	10.0	11.0	12.0	14.0	16.0	18.0	19.0	21.5	20.0	20.0	21.0	21.0	22.0	22.0	23.0	22.0	24.0
	No.10	8.0	10.0	9.0	10.5	12.5	13.0	15.5	14.0	16.0	19.0	20.0	19.0	20.0	22.0	23.0	22.5	21.0	20.0	23.0
Leucin Administered Group ♂	No. 1	12.0	13.0	13.0	16.0	16.0	18.0	16.0	17.0	20.0	20.0	22.0	20.0	22.0	23.0	24.0	22.0	23.0	22.0	
	No. 2	8.0	10.0	10.0	12.0	12.0	13.0	14.0	14.0	15.0	16.0	21.0	21.0	20.0	21.0	23.0	20.0	20.0	21.0	
	No. 3	7.0	8.0	7.0	10.0	12.0	11.0	12.0	14.0	16.0	16.0	18.0	20.0	19.0	18.0	19.0	18.0	20.0	20.0	
	No. 4	12.0	11.0	10.0	15.0	17.0	18.0	20.0	18.0	20.0	20.0	24.0	24.0	25.0	21.0	23.0	24.0	23.0	24.0	
	No. 5	14.0	12.0	10.0	10.0	10.0	11.0	12.0	12.0	16.0	16.0	16.0	18.0	20.0	20.0	22.0	22.0	23.0	25.0	
	No. 6	10.0	10.0	16.0	18.0	20.0	20.0	18.0	21.0	24.0	26.0	24.0	25.0	25.0	23.0	25.0	26.0	26.0	28.0	30.0
	No. 7	12.0	18.0	20.0	21.0	23.0	22.0	22.0	24.0	23.0	24.0	21.0	22.0	23.0	26.0	26.0	26.0	27.0	26.0	28.0
	No. 8	9.0	1 50	20.0	22.0	24.0	25.0	25.0	26.0	23.0	23.0	22.0	23.0	26.0	25.0	27.0	25.0	26.0	26.0	32.0
	No. 9	12.0	12.0	16.0	19.0	21.0	22.0	24.0	28.0	26.0	26.0	25.0	26.0	25.0	27.0	24.0	23.0	25.0	24.5	32.0
	No.10	12.0	12.0	16.0	16.0	16.0	18.0	20.0	22.0	24.0	22.5	23.0	23.0	22.0	23.0	22.0	21.0	23.0	24.0	26.0

Table 8 Body Weight in the Leucin Administered and Control Groups.

	Days	Bofore	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	70	140
Control Group ♀	No. 1	8.0	10.0	11.5	13.0	13.5	16.0	17.0	16.0	16.0	17.5	18.0	18.0	18.0	19.5	20.0	20.5	20.0	20.5	
	No. 2	8.0	9.5	12.0	13.5	14.0	16.0	15.0	16.0	18.0	16.5	17.5	18.0	17.5	18.0	18.5	18.5	18.0	18.0	
	No. 3	9.0	10.0	13.0	14.0	16.0	16.0	15.0	18.0	17.0	18.0	19.0	19.5	20.0	20.0	19.5	19.5	20.0	20.0	
	No. 4	8.0	9.5	12.0	14.0	13.0	14.0	17.0	16.5	18.0	18.0	18.5	20.0	20.0	20.5	20.5	20.0	20.0	20.0	
	No. 5	8.0	10.0	12.0	12.0	14.0	19.0	19.0	20.0	20.0	18.0	21.0	23.0	22.0	21.0	22.0	22.0	21.0	23.0	
	No. 6	7.0	10.0	11.5	13.5	14.5	15.5	15.0	18.0	16.0	18.0	18.0	18.0	19.0	20.0	22.0	20.0	20.0	21.0	26.0
	No. 7	9.0	10.0	11.5	12.0	15.0	14.0	15.0	15.0	17.0	18.0	17.0	18.0	18.0	19.0	20.0	19.0	19.0	19.0	21.0
	No. 8	8.0	10.0	11.0	12.0	14.0	14.5	14.0	15.0	16.0	16.0	18.0	18.0	18.0	18.5	19.0	20.0	20.0	20.0	22.0
	No. 9	9.0	10.0	11.5	12.5	14.0	16.0	18.0	16.0	17.0	18.0	20.0	20.0	19.0	18.0	18.5	19.0	19.5	21.0	23.0
	No.10	8.0	9.0	11.0	10.5	13.0	14.5	15.0	14.5	14.5	15.0	17.5	19.0	20.0	21.0	21.0	20.0	20.0	21.0	23.0
Leucin Administered Group ♀	No. 1	7.0	8.0	9.0	12.0	13.0	12.0	12.0	13.0	14.0	17.0	20.0	21.0	20.0	20.0	21.0	21.0	21.0	22.0	
	No. 2	12.0	12.0	12.0	13.0	12.0	14.0	17.0	16.0	18.0	20.0	18.0	20.0	18.0	20.0	22.0	20.0	20.0	20.0	
	No. 3	14.0	10.0	10.0	12.0	13.0	12.0	12.0	14.0	14.0	16.0	16.0	20.0	18.0	19.0	20.0	20.0	21.0	21.0	
	No. 4	12.0	13.0	14.0	15.0	17.0	20.0	20.0	20.0	24.0	25.0	25.0	27.0	24.0	26.0	26.0	25.0	27.0	26.0	
	No. 5	12.0	12.0	16.0	16.0	16.0	16.0	22.0	22.0	24.0	23.0	23.0	23.0	22.0	23.0	22.0	24.0	22.0	24.0	
	No. 6	12.0	13.0	18.0	18.0	18.0	20.0	20.0	21.0	23.0	22.0	21.0	23.0	23.0	23.0	22.0	24.0	23.0	24.0	26.0
	No. 7	12.0	12.0	12.0	13.0	12.0	14.0	17.0	16.0	18.0	20.0	18.0	20.0	18.0	20.0	22.0	20.0	20.0	20.0	24.0
	No. 8	6.0	8.0	10.0	8.0	10.0	12.0	16.0	16.0	18.0	17.0	19.0	20.0	20.0	20.0	21.0	22.0	23.0	23.0	21.0
	No. 9	12.0	16.0	17.0	20.0	22.0	20.0	20.0	20.0	20.0	20.0	19.0	19.0	22.0	20.0	22.0	21.0	22.0	24.0	25.0
	No.10	14.0	15.0	17.0	16.0	20.0	20.0	20.0	20.0	19.0	19.0	18.0	20.0	22.0	22.0	23.0	22.0	23.0	23.0	27.0

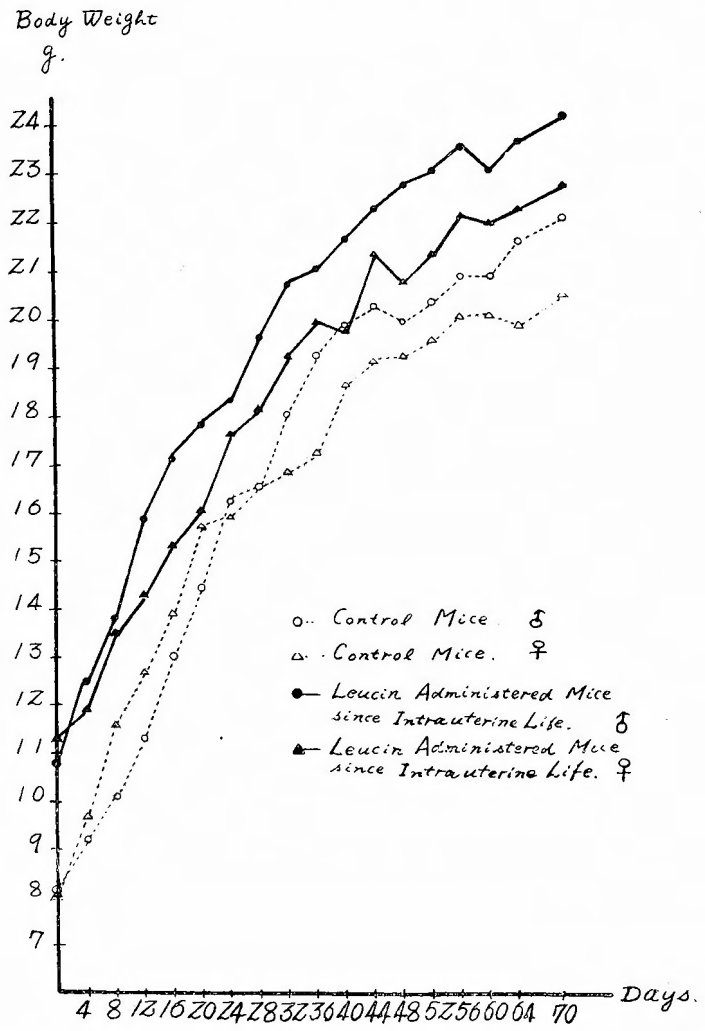

Fig. 22 Body Weight Increase Curve of Both Leucin Administered and Control Mice.

Table 9 Percentages of Three Kinds of Cells in Adenohypophysis of Mice Administered Leucin since Intrauterine Life.

		Acidophilic Cell	Chromophobe Cell	Basophilic Cell
Control	♂	50.2	45.4	4.4
Leucin Administered for 10 Weeks since Intrauterine Life	♂	60.4	35.2	4.4
Leucin Administered for 20 Weeks since Intrauterine Life	♂	61.2	33.7	5.1
Control	♀	49.0	45.5	5.5
Leucin Administered for 10 Weeks since Intrauterine Life	♀	56.8	38.2	5.0
Leucin Administered for 20 Weeks since Intrauterine Life	♀	58.6	34.6	6.8

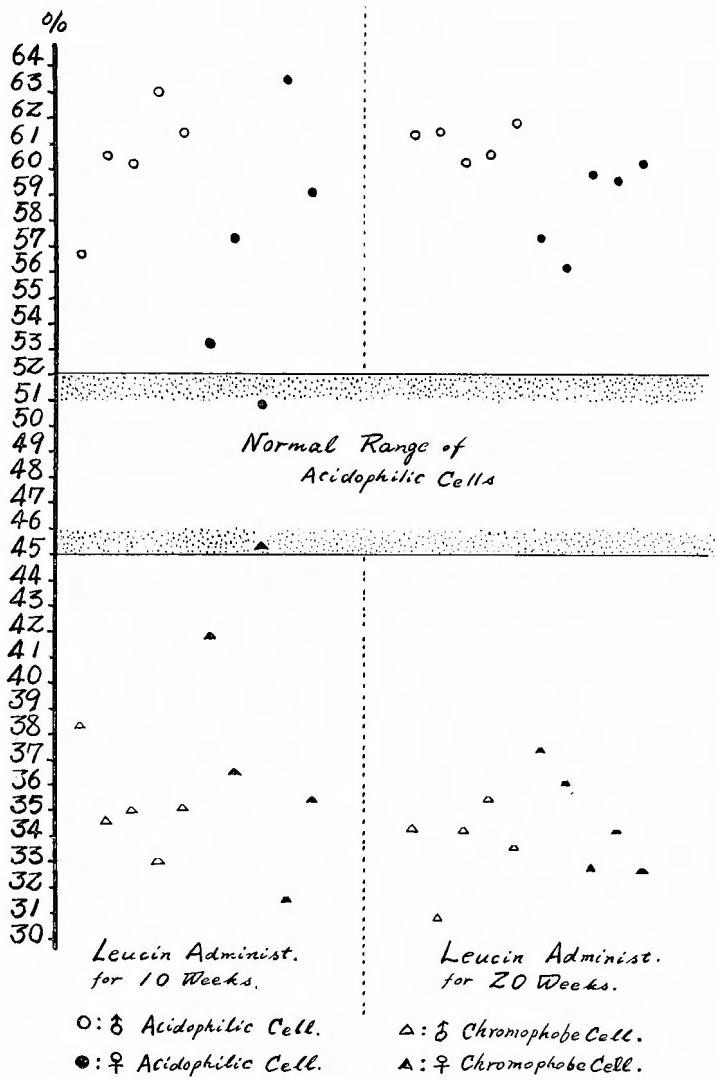

Fig. 23 Percentages of Acidophilic and Chromophobe Cells in Adenohypophysis of Mice Administered Leucin since Intrauterine Life.

commencement of this experiment (one month after birth). After 20 weeks, as in the previous experiment, they showed a much greater difference as compared with the control groups : The average weight of the male control groups was 24.4 g, the maximum being 28.0 g and the minimum 21.0 g, whereas the males of the leucin administered groups showed an average of 29.6 g with the maximum of 32.0 g and the minimum of 28.0 g. The mean value of the female control groups was 23.0 g with the maximum of 26.0 g and the minimum of 21.0 g, whereas the leucin administered female groups showed an average of 25.2 g with the maximum of 27.0 g and the minimum of 24.0 g (Fig. 22. Tables 7 and 8).

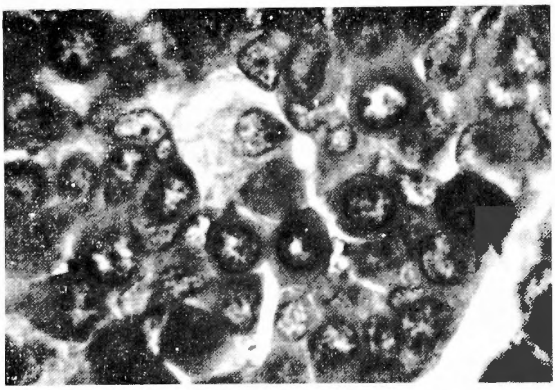

Fig. 24 Hypophysis in Mice Administered Leucin for 20 Weeks since Intrauterine Life. Halmi's Stain × 1000

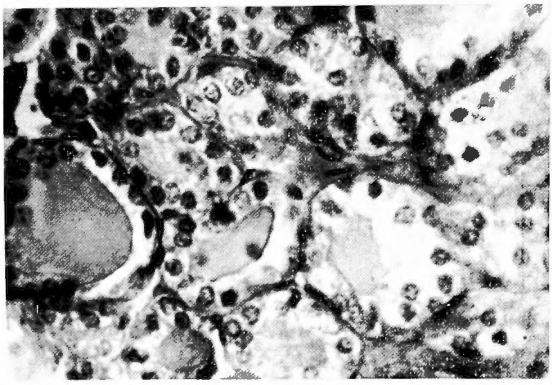

Fig. 25 Thyroid Gland in Mice Administered Leucin for 20 Weeks since Intrauterine Life. H. E. Stain × 400

IV. ADRENAL CORTEX

II. HYPOPHYSIS

Fixation and staining were the same as previously mentioned. In the anterior lobe of the hypophysis there was a considerable increase in α-cells and a decrease in γ-cells in groups to which leucin was administered for 10 weeks and 20 weeks respectively. These changes were more outstanding than in previous experiments. However, neither hypertrophy nor atrophy of the cells and no appearance of any abnormal cell were noticed (Table 9, Figs. 23 and 24).

III THYROID GLAND

Even if a large amount of leucin had been administered to the mice since the intrauterine life, nothing more than a slight change appeared in the thyroid gland. In the mice administered with leucin for 10 weeks, 3 out of 10, and in the mice administered with leucin for 20 weeks, 4 out of 10, showed an increase in height of their epithelial cells, but no change was found in the blood vessels or in the amount of colloid (Fig. 25).

Table 10 Mean Value of Weights of Suprarenals in Both Leucin Administered and Control Groups.

		10 Weeks			20 Weeks		
			Suprarenals			Suprarenals	
		Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
control	♂	22.4	6.6	0.29	24.4	5.0	0.20
Leucin Administration	♂	22.4	6.5	0.29	29.6	7.7	0.26
Control	♀	20.3	13.1	0.65	23.0	12.0	0.50
Leucin Administration	♀	22.6	10.1	0.45	25.2	15.6	0.62

The weight of the adrenal gland and its ratio to body weight showed no remarkable increase or decrease in each of the experimental leucin groups as compared with the control groups (Table 10).

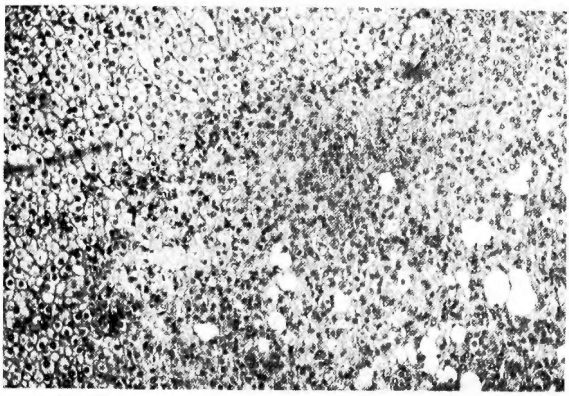

Fig. 26 Adrenal Gland in Female Mice Administered Leucin for 10 Weeks since Intrauterine Life. H. E. Stain × 100

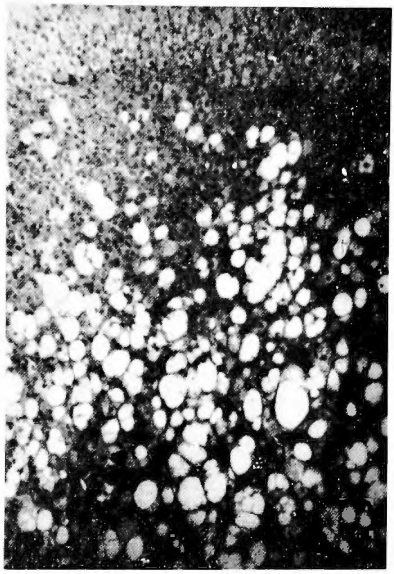

Fig. 27 Adrenal Gland in Female Mice Administered Leucin for 20 Weeks since Intrauterine Life. H. E. Stain × 100

Microscopically, as in the previous experiment, adrenal glands of both the males and the females revealed marked hypertrophy of the cells in the fascicular zone, and the decrease in the lipoid of these cells and hyperplasia and vacuole formation were found in the X-zone of female mice. These changes were more remarkable in mice receiving leucin administration since their intrauterine life (Figs. 26 and 27).

V. GONAD

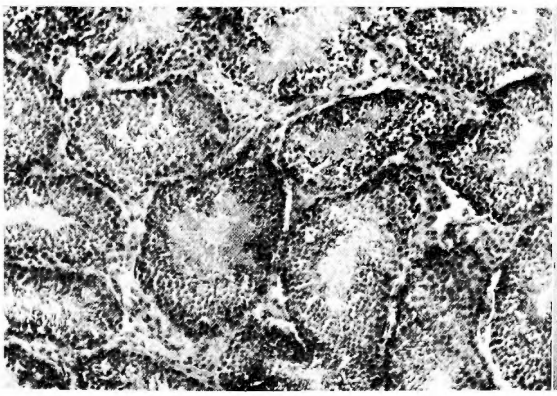

Fig. 28 Testis in Mice Administered Leucin for 10 Weeks since Intrauterine Life. H. E. Stain × 100

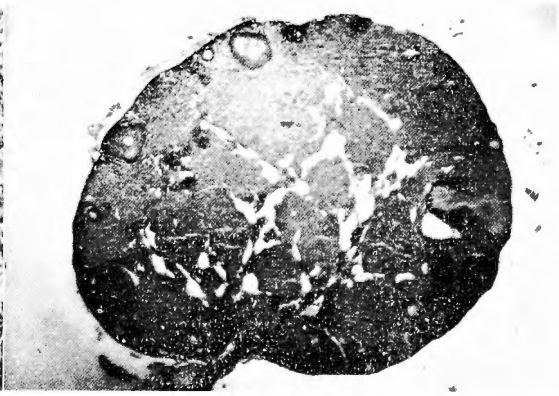

Fig. 29 Ovarium in Mice Administered Leucin for 20 Weeks since Intrauterine Life. H. E. Stain × 20

The weights of the ovarium and the testis and their ratio to body weight in the experimental leucin groups did not show any significant difference from those in the control groups (Tables 11 and 12).

Histological examination revealed a tendency to increase in the interstitial Leydig cells in the testis, but no change in the seminiferous tubles or in the spermatogenesis. In the ovarium, luteinization was exceedingly prominent. No outstanding change in the follicles was found (Figs. 28 and 29).

Table 11 Mean Value of Weights of Testes in Both Leucin Administered and Control Group.

	10 Weeks			20 Weeks		
		Testes			Testes	
	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body weight $\left(\frac{mg}{g}\right)$
Control	22.4	179	8.00	24.4	201	8.24
Leucin Administration	22.4	209	9.33	29.6	210	7.09

Table 12 Mean Value of Weights of Ovaria in Both Leucin Administered and Control Groups.

	10 Weeks			20 Weeks		
		Ovarium			Ovarium	
	Mean Velue of Body Weights (g)	Meight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$	Mean Value of Body Weights (g)	Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	20.3	22.4	1.10	23.0	19.0	0.83
Leucin Administration	22.6	16.4	0.73	25.2	23.8	0.95

VI. BONE

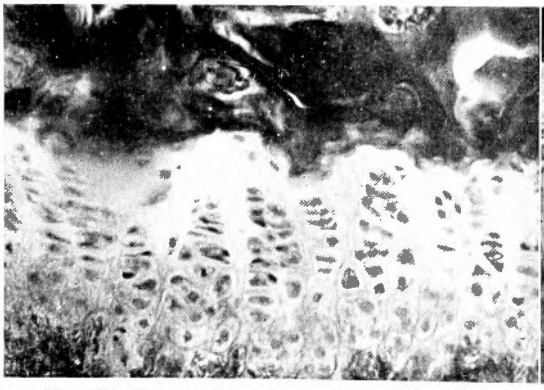

Fig. 30 Bony Tissue in Control Mice. H. E. Stain × 200

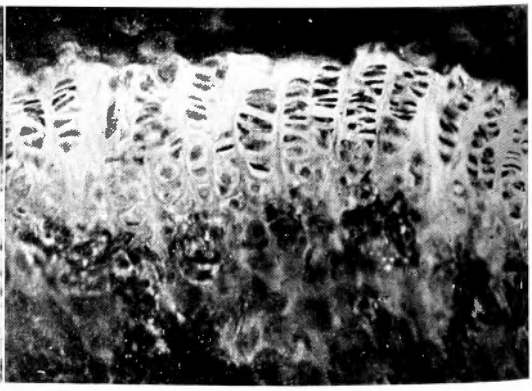

Fig. 31 Bony Tissue in Mice Administered Leucin for 10 Weeks. H. E. Stain × 200

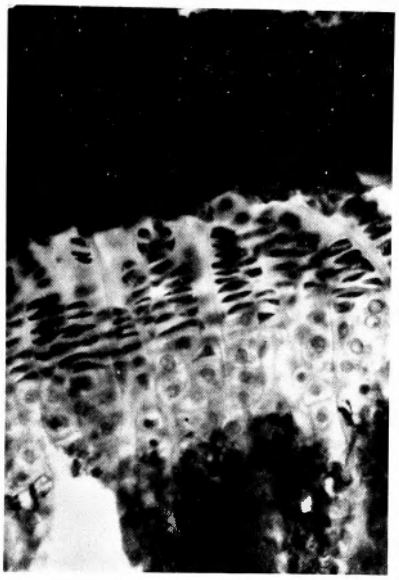

Fig. 32 Bony Tissue in Mice Administered Leucin for 20 Weeks. H. E. Stain ×200

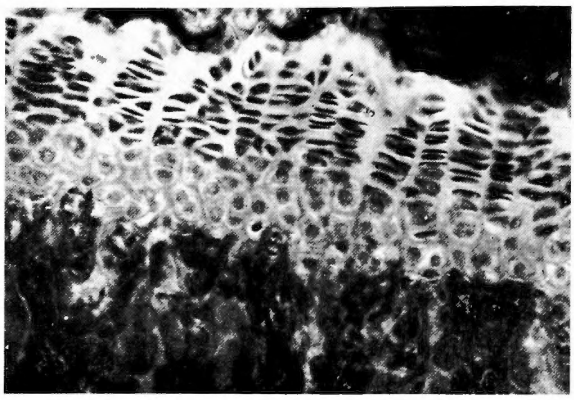

Fig. 33 Bony Tissue in Mice Administered Leucin for 20 Weeks since Intrauterine Life. H. E. Stain × 200

The proximal end of the tibia was taken and decalcified in 10% trichloroacetic acid, imbedded in celloidin and stained by hematoxylin-eosin.

In the 10 week leucin administered mice, the tibia showed a wider epiphyseal line as compared with that of the control mice, and also an advanced differentiation of the cartilage cell columns in the epiphyseal line with several slightly calcified areas in the cartilage.

In the 20 week leucin group, though there was no great difference in the width of the epiphyseal line from that of the 10 week group, the columns of cartilage cells became narrower and the ossifying tissue appeared more abundnatly in the cartilage.

Moreover, in both the 10 week group and the 20 week group of the mice which had received administration of leucin from the intrauterine life, each column of cartilage cells of the epiphyseal line became sparse and hyaloid tissue was found to invade the intercolumnar space. The process of calcification was remarkably advanced and accompanied by hyperactive ossification. This change was much more noticeable in the 20 week leucin administered group (Figs. 30, 31, 32 and 33).

COMMENT

The long-term administration of a large amount of leucin increases the body weight considerably, and promotes the bony growth in the epiphyseal line. If leucin was administered since the intrauterine life, the body weights one month after birth were exceedingly larger, a fact indicating that leucin brings about the most remarkable effect on the embryonal body growth.

Moreover, an increase in α-cells in the adenohypophysis was recognized to be more prominent when leucin was administered for long periods. This seemed to be roughly parallel in grade with an increase in body weight. In the adrenal cortex, as was seen in the case of ACTH administration for a long time, the hypertrophy of the cells in the fascicular zone and the decrease in lipoid in those cells were noticed, both changes being the definite evidences of the hyperactivity of the adrenal cortex. Moreover, an interesting change is that prominent hypertrophy and hyperplasia, and vacuole formation of the X-zone in female mice were noticed. Such changes were more prominent in those which had received administration of leucin for long periods.

In the gonad, an increase in the interstitial cells in the testis and the increased luteinization in the ovarium were noticed. There was nothing particular in the change of the thyroid gland.

EXPERIMENT II

Since Stokstand announced that aureomycin effected the accelerated body growth of young animals (1949), similar effects were reported one after another of many other antibiotics.

In 1955, Ozawa administered aureomycin, achromycin and penicillin to young mice orally and/or parenterally and noticed that this accelerated the body growth. He assumed that the effect was due to the hyperfunction of the pituitary-adrenocortical system.

In this experiment I have orally administered 10 mg of achromycin every day to mice one month after birth, and have weighed them in the early morning before feeding every fourth day, and have killed them after forty days to examine histoiogically the endocrine organs and bones.

RESULTS OF EXPERIMENT

I. BODY WHIGHT

Table 13 Body Weight in the Achromycin Administered and Control Groups.

Days		Before	4	8	12	16	20	24	28	32	36	40
Control Group	No. 1	8.0	10.0	10.0	11.0	14.0	14 0	15.5	16.0	18.0	18.0	20.0
	No. 2	9.0	10.0	11.0	12.0	12.0	14.5	15.0	16.5	18.0	18.0	21.5
	No. 3	7.0	9.5	11.5	12.0	14.0	15.0	16.0	17.0	18.5	19.0	22.5
	No. 4	8.0	10.0	11.0	11.0	12.5	13.0	14.0	15.0	16.5	18.5	20.0
	No. 5	7.0	8.5	10.0	11.0	11.5	12.5	15.0	16.0	19.0	20.0	21.5
	No. 6	10.0	11.0	12.0	12.5	11.0	14.0	17.0	17.0	18.5	20.5	21.5
Achromycin Administered Group	No. 1	10.0	11.0	10.0	13.0	15.0	16.0	16.0	18.0	20.0	20.0	21.0
	No. 2	9.0	11.0	12.0	12.5	15.0	16.0	17.0	20.0	20.0	20.0	21.5
	No. 3	10.0	10.0	11.0	12.0	13.0	16.0	16.0	18.0	20.0	21.0	23.0
	No. 4	8.0	11.0	11.0	12.5	13.0	17.0	16.5	18.0	18.0	20.0	23.0
	No. 5	9.0	11.0	12.0	14.0	13.0	15.0	16.0	17.0	19.0	20.0	23.0
	No. 6	10.0	10.0	11.5	12.5	15.0	17.0	18.0	20.0	19.5	23.0	25.0

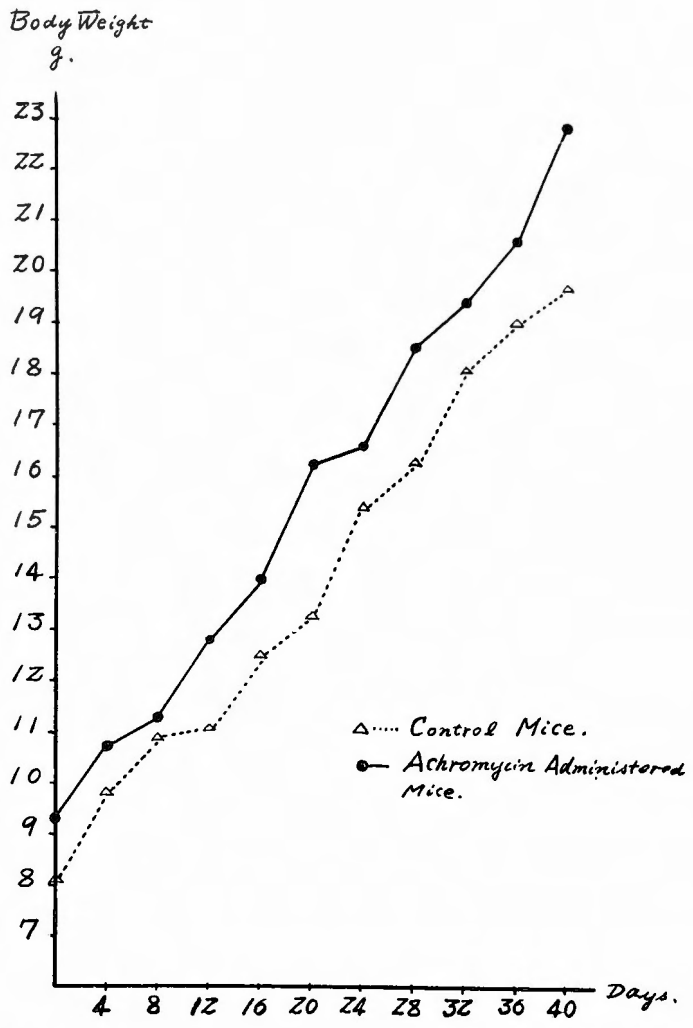

Fig. 34 Body Weight Increase Curve of Both Achromycin Administered and Control Mice.

The degree of the increase in body weight during the period of 40 days from the beginning of this experiment was almost the same as that of OZAWA's experiment. The experimental group to which achromycin had been administered began to show a greater increase in body weight than the control group from the third week after commencement of the experiment. The increase was almost the same in degree as that obtained by the leucin administration (Fig. 34 and Table 13).

II. HYPOPHYSIS

Some increase in the eosinophilic cells and decrease in the chromophobe cells of the adenohypophysis were seen in the achromycin administered groups, though the changes were not so marked. Besides, there was no change in the morphology of the cells (Table 14).

Table 14 Percentages of Three kinds of Cells in Adenohypophysis of Achromycin Administered Mice.

	Acidophilic Cell	Chromophobe Cell	Basophilic Cell
Control	50.2	45.4	4.4
Achromycin Administration	52.1	42.7	5.2

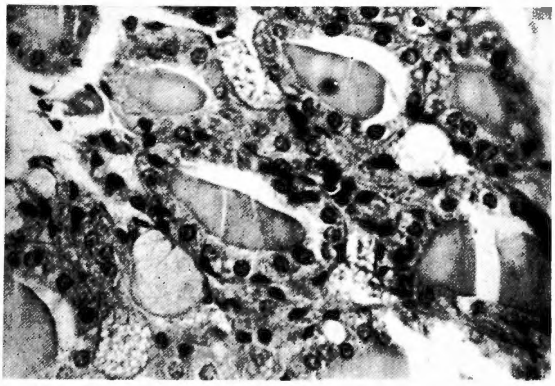

Fig. 35 Thyroid Gland in Mice Administered Achromycin. H. E. Stain × 400

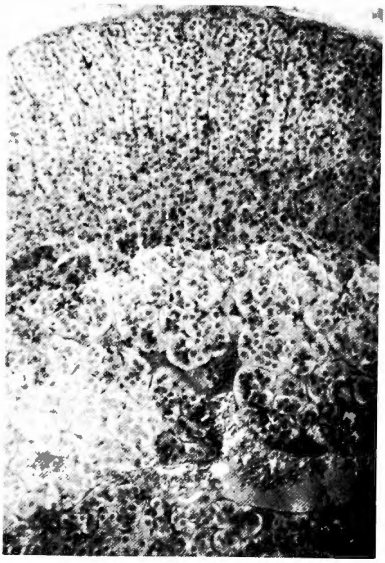

Fig. 36 Adrenal Gland in Mice Administered Achromycin. H. E. Stain × 100

III. THYROID GLAND

In the achromycin administered mice, no histological change took place in the thyroid gland (Fig. 35).

IV ADRENAL CORTEX

The weights of the adrenal gland and their ratio to the body weight in the achromycin administered groups showed, though slight, a tendency to decrease in comparison with those of the control groups. But histologically, no special change could be found (Table 15 and Fig. 36).

Table 15 Mean Value of Weights of Suprarenals in Both Achromycin Administered and Cotnrol Groups.

	Mean Value of Body Weights (g)	Suprarenals	
		Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	19.7	5.3	0.27
Achromycin Administration	22.8	3.3	0.14

V. GONAD

The testicular weights and their ratio to the body weights of the achromycin administered mice showed some decrease in comparison with the control group. But histologically, no change could be noticed of the interstitial cells, of the seminiferous tubles and of the spermatogenesis (Table 16 and Fig. 37).

Table 16 Mean Value of Weights of Testes in Both Achromycin Administered and Control Groups.

	Mean Value of Body Weights (g)	Testes	
		Weight (mg)	Ratio to Body Weight $\left(\frac{mg}{g}\right)$
Control	19.7	170	8.63
Achromycin Administration	22.8	123	5.49

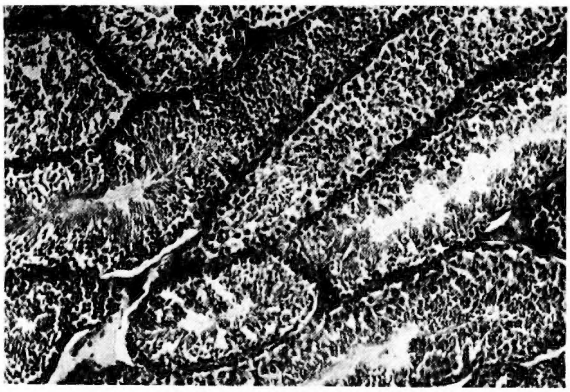

Fig. 37 Testis in Mice Administered Achromycin. H. E. Stain × 100

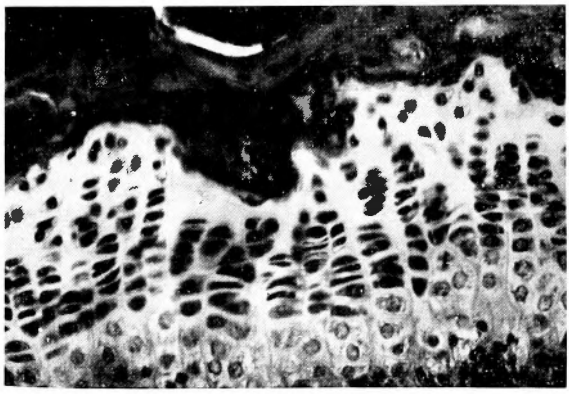

Fig. 38 Bony Tissue in Mice Administered Achromycin H. E. Stain × 200

VI. BONE

The proximal end of the tibia was histologically examined, but there was no outstanding difference in the width of the epiphyseal line, the differentiation grade of the cartilage cell columns, etc., between the achromycin administered group and the control group and, moreover, no histological change of bony growth could be found in the achromycin group (Fig. 38).

COMMENT

It is certain that the administration of achromycin to mice causes some increase in the body weight, and in the eosinophilic cells of the hypophysis, with a decrease in the chromophobe cells, but no particular histological change in other endocrine organs. No sign of hyperactivity of bony growth could be found.

DISCUSSION

The anterior pituitary cells have been classified into three kinds of cells, namely the eosinophilic cells (α-cells), the basophilic cells (β-cells) and the chromophobe cells (γ-cells). Recently, HALMI (1950, 1952), and PURVES and GRIESBACH (1951) described that the basophilic cells of the hypophysis were to be divided into two types of cells from the affinity of the granule for aldehyde fuchsin or

light green. The one is a β-cell in a narrow sense which has the affinity for aldehyde fuchsin, and the other is a δ-cell which can be easily stained with light green.

However, it has not been decided what kind of pituitary hormones originates from each kind of adenohypophysial cells.

Benda and Fischer (1910), and Bailey and Davidoff (1925), etc. reported, according to the histological findings of the anterior lobe of the pituitary gland in the case of acromegaly, that the growth hormone is secreted probably from α-cells, and this assumption seems to be definite.

In regard to thyrotrophin, Zeckwer and coworkers (1935) and Morris (1951) claim that the thyroidectomy cells (T-cells) originate from the α-cells, and Scharf and Forster (1954) claim that they originate from the γ-cells, or Purves and Griesbach (1954) and Halmi (1952) claim that they originate from the δ-cells, and thus in any case, nothing is definitely known at present; recently, the theory that thyrotrophin is secreted from δ-cells seems to be prevailing.

As regards gonadotrophin, there is a theory which claims that the β-cells secrete gonadotrophin, because the amount of gonadotrophin increases in the urine after castration and, at the same time, β-cells increase in the hypophysis. On the other hand, Wolfe (1935) or Shadwick (1936) reported that the α-cells of hypophysis in rats, in the stage of oestrus, were filled with granules which were dyed with orange G, and that during their stage of diestrus, the decrease in the number and in the stainability of these granules was acknowledged, and on the other hand, the β-cells, during their stage of proestrus, carried a large amount of basophilic granules which extremely decreased during the stages of oestrus and metestrus. Therefore, it has been assumed that FSH is secreted from the β-cells and LH from α-cells.

As regards ACTH, there is no definite theory at present. Finerty (1952) in an experiment of stress given by hot water, reported that there was a slight increase in basophilic substance and an outstanding increase in granules stainable with the acid hematein in the protoplasm of α-cells, but no change whatsoever in the β-cells. Ever since, a theory that ACTH was secreted from α-cells became prominent. Moreover, Herbaut (1952) succeeded in separating the granules from α-cells using a centrifugal method and acknowledged an ACTH-like function as well as an LH-like function in these granules. He also reported that he found a TH-like as well as an FSH-like function in the granules separated from the β-cells by the same way.

In leucin administered mice, I found an outstanding increase in the number of α-cells and a decrease in that of γ-cells in the adenohypophysis, but noticed no definite change in the number of β-cells. In addition, I found, in the adrenal cortex, a hypertrophy of the fascicular zone and a decrease in the lipoid drop within the cells. These findings of the adrenal cortex are quite similar to the change in the adrenal cortex of the animals administered ACTH for long periods. Also, I found a tendency to increase in the interstitial Leydig cells in the testis,

and that to increase in corpora lutea in the ovarium. These changes seem to have an intimate relation with the increase in the α-cells of the hypophysis, and to support the theory that ACTH and LH are secreted from α-cell.

CONCLUSION

I. The oral administration of a large amount of leucin brought about the increase in body weight to some extent and, at the same time, the promotion of the bony growth in the epiphyseal line. The increase in body weight was much more remarkable when leucin was administered since the intrauterine life.

2. The eosinophilic cells of the adenohypophysis showed a prominent increase in the leucin administered mice but no morphological change of the cells was noticed.

3. In the adrenal cortex, the hypertrophy of the cells in the fascicular zone and the decrease in lipoid within those cells were observed. These findings were similar to those gained when ACTH was administered to the animal for long periods. In addition, in the case when leucin was administered to female mice, a marked hyperplasia of cells and a vacuole formation in the X-zone were found.

4. In the testis, an increase in the interstitial Leydig cells was noticed, and in the ovarium corpora lutea were found to increase in number.

5. As regard the thyroid gland, no special change was noticed.

6. In the experiment of achromycin administered mice, some increase in the body weight and some increase in the α-cells of the hypophysis were found but no marked histological change was noticed in other endocrine organs.

From the above findings, I uphold the theory that ACTH and LH are secreted from α-cells.

Thanks are expressed to Dr. Naoki Kageyama for his kind suggestions during this study.

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和 文 抄 録

経口的ロイシン大量投与マウスに於ける内分泌臓器並びに骨の組織学的検索

京都大学外科学第1講座 (指導：荒木千里教授)
今 井 昭 和

ACTH或は副腎皮質ホルモンがアミノ酸の代謝と密接な関係を有する事は F. L. Engel 及び若干の人々により報告されているが，逆に一定のアミノ酸が或種のホルモン分泌に関係を有すると云う事は，M. Harada et al によるヒスチヂン及びロイシンと下垂体の関係を論じた以外には無い様である．

私は生後1ヵ月のマウスに大量のロイシンを経口的に長期間の投与を試みたところ，若干の体重増加を認めると共に，脛骨の骨端線部組織像に於て骨生長の促進像を認め，同時に脳下垂体前葉に於ては著明な酸好性細胞の増加，嫌色素性細胞の減少を認めた．更に副腎皮質に於ては束状帯細胞の肥大及びリポイドの減少を認め，これは ACTH 長期投与における副腎皮質の像と一致した．更に又，性腺の変化として，睾丸の Leydig 細胞の増加，卵巣の黄体形成促進像が認められた．かゝる副腎皮質及び性腺の変化は脳下垂体に於ける酸好性細胞の増加と密接な関係を有するものと思われる．

更に興味ある変化として，胎生時よりロイシンの投与を行つた場合，生後1ヵ月目の体重にかなり著明な増加が認められた．

更に又，ロイシン投与群の雌性マウスの副腎皮質に於て X-Zone の著明な増殖肥大，及び空胞形成が認められたが，その発生機構については今後研究にまちたい．

又一方，種々の抗生物質が幼弱動物の発育を促進する事が知られているが，私は Achromycin の少量を経口的に投与し，内分泌臓器及び骨の組織学的検索を行つたが，Achromycin 投与に際しては若干の体重増加と，下垂体に於ける酸好性細胞の増加以外には特別変つた変化は認められなかつた．