STUDIES ON ADRENOCORTICAL FUNCTION IN INSULIN-TREATED COMPLETELY DEPANCREATIZED DOG

by

KOTARO AIKAWA

From the 2nd Surgical Division, Kanazawa University Medical School
(Director; Prof. Dr. ICHIO HONJO)

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CONTENTS

I. INTRODUCTION

Concerning the relationship between diabetes mellitus and the pancreas, THOMAS COWLEY[1] first noticed its disturbance as a cause of diabetes, and after that ROKITANSKY observed the mutual relationship between diabetes and the pancreas, although there has remained a problem unsolved.

Since in 1899 von MEHRING and MINKOWSKI[2] confirmed the development of diabetes mellitus in the completely depancreatized dog, many studies as regards diabetes have made

rapid advance. Following the success of BAILEY and BAILEY[3] using alloxan in 1943, experimental studies on diabetes have come to be carried out with ease.

DRAGSTEDT[4] observed the development of fatty liver in totally depancreatized dogs and noticed the deficiency symptom of total pancreatectomy. In 1943, ROCKEY[5] tried for the first time clinically total pancreatectomy for pancreatic cancer, and then PRIESTLY[6] reported a case of total pacreatectomy for hyperinsulinism due to islet-cell adenoma which survived for 5 years. In this case also the deficiency symptom of total pancreatectomy was the problem of utmost importance.

The deficiency symptom of total pancreatectomy can be classified as follows;

1) The deficiency of exocrine function of the pancreas, i.e, loss of salts and digestive ferments.
2) The deficiency of endocrine function of the pancreas, the development of characteristic diabetes which is due to the total pancreatectomy, i.e, the loss of insulin and glucagon. Although there are many reports[7][9][37][42] concerning the correlation between pancreatic diabetes and other endocrine functions, the majority of these deal with the internal diabetes mellitus or alloxan diabetes, and the results do not come to an accordance.

As to the adrenal cortex of the totally depancreatized animals, there are several brief discriptions, most of which contain the problems to be solved. Supposedly these experimental results might have been influenced by the degree of diabetes according to whether or not insulin was administered or they had some complication of infection and digestive disturbances.

In the present experiment, the adrenocortical response in completely depancreatized dogs was studied under the condition in which the blood sugar level was controlled to be about 200 mg/dl with insulin administration and the digestion and absorption was improved with pancreatin etc.

II. METHOD OF EXPERIMENT

1. Material:

Adult mongrel dogs of both sexes were employed for this experiment.

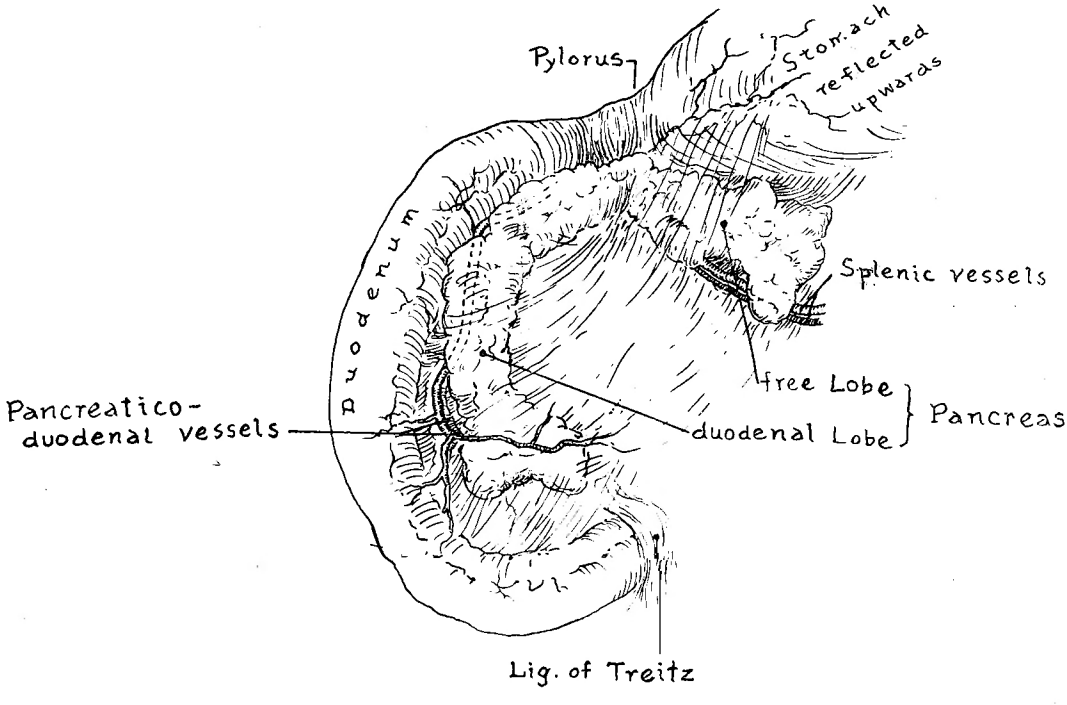
2. Method:

Since the adrenocortical function is participated in various metabolism, there are many various methods applicable in adrenocortical function test. Recently, it is reported[8][9][11][37][42] that the estimation of the glucocorticoid level, especially of the 17-OHCS, indicates the adrenocortical function most correctly, in the case in which the disturbance of carbohydrate metabolism, as diabetes mellitus, is playing an important role.

As serious diabetes developed following total pancreatectomy, in this experiment, the levels of free plasma fluorogenic corticoids (KENDALL's Compound F-like substance) were estimated by SWEAT-TAKEDA's method[13][11]. And as BLISS[10] and others[12][13][14][15][38] reported that the response of 17-OHCS level to ACTH is a good indication to estimate the adrenocortical reserve, the fluctuation of Cpd-F level after the administration of ACTH was observed. Moreover the research was made on the relationship between the Cpd-F level and the blood sugar level, and on the influence of insulin upon the adrenal cortex before

and after total pancreatectomy. At the same time circulating eosinophils and the rate of decrease in eosinophils, and adrenal weight were measured and histological findings of adrenal cortex were examined.

A : Total pancreatectomy in dogs[16].



After dogs were bred at least for 2 or 3 weeks, the blood examination was performed and then the dog was operated on. Cocktelin H 50 mg was injected intramuscularly as a premedication, and the operation was performed as follows ; Under the anesthesia of isozol about 10 to 20 mg/kg, an upper median incision was made. The pancreas in the dog is free from retroperitoneal wall and consisted of two parts, duodenal lobe and free lobe. The pancreas was completely removed by isolating duodenal lobe from the duodenal wall by tying and cutting off the branches of the pancreaticoduodenal vessels and by isolating free lobe from the mesenterium by tying and cutting off the branches of the gastrolienal vessels.

B ; Postoperative regulation of the blood sugar level.

Although hyperglycemia, of 200 to 400 mg/dl of blood sugar level, developed several hours after total pancreatectomy, insulin was first injected after the dog was awakened from anesthesia and recovered its appetite. As a usual dose, 1 to 5 units of insulin per kg a day was used according to YAO's[17] results, and as a control group 0 1 to 0 5 units of insulin per kg was injected a day. And it was assured that fatty infiltration in liver was observed in the latter but not in the former by Sudan III staining.

C ; Estimations.

The blood sample was taken in fasting state from the femoral vein without anesthesia.

1) Free plasma fluorogenic corticoids (Cpd-F like substance) was estimated by SWEAT-TAKEDA's[18)19)] method. Procedures are as follows;

Reagents:

1. Chloroform; 100 mg of 2,4-dinitrophenylhydrazine is added to 1 L of chloroform, refluxed for several hours at 70°C and then redistilled.
2. Petroleum ether; 1/10 volume of conc. sulfric acid is added to petroleum ether, shaken and distillated between 45°C and 50°C.
3. Ethanol; 5 g of zinc powder is added to 1 L of absolute ethanol, refluxed to boiling for 3 hours, and then redistilled.
4. 0.1 N. NaOH;
5. Phosphoric acid; guaranteed.
6. Silica gel; for chromatography.
7. Phenylhydrazine hydrochloride.

Extraction of corticosteroids from plasma;

Four cc of sample of heparinized plasma is vigorously shaken (ca 600 times) for extraction with 5 volumes of chloroform once, in a seperatory funnel. Chloroform layer is washed with 0.1 volume of 0.1 N. NaOH and distilled water respectively, and transferred into a stoppered test tube and the solvent distilled off in vacuum to dryness at about 50°C. Then the residue is dissolved in 1 cc of 70 % ethanol solution and partitioned between petroleum ether, and this procedure is repeated three times. The ethanol layer is distilled off in vacuum to dryness and the residue dissolved in a small amount of chloroform (0.5cc).

Silica Gel Column Chromatography (Ultramicrotechnique):

Preparation of Column; A small piece of filter paper is put on the bottom of the column to support the adsorbent. One hundred mg of Silica Gel is settled, and 4 cc of 10% EtOH-CHCl, next 4 cc of chloroform are poured into the column for cleaning. The chloroform extract from the material is transfered into the column. After the chloroform extract is adsorbed completely by Silica Gel, additional 3 cc of chloroform is let through the column. Eight cc of 1% and 5% EtOH-CHCl are poured into the columr, and these effluents are collected in a 20 cc test tube and are evaporated to dryness below 50°C. Four cc of 85 % phosphoric acid is added to each residue of EtOH-CHCl effluents, which is well mixed and the solution is heated in boiling water for 25 min. After rapid colling in tapped water, fluororescence which is induced by steroids in each fraction, is estimated by a fluorometer of BECKMANN-type. Intensities of fluorescence are measured on a microammeter, comparing with that of standard hydrocortisone (2γ) and adjusting phosphoric acid alone to zero. As a light source a tungsten lamp is used and the primary filter is K-7 (450 mμ), the secondary filter is YA-3 (520 mμ). In parallel with these procedures, a blank test should be carried out in the same manner, using the reagents alone.

The concentration of the corticosteroids is calculated according to the following formula.

$$\text{Fluorogenic corticoids} = (A-B) \times 2/100 \times 100/P \quad (\gamma/dl)$$

A; The intensity of fluororescence in the sample.

B; Blank.

P; Plasma volume.

Remarks; The intensity of fluororescence produced by corticosterone is estimated to

be equal to 50 % of that by hydrocortisone, therefore the reading of Cpd-B fraction should be duplicated when hydrocortisone is used as a standard sample. Since the level of Cpd-B fraction did not show a credible tendency in the present experiment, it was not adopted.

2. Blood sugar level was determined by SOMOGYI-NELSON's method[29].

3. Circulating eosinophils were calculated by the FUCHS-ROSENTHAL's hemocytometer, using HINKELMANN's solution.

4. Total cholesterol and esterified cholesterol were determined by Bloor's Acetic anhydride method[31].

D ; Histological examinations.

As soon as the dog died postoperatively or after slaughter with rapid intravenous injection of a large dose of isozol, laparotomy was performed and the adrenal glands were taken out from the surrounding adipose tissue in a state as fresh as possible. The adrenals were weighed in the torsion balance, which were then fixed in 10 % neutral formalin solution, and were put on the following histological examination.

1) Hematoxylin-eosin double staining.
2) Sudan III staining.
3) Ketosteroid staining by the ASHBEL-SELIGMAN's method[1].

III. RESULTS

(I) Changes of plasma Cpd-F level after total pancreatectomy.

Before the operation plasma Cpd-F level was estimated in a fasting state The post-operative estimation was commenced with an interval of 2 weeks from the 10 th day or the 14 th day after the operation, since the influence of the surgery seemed to have disappeared and the blood sugar level was relatively stable towards the 10 th day to the 2 nd week.

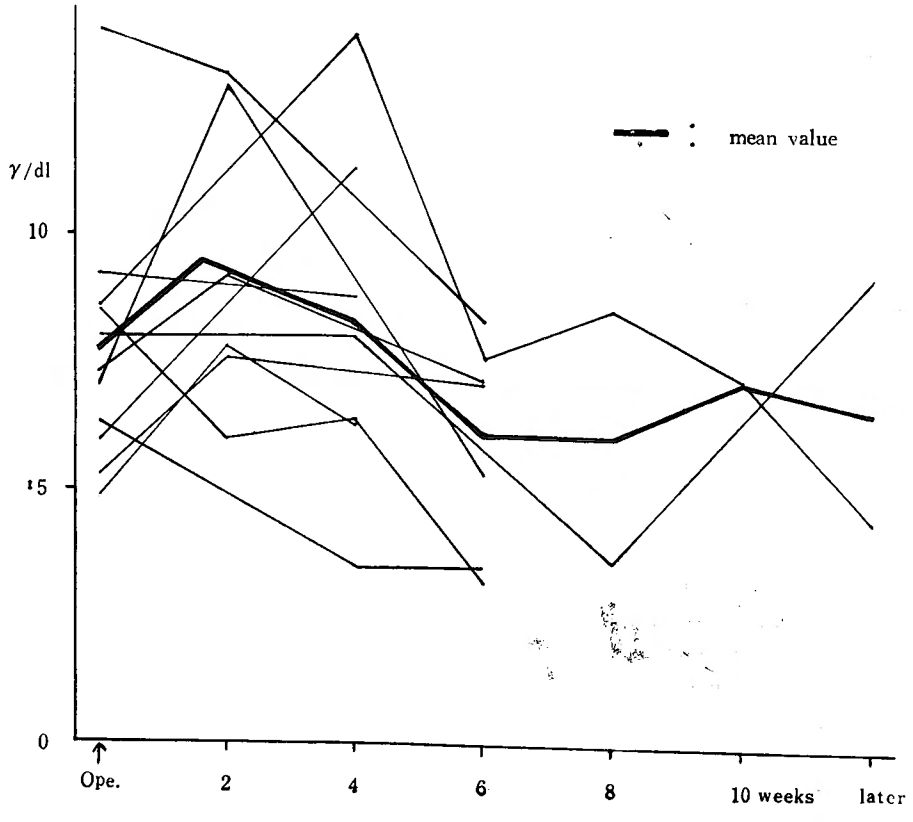
The level of plasma Cpd-F in dogs was reported to be 8 0 to 16 7 γ/dl by Takeda. In the present experiment, the level was estimated to be 4 9 to 14 0 γ/dl, 7 74 γ/dl on the average, being slightly lowered than Takeda's report. After the total pancreatectomy (Table 1, Fig. 1), the highest level on the average was observed towards the postoperative 2nd week, i. e , 9.45 γ/dl, which maintained as high a level as 8 12 γ/dl until the 4th week, and decreased to approximately 6.0 γ/dl thereafter. In some cases, the dogs could survive relatively long, and the level showed reelevation. It was supposedly because the physiological condition were so much influenced by various factors after the operation that the individual deviation of the level was relatively marked, and a clear constant tendency was hardly observed.

After total pancreatectomy, there was noticed a period of adrenocortical hyperactivity due to the mobilisation of glucocorticoid for gluconeogenesis from protein, which was caused by hyperglycemia, glycosuria and consumption of glucose[13][15]. However, when the blood sugar level was controlled to some extent by the administration of insulin and at the same time the loss of body weight was being prevented by the well-managed digestion and absorption with pancreatin, the adrenocortical function, which once accelerated, seems to be lowered corresponding to hyperglycemia.

Table. 1 Fluctuation of the plasma Cpd-F level after total pancreatectomy

Dog No.	Sex	Plasma Cpd-F level (γ/dl)						
		Before ope.	Week after operation					
			2	4	6	8	10	later
No. 40	♂	8.6		13.9	7.6	8.5	7.2	4.5
42	♂	6.3		3.5	3.5			
45	♀	8.0		8.0		3.6		9.2
51	♂	8.5	6.0	6.4	3.2			
53	♂	4.9	7.8	6.3				
54	♀	14.0	13.2		8.3			
56	♀	9.2		8.8				
58	♀	5.3	7.6	7.3	7.1			
66	♂	6.0		11.3				
67	♀	7.1	12.9		5.3			
77	♀	7.3	9.2		7.1			
Mean		7.74	9.45	8.18	6.01	6.05	7.2	6.53

Fig. 1 Changes of the plasma Cpd-F level after total pancreatectomy



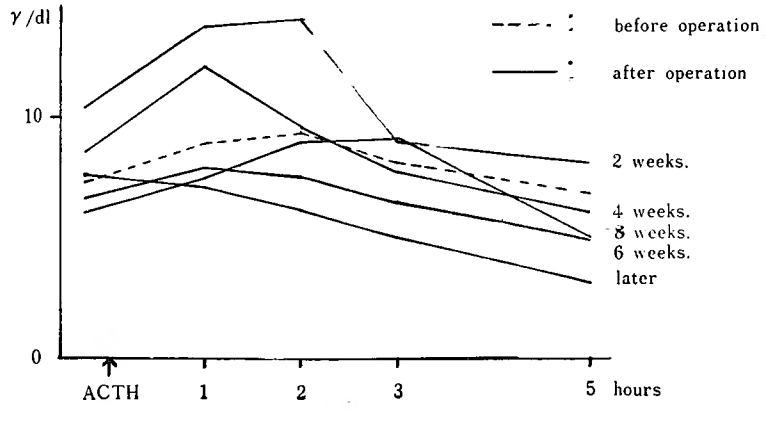
(II) Fluctuations of plasma Cpd-F level after ACTH administration.

Ten units of ACTH Schering diluted in 50 cc of physiological salt solution was injected intravenously into the femoral vein gradually, and blood samples were taken before and in the 1, 2, 3, and 5th hour after ACTH administration and the amount of Cpd-F was measured. The dogs were fasted during the examination. (Table 2, Fig. 2) Before the

Table. 2 Fluctuation of the plasma Cpd-F level after ACTH administration (Before total pancreatectomy)

Dog No.	Sex	Plasma Cpd-F level (γ/dl)				
		Before ACTH adm.	Hours after ACTH administration			
			1	2	3	5
No. 3	♂	9.5	10.8	11.9	11.3	7.2
5	♂	3.0	7.4	2.9	4.7	3.1
13	♂	5.7	6.5	7.6	9.6	10.3
16	♂	5.9	9.5	8.2	7.0	5.2
34	♂	4.7	6.7	8.6	12.7	7.4
40	♂	8.6	8.0	10.6	9.2	3.0
42	♂	6.3	8.2	11.4	14.5	9.8
45	♀	8.0	9.8	6.7	7.2	7.2
47	♀	8.8	10.8	11.3	8.8	7.8
53	♀	4.9	8.9	8.2	3.2	6.2
56	♀	9.2	7.9	12.5	6.2	5.9
66	♂	6.0	9.2	9.1	7.1	5.2
67	♀	7.1	7.3	8.8	5.8	6.1
77	♀	7.3	10.2	7.3	7.5	7.0
54	♀	14.0	16.0	16.5		11.8
Mean		7.26	8.88	9.44	8.20	6.88

Fig. 2 Fluctuations of the plasma Cpd-F level after ACTH acministration (Mean value)



operation, the level of Cpd-F was 7.3 γ/dl on the average and elevated to the peak of 9.44 γ/dl at the 2nd hour after ACTH administration, and then showed the lower value

than that before administration of ACTH at the 5th hour. In all cases, almost the similar tendency was observed. In depancreatized dogs about 2 weeks after the operation, the level of Cpd-F showed relatively a high value of 10 38 γ/dl on the average, and elevated to the peak of 14 12 γ/dl at the 2nd hour after ACTH administration (Table 3, Fig. 2), showing increased adrenocortical reserve. But at the 3rd hour after ACTH administration, the value was so markedly lowered than the level before ACTH administration that the steadfast hyperactivity of the adrenal cortex was hardly recognized. In the 4th week after the operation (Table 4, Fig. 2) the level was estimated to be 8.55 γ/dl and elevated to 12.18 γ/dl at the lst hour after ACTH administration, and it gradually decreased later. In the 6th week (Table 5, Fig. 2), the level was lowered and shifted slightly, that is, from 6.66 to 7.93 γ/dl at the lst hour after ACTH administration. In the 8th week (Table 6, Fig. 2), the level was also lowered, 6.05 γ/dl on the average and the elevation to 9.2 γ/dl at the 3rd hour after ACTH administration, suggesting the slight convalescence of adrenocortical reserve. It is suggested that the animals of this group could happen to survive relatively for long periods owing to their remained vitality. After the 8th postoperative week (Table 7, Fig. 2), the adrenocortical reserve was supposed to have vanished. Comparing the individuals with each other case, for example, in No. 40 and No. 45, it

Table. 3 Fluctuation of the plasma Cpd-F level after ACTH administration (2 weeks after operation)

Dog No.	Sex	Days after ope.	Plasma Cpd-F level (γ/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 53	♂	16	7.8	9.3	10.7	7.2	7.6
54	♀	13	13.2	18.5	18.0	10.2	10.4
56	♀	16	8.8	9.2	10.3	7.3	4.8
67	♀	12	12.9	14.7	15.8	14.0	10.9
77	♀	15	9.2	17.2	15.8	6.2	7.3
Mean			10.38	13.78	14.12	8.98	8.2

Table. 4 Fluctuation of the plasma Cpd-F level after ACTH administration (4 weeks after operation)

Dog No.	Sex	Days after ope.	Plasma Cpd-F level (γ/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	25	13.9	21.2	10.4	11.1	3.2
42	♂	28	3.5	6.5	6.9	9.2	9.1
45	♀	27	8.0	13.1	9.0	6.8	6.2
53	♂	29	6.3	9.5	10.2	5.9	5.4
54	♀	30	8.3	9.5	12.0	7.2	8.0
66	♂	26	11.3	10.3	9.2	6.5	4.6
Mean			8.55	12.18	9.61	7.78	6.08

Table. 5 Fluctuation of the plasma Cpd-F level after ACTH administration (6 weeks after operation)

Dog No.	Sex	Days after ope.	Plasma Cpd-F level (γ/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	40	7.6	7.4	9.8	10.1	6.2
67	♀	39	5.3	6.7	4.9	5.1	3.8
77	♀	38	7.1	9.7	8.0	4.4	4.9
Mean			6.66	7.93	7.56	6 53	4.96

Table. 6 Fluctuation of the plasma Cpd-F level after ACTH administration (8 weeks after operation)

Dog No.	Sex	Days after ope.	Plasma Cpd-F level (γ/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	54	8.5	9.7	11.9	11.0	4.3
45	♀	58	3.6	5.3	6.1	7.4	5.8
Mean			6.05	7.5	9.0	9.2	5.05

Table. 7 Fluctuation of the plasma Cpd-F level after ACTH administration (later than 8 weeks after operation)

Dog No.	Sex	Days after ope	Plasma Cpd-F level (γ/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 45	♀	71	9.2	6.3	8.9	5.1	2.2
77	♀	82	5.9	7.9	3.5	5.1	4.2
Mean			7.55	7.1	6.2	5.1	3.2

was found that they maintained the relatively increased adrenocortical reserve in the 4th week after the operation, while No. 42 showed decreased reserve already in the 4th week suggesting the early emaciation and short survival due to shock.

(III) Fluctuation of the blood sugar level after ACTH administration.

It was recognized that ACTH showed a slight diabetogenic action in normal organisms[24)25)29)]. In the present experiment, the blood sugar level elevated slightly, namely 72 mg/dl to 82 mg/dl on the average at the 2nd hour after ACTH administration (Table 8). This determination was performed simultaneously with the examination in (II). In the 2nd week after total pancreatectomy (Tab. 9), the level was estimated to be 239.6 mg/dl on the average, then elevated markedly to 286.0 mg/dl at the 2nd hour after ACTH administration. In the 4th week (Table 10), the level showed 224.3 mg/dl on the average and elevated to 247.5 mg/dl at the 3rd hour after ACTH administration. After 6 weeks postoperatively (Table 11, 12, 13), the level showed a slight elevation

Table. 8 Fluctuation of the blood sugar level after ACTH administration (Before total pancreatectomy)

Dog No.	Sex	Blood sugar level (mg/dl)				
		Before ACTH adm.	Hours after ACTH administr.			
			1	2	3	5
No. 3	♂	85	81	98	106	92
5	♂	58	65	48	71	62
13	♂	70	71	73	70	75
16	♂	61	78	62	60	62
34	♂	52	60	51	56	50
40	♂	63	64	69	63	65
42	♂	62	64	68	74	73
45	♀	50	51	53	69	63
47	♀	81	93	112	85	85
53	♀	89	103	108	92	90
54	♀	109	115	159	145	99
56	♀	93	94	106	98	98
66	♂	69	76	79	80	73
67	♀	55	72	68	58	56
77	♀	78	82	83	92	79
Mean		71.66	77.93	81.86	81.2	74.8

Table 9 Fluctuation of the blood sugar level after ACTH administration (2 weeks after operation)

Dog No.	Sex	Days after ope	Blood sugar level (mg/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 53	♂	16	184	297	298	278	284
54	♀	13	246	272	266	258	270
56	♀	18	243	235	240	236	205
67	♀	12	310	246	348	320	298
77	♀	15	216	272	278	250	262
Mean			239.6	264.4	286.0	268.4	263.8

after ACTH administration. Observing the dogs of two groups divided according to the degree of blood sugar level response to the administration of ACTH (Fig. 3), i. e., the group of elevated adrenocortical reserve and that of the lowered reserve, it was noticed that in the former the blood sugar level showed 249.6 mg/dl before ACTH administration and an elevation of ca. 45 mg/dl after ACTH administration, while in the latter the level of 254 5 mg/dl before ACTH administration and showed little elevation, as compared to the former, after ACTH administration, rather showing tendency of a gradual decrease.

(IV) Circulating eosinophils in the peripheral blood and its rate of decrease (Thorn's test). (Table 17)

Table. 10 Fluctuation of the blood sugar level after ACTH administration (4 weeks after operation)

Dog No.	Sex	Days after ope.	Blood sugar level (mg/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	25	128	97	128	125	113
42	♂	28	324	314	290	280	238
45	♀	27	140	175	218	268	270
53	♂	29	224	286	268	272	298
54	♀	30	312	346	338	348	352
66	♂	26	218	226	208	192	179
Mean			224.3	240.6	241.6	247.5	241.6

Table. 11 Fluctuation of the blood sugar level after ACTH administration (6 weeks after operation)

Dog No.	Sex	Days after ope.	Blood sugar level (mg/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	41	264	262	296	270	282
67	♀	39	308	292	290	252	258
77	♀	38	234	342		358	344
Mean			268.6	298.6	293.0	293.3	294.6

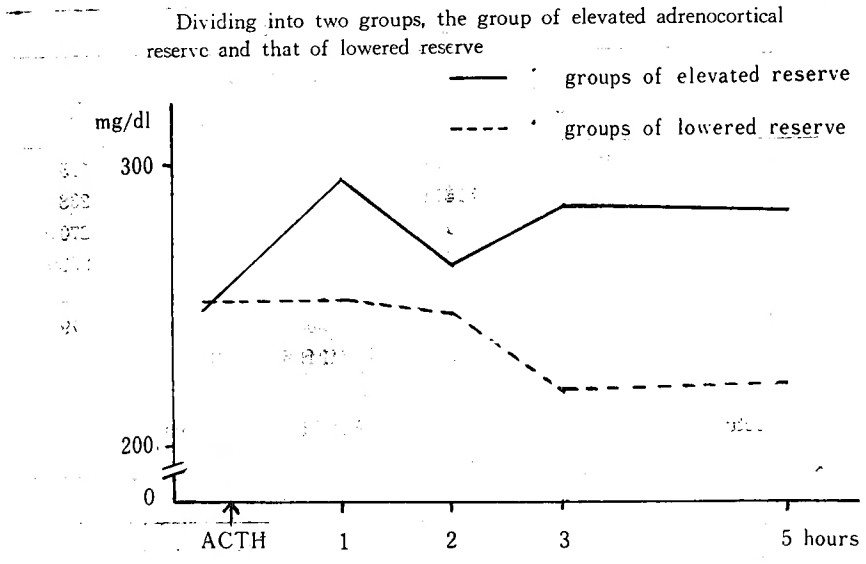
Table. 12 Fluctuation of the blood sugar level after ACTH administration (8 weeks after operation)

Dog No.	Sex	Days after ope.	Blood sugar level (mg/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 40	♂	54	352	382	390	362	372
45	♀	58	318	310	340	366	386
Mean			335	346	365	364	379

Table. 13 Fluctuation of the blood sugar level after ACTH administration (later than 8 weeks after operation)

Dog. No.	Sex	Days after ope.	Blood sugar level (mg/dl)				
			Before ACTH adm.	Hours after ACTH administr.			
				1	2	3	5
No. 45	♀	71	208	204	186	172	174
77	♀	82	284	302	304	264	276
Mean			246	253	245	218	225

Fig. 3. Fluctuations of the blood sugar level aftr ACTH administration (Mean value)



The count of circulating eosinophils of dogs was markedly different in each individual because the dogs were often inhabited by parasites especially by a filaria. The eosinophils counts are calculated to be about 1000 to 2000 per mm^3, 1528 per mm^3 on the average before the operation. After total pancreatectomy the count of eosinophils decreased rapidly to 546 per mm^3 on the average in the 4th week after the operation, 122 per mm^3 in the 8th week and eosinophils disappeared after the 9th postoperative week. It is suggested that the decrease in eosinophils may not be due to the adrenocortical hyperactivity, but to the adrenocortical stimulation by a large dose of insulin injected twice a day. This suggestion is assured by the fact that in the normal dogs a decrease of about 75% in eosinophil count was observed after insulin administration. Moreover, since the large dose of insulin was used after pancreatectomy day by day, it would have been impossible to recover the count of eosinophils in the dogs. In addition to this factor, the progressing anemia may promote eosinopenia after the operation.

In THORN's[27)28)] test, the rate of decrease in eosinophils after ACTH administration showed about 87.5% before the operation, and remained to be about 80% after total pancreatectomy. As high a degree of adrenocortical insufficiency as observed in cases of Addison's disease may exist when Thorn's test shows the lowerd value below 50%. After total pancreatectomy, there could not be observed such an intense cortical insufficiency as stated above.

(V) Fluctuation of Cpd-F level after insulin administration.

It is recognized that insulin activated the adrenal cortex[14)29)30)53)]. In this point of view, the response of adrenocortical excretion to insulin was determined before and after total pancreatectomy, because the blood sugar level was controlled by insulin day by day after total pancreatectomy. Twenty units of insulin was injected intravenously, and the blood sample was taken 30 minutes, 1, 2 and 3 hours after the injection, and their values

of Cpd-F was compared with that estimated before the injection. Before total pancreatectomy, (Table 14, Fig. 4), the level of Cpd-F was 6 44 γ/dl on the average and elevated to the peak of 10 56 γ/dl at the lst hour after insulin administration. After the operation Cpd-F level was 5 78 γ/dl on the average and elevated to the peak of 10.15 γ/dl at the 2nd hour after insulin administration (Table 15, Fig. 5). However, examining the results in each case, it was observed that the level was kept elevated at the 3rd hour after insulin administration. It was recognized that the elevation of Cpd-F level after the operation was not so distinct but showed a tendency of a prolonged elevation than before the operation. After total pancreatectomy, the blood sugar level showed the prolonged lowering at the 3rd hour after insulin administration, as is proved in the following experiments. It is suggested that the change of the Cpd-F level may be related to the blood sugar level after insulin administration.

(VI) Fluctuation of the blood sugar level after insulin administration. (Table 14).

Before the operation the blood sugar level decreased markedly after the intravenous injection of 20 units of insulin, that is, it was 77.6 mg/dl on the average and decreased to 31.4 mg/dl at the lst hour after the injection, and then increased gradually but did not recover up to the first value even at the 3rd hour after the injection. After the operation (Table 15) the fasting blood sugar level was 348.4 mg/dl on the average and decreased to 110 mg/dl at the 3rd hour after insulin administration. In a few cases the decreased blood sugar level showed a tendency of slight recovery at the 3rd hour after insulin injection. It is suggested that such a distinct prolonged decrease of the blood sugar level may prompt the continuous release of the corticoids.

(VII) Insulin shock.

As HONJO[31][32] and others[4][33] reported, it is not until the postoperative 5th week that the totally depancreatized dog comes to fall easily into the insulin shock, and accordingly it is necessary to reduce the dose of insulin. In the present experiment, the level of Cpd-F

Table. 14 Fluctuation of the plasma Cpd-F level and blood sugar level after insulin administration
(Before total pancreatectomy)

Dog No.	Sex		Before insulin adm.	Hours after insulin administr.			
				30 mns.	1	2	3
25	♂	Blood sugar	83 mg/dl.	37	16	20	67
		Cpd-F	7.8 γ/dl	8.4	7.0	9.1	7.2
56	♀	Blood sugar	74	28	20	32	66
		Cpd-F	5.8		9.8	7.8	5.2
66	♂	Blood sugar	85	65	64	45	38
		Cpd-F	7.0		12.0	9.8	7.5
77	♀	Blood sugar	78	41	23	43	50
		Cpd-F	5.3	10.5	11.9	10.7	11.7
82	♀	Blood sugar	68	40	29	36	62
		Cpd-F	6.3		12.1	7.1	8.4
Mean		Blood sugar	77.6	42.2	31.4	35.2	56.6
		Cpd-F	6.44	9.45	10.56	8.9	8.0

Fig. 4 Fluctuations of the Cpd-F level after insulin administration (Before total pancreatectomy)

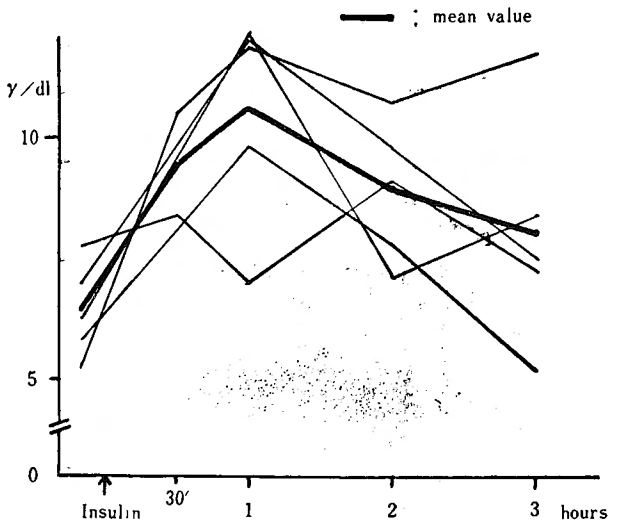


Table. 15 Fluctuation of the plasma Cpd-F level and blood sugar level after insulin administration (After total pancreatectomy)

Dog No.	Sex	Days after ope.		Before insulin adm.	Hours after insulin administr.			
					30 mns.	1	2	3
56	우	28	Bl. sugar Cpd-F	354 mg/dl 7.0 γ/dl	328 9.5	147 10.9	84 12.4	54 12.2
66	숫	24	Bl. sugar Cpd-F	390 6.5		250 10.7	308 10.9	155 13.0
77	우	11	Bl. sugar Cpd-F	220 2.6	180 7.4	95 8.4	56 8.8	52 9.5
77	우	56	Bl. sugar Cpd-F	364 7.3	210	184 10.2	115	87 5.3
82	우	1	Bl. sugar Cpd-F	414 5.5	392	222 7.0	196 8.5	198 8.6
Mean			Bl. sugar Cpd-F	348.4 5.7	277.7 8.45	199.4 9.44	151.8 10.15	110.0 9.72

and blood sugar level at the time of insulin shock were estimated, and the time interval from the insulin injection to the developement of insulin shock was observed (Table 16). It was about 6 hours on the average until the disclosure of insulin shock after insulin administration. The blood sugar level was 23.7 mg/dl on the average at the moment of insulin shock and simultaneously the Cpd-F level showed as low a level as 3.6 γ/dl. As the insulin test showed, it was observed that after total pancreatectomy the Cpd-F level showed a prolonged increase after insulin administration, while the blood sugar level decreased distinctly. Nevertheless at the moment of insulin shock the Cpd-F level dec-

Fig. 5 Fluctuations of the Cpd-F level after insulin administration (After total pancreatectomy)

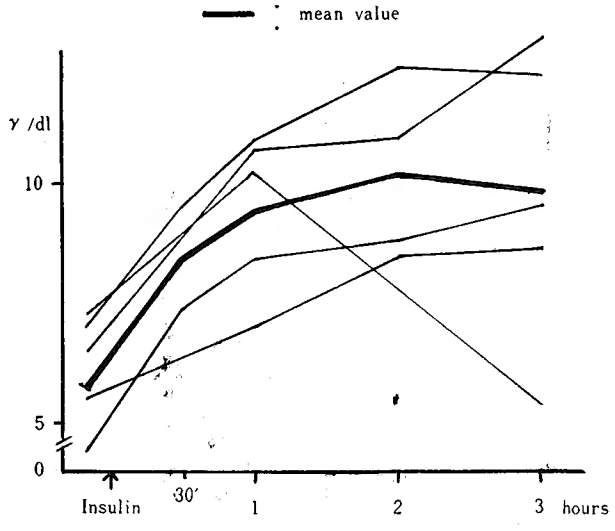


Table. 16 Insulin shock

Dog No.	Sex	Days afteer ope.	Hours from insulin adm. to insulin shock	Blood sugar	Plasma Cpd-F level
40	♂	36	ca 6 hours	23 mg/dl	4.3 γ/dl
80	♀	32	ca 7 hours	20	3.2
84	♀	28	ca 5 hours	28	3.4
Mean			ca 6 hours	23.7	3.63

reased, accompanying the lowering of blood sugar level. Therefore, it is suggested that the insulin shock occurs readily, because the adrenal cortex is exhauste and gluconeogenesis in the liver is reduced after a prolonged release of corticoids due to the prolonged hypoglycemia.

(VIII) Adrenal weights.

The adrenal weights of dogs vary in wide range individually, being 1175 mg on the average in males and the ratio of adrenal weight to the body weight was 126 8 mg/kg on the average. In females these were 1312 mg and 140 1 mg/kg. After total pancreatectomy, the ratio of the adrenal weight to the preoperative body weight decreased slightly, i e., 110 8 mg/kg in the males and 121.4 mg/kg in females. Individual deviation of postoperative adrenal weights is also distinct. It may be suggested, however, that the adrenal weight seems to decrease somewhat in a mean value after total pancreatectomy (Table 18).

(IX) Some other blood examinations.

More than 4 weeks after total pancreatectomy, serum protein was determined to be lowered gradually to about 5.0 or 6.0 g/dl, at the same time hematocrit ratio also lowered

Table. 17 Circulating eosinophils and its rate of decrease.

Dog No.	Sex		Before ope.	Weeks after ope. Till 4 wks	Till 8 wks	More than 8 wks
40	♂	Eosinophils Rate of decr.	808 per mm³. 88.4%	831 94.6	212 82.3	50
42	♂	Eosinophils Rate of decr.	4612 78.3	334 80.0		
45	♀	Eosinophils Rate of decr.	2250 84.7	794 83.4		31 80.0
47	♀	Eosinophils Rate of decr.	156 4.0	19		
53	♂	Eosinophils Rate of decr.	1175 90.3		49 87.0	
54	♀	Eosinophils Rate of decr.	794 94.4		100 81.2	
66	♂	Eosinophils Rate of decr.	1413 97.3		75 83.3	
77	♀	Eosinophils Rate of decr.	1013 82.7	744	175 75.0	56
Mean		Eosinophils Rate of decr.	1527.6 87.5	546.4 86.0	122.2 81.7	45.6 80.0

to about 30 %. Icterus index ranged from 5 to 10, and B.S.P. test showed the figures from 0% to 2.5% 15 minutes after the injection of bromsulphalein. Total cholesterol was estimated to be about 250 mg/dl, esterified cholesterol about 150 mg/dl, and cholesterol ester ratio about 60 %. In short, it is suggested that the liver function of totally depancreatized dogs is not so much disturbed.

(X) Histological observations of the adrenal cortex.

In present experiment histological findings of the adrenal cortex of mouse and rabbit were referred to those of depancreatized dogs, because the most of the histological studies [34)35)] on the adrenal cortex, hitherto been reported, were usually made on these animals.

A) Hematoxylin-eosin double staining :

Fig. 6 ; No. 40, male, survived for 77 days. The glomerular zone was relatively normal, and demarcated distinctly from the fascicular zone, while the transitional zone was not evident. The outer fascicular zone was relatively more lucid than the inner area, and seemed to be filled with fat granules, though the sudanophile granules were rather diminished as mentioned below in the cortex on Sudan III staining. Cellular cords of the fascicular zone were arranged irregularly and sinusoidal capillaries were not also remarkable. Individual cells were observed indistinctively, and the degenerating process was noted in cells, demonstrating faintly reticulated structures and/or disappearance of protoplasm.

Fig. 7 ; No. 41, female, survived for 16 days. Numerous vacuolarized cells were observed in fascicular zone. The interspace between cellular cords dilated and sinusoidal cells were distinctly observed, though individual cells were rather atrophic, and therefore, it is suggested that this finding did not indicate an adrenocortical hyperfunction but rather

Table. 18 Change of adrenal weight after total pancreatectomy.

(male)

Control				Totally depancreatized dog				
No.	Body weight (kg.)	Adrenal weight (mg.)	Ratio of adrenal weight to body weight (mg./kg.)	Dog No.	Body weight (kg.)	Adrenal weight (mg.)	Ratio of adrenal weigt to body weight (mg./kg.)	Days after ope.
1	3.3	490	148 5	21	14.0	1550	110.7	12
2	9.5	1010	106.3	30	10.0	870	87.0	8
3	13 5	1050	78 5	34	6.0	1180	196.6	14
4	8.0	860	107.5	40	15.5	1220	78.7	77
5	6.3	1110	176.2	42	10.5	1390	132.4	32
6	12.5	1740	139.4	50	9.0	1610	173.3	35
7	10.5	1480	140.9	52	13 5	1490	110.4	8
8	6.0	1440	240.0	53	12.5	1420	113.6	40
9	8.0	1180	147.5	55	14.0	1360	97.1	34
10	15.0	1280	85.3	66	11.0	1140	103.6	47
11	9.5	1190	125.2	70	12.5	820	65.6	27
12	5.3	690	130.2	71	13.0	1340	103.0	10
13	13.0	1750	134.6	72	10.0	1530	153.0	16
				75	10.5	920	87.6	62
				86	10.5	1290	122.8	26
Mean	9.3	1175	126.8		11.5	1275	110.8	

(female)

1	5.5	730	132.6	31	8.5	1390	163.5	28
2	8.0	980	122.5	39	12.0	1500	125.0	14
3	6.5	1150	176.9	45	17.0	1470	86.5	78
4	15.0	970	64.6	49	14.0	1310	93.5	35
5	9.5	2050	215.7	47	10.5	2060	196.2	9
6	9.0	1350	150.0	54	11.0	1070	97.3	36
7	6.0	740	123.3	56	10.5	1680	160.0	36
8	11.0	1690	153.6	67	9.0	1080	120.5	41
9	16.0	1750	109.3	73	8.0	950	118.7	91
10	8 5	1090	128.2	74	8.0	950	118.7	90
11	11.0	2250	204 5	77	14.5	1950	134.5	121
12	6.0	990	165.0	80	8.5	770	90.6	36
				85	10.5	1430	136.2	22
				87	10.0	870	87.0	34
Mean	9.3	1312	140.1		10.9	1320	121.4	

a markedly stimulated state.

Fig. 8; No. 66, male, survived for 47 days. The glomerular zone was normal and a thickened transitional zone with flattened nuclei was observed beneath the glomerular zone. In the outer fascicular zone, its cells showed a honeycomb-like structure and a hollow-like deficit of cellular contents, suggesting atrophic changes of the adrenal cortex.

Fig. 9; The high-power photomicrograph of the Fig. 8. This is a typical finding in the adrenal cortex of totally depancreatized dogs. Insulin shock developed in this case.

Fig. 10 and Fig. 11; No. 82, male, survived for 82 days. Fig. 10 is the photomicrograph of the right adrenal gland which was extirpated on the 19th day after total pancreatectomy, and Fig. 11 is the photomicrograph of the left adrenal gland which was extirpated when the animal died on the 82nd day after the operation. A characteristic finding was observed in Fig. 10, that is, the glomerular zone was normal and the transitional zone was absent, and the fascicular zone was generally lucid and showed a mosaic arrangement which resembled closely to the finding of hyperfunctioning adrenal cortex due to the continuous administration of ACTH. Fig. 11 shows apparently alike as seen in the other dogs operated on, showing a finding of atrophy, outer cells of the fascicular zone having less content and its nucleus deviated to the border. Namely, the right adrenal cortex extirpated on the 19th day after total pancreatectomy shows a finding of hyperfunction while the one extirpated on the 82nd postoperative day shows atrophy.

Fig. 12; No. 87, female, survived for 34 days. In this case a relatively small dose of insulin was injected, and therefore, as shown in Fig. 13, fatty infiltration was observed in the liver. In the adrenal cortex, the capsule and the glomerular zone were slightly thickened, and cellular cords in the fascicular zone were arranged regularly, though the outer area of the fascicular zone was rather atrophic.

Fig. 14; No. 88, male, survived for 33 weeks. This was an exceptional case, that is, the adrenal gland increased in its weight, and its cortex showed a relatively normal appearance. The developement of capsular adenoma was observed here and there. It is reported by MORI[34] that adenoma of this sort was apt to develop following the continuous administration of insulin in normal animals.

B) Sudan III staining:

Fig. 15; No. 80, female, survived for 36 days. While normal deposition of fat was observed in the glomerular zone, a high degree of reduction in fat amount was noticed in the fascicular zone. Although this case seemed to be highly emphasized one, the sudanophile granules decrease, at any rate, in the adrenal cortex after total pancreatectomy.

Fig. 16; No. 87, female, survived 34 days. A decrease in the sudanophile granules in both the glomerular and fascicular zone was observed, and especially the relatively lucid zone, so-called clear zone or sudanophobe zone was observed beneath the glomerular zone. It is supposed that this clear zone would be a sign of hypofunction of the adrenal cortex.

C) Ketosteroid staining by the Ashbel-Seligman's method:

Fig. 17; No. 45, female, survived for 78 days. It is noteworthy that the outer area of the fascicular zone was coloured blue deeply, though there have been much to be discussed[30] about this staining method.

IV. DISCUSSION

It has been recommended[9)11)] to determine the level of glucocorticoid especially that of hydrocortisone (KENDALL's Compound F), that occupies most part of the glucocorticoids, as a most reliable indication of adrenocortical function in diabetes mellitus, since it reveals a disturbance of carbohydrate metabolism as its chief symptom.

NELSON[11] and others[10)12)13)14)15)38], moreover, determined the fluctuation of the plasma 17-OHCS level after the administration of ACTH in order to estimate the adrenocortical reserve. Besides these, the change and the rate of a decrease in circulating eosinophils have been considered to be of similar significance[25)27)28].

There have been many reports concerning the relationship between diabetes mellitus and the function of the adrenal cortex. But their results do not come to an accordance. That is, some investigators[39] insist on the declined adrenocortical function in diabetes, judging from the calculated values of steroid excretion in urine and the change in number of circulating eosinophils, while others assert that the function is accelerated or remains in normal range[40)41)42]. Many of these reports have no comment as to the administration of insulin and its dose. In recent years, however, it is widely accepted that the steroid excretion in urine in diabetics remains in normal range when the blood sugar level is controlled well with insulin and that it is augmented in severer cases and in those accompanied with infections or cardiovascular complications[43]. On the other hand, MARTIN[44] and others reported a case of diabetic patient with pituitary insufficiency.

There have been many reports of experimental studies[50)58] on diabetic animals, most of them reporting an increases in steroid excretion in urine or in adrenal weight. Thus the adrenocortical function in diabetes seems to be accelerated at least in these experimental studies, although the animals were not administered with insulin in most of these experiments.

Since von MEHRING and MINKOWSKI ascertained the developement of diabetes mellitus in totally depancreatized dogs in 1899, an experimental study on diabetes mellitus has become feasible without so much difficulty as before. In 1943 DRAGSTEDT et al recognized the developement of fatty liver and the occurence of insulin shock after total pancreatectomy, thence the pathophysiology following this operation has become a problem of much discussion. It has been widely recognized that the insulin shock, which is apt to occur more than 4 weeks after total pancreatectomy, may be caused by increased sensitivity to insulin. Dragstedt et al suggested that fatty liver may cause the insulin shock after the operation, and yet observed that the insulin shock may be induced without fatty liver. Some others insist that the endocrine glands, which counteract to insulin, should play an important role. Formerly KRAUS[45] reported the appearance of atrophy of the anterior pituitary and the adrenal cortex in a totally depancreatized cat. HASEGAWA[46] observed that the chromophil cells of the anterior pituitary lobe decrease in number after total pancreatectomy, and suggested that this decrease in chromophil cells might be interpreted as a sign of pituitary hypofunction, and that the experimental dogs are coming near to the HOUSSAY's dog[47)48)49]. MCARTHUR[52)53] et al reported that acidosis decreases in its grade after adrenalectomy in a totally depancreatized dog. Based on the calculation of assimilation index of glucose, YAO[17] concluded that the insulin sensitivity did not increase in a totally depancreatized dog, but, on the contrary, decreased, and also he assumed that an attack of insulin shock might be due to hypoglycemia unresponsiveness induced by anterior pituitary hypofunction. MARTIN[44] expressed the similar opinion upon insulin shock in diabetics as YAO's. YAMAMOTO[54] reported that fatty liver did not develop in depancreatized dogs in which an adequately large dose of insulin was given after the

operation and nevertheless occurrence of insulin shock was noticed. As HASEGAWA reported, the differences between diabetes after total pancreatectomy and internal medical diabetes are as follows; In the former, glucagon is lost and a starvation-like state is induced and the activity of the anterior pituitary lobe (anti-insulin system) is lowered. However, it is also suggested that the condition of the hypophysis-adrenal system might not differ essentially between these two sorts of diabetes in the point of counteracting the hyperglycemic condition. At any rate the occurrance of insulin shock after total pancreatectomy, although not in every case, may suggest the destiny of the adrenal cortex after the operation.

In 1957, NOMURA[55] reported in detail on the adrenocortical histology of the totally depancreatized dog. In her experiment, the Holocrine-like finding of the tissue was observed in the fascicular zone after total pancreatectomy. From this finding she suggested that the function of the adrenal cortex was accelerated especially from the 10 th to the 14 th postoperative day. In the present experiment, the finding of hyperactivity in the adrenal cortex was confirmed on the 19 th day after total pancreatectomy in a case which survived for about 80 days, in the most of other cases were observed the devastation of the tissue in the outer part of the fascicular zone.

It is recognized[14][29][30][53] that insulin itself activated the adrenal cortex and brings forth hypertrophy of the adrenal cortex in normal animals when insulin is injected successively, and there are many investigations reporting that administration of insulin increases 17-OHCS level in blood and urine. On the other hand, it was reported[13] that in the course of insulin therapy for diabetes the abnormal adrenocortical activity was corrected. Thus it may be commented that there exists an intricate interrelationship between insulin and the adrenal cortex. In the present steps, it would go too far to judge the adrenocortical activity according to the insulin test. In the present experiment also, Cpd-F level showed a prolonged increase after the administration of insulin in a totally depancreatized dog which is due to the prolonged hypoglycemia. It may be suggested that the adrenal cortex is exerting continuous response to the rapid changes of blood sugar level and insulin shock appear when the changes of blood sugar level comes to surpass the limits of adrenocortical activity. MATSUI[11] and others reported that 17-OHCS level in urine shows either a conspicuous or slight elevation in both hyperglycemic coma and hypoglycemic shock MARKS[10] et al observed that in insulin coma therapy for paranoid schizophrenic patient the urinary excretion of corticosteroids shows a moderate increase in the course of therapy and shows a slight decrease after the therapy. In the present experiment it is assumed that, as corticoids level which showed a prolonged increase after insulin administration had already been exhausted at the time when the shock occurred, corticoids level and blood sugar level were reduced. This assumption may be adopted as one of the reasons of the existence of hypoglycemic unresponsiveness which was asserted by YAO and others. It is well recognized today that the eosinophil counts indicate the adrenocortical activity and not merely ACTH activates the adrenal cortex, but muscular labour, surgery, adrenalin, insulin and electric shock etc. also stimulate the adrenal cortex and mobilize glucocorticoid into blood stream and consequently bring forth a decrease in number of eosinophiles. In the present experiment, THORN's test showed the rate of decrease of about 85% in dogs and did not seem to be lowered below 80% after total pancreatectomy, but later it became

difficult to judge the figures because the absolute value of eosinophils decrease so much at that time. This decrease in number of eosinophils may be due to the increased secretion of glucocorticoid caused by violent fluctuations of blood sugar level after the administration of insulin which were given twice a day without permitting the recovery of eosinophil counts.

As is described above, the adrenocortical hyperactivity developed in the early period after total pancreatectomy is due to the absolute deficiency of insulin and glucagon, and disturbance of digestion and absorption. The rapid emaciation occurs as a result of metabolic disturbances forming a vicious circle[57]. When the blood sugar level is controlled as correctly as possible by insulin, the hormone balance would be maintained in a state of lowered adrenocortical activity. However, it is suggested that such a hormone balance is so unstable that it is apt to be disturbed easily by the occurrence of slight complication.

V. CONCLUSION

The adrenocortical function was studied in a completely depancreatized dog whose blood sugar level was controlled at about 200 mg/dl with insulin of 1 to 5 units per kg a day. In these dogs, the plasma Cpd-F level, the blood sugar level, eosinophil counts, the adrenal weight were estimated and the histological findings of the adrenal cotrex were examined with the results as follows:

1) Plasma Cpd-F level;

The plasma Cpd-F level and its response to ACTH were found to be higher until about the 4th postoperative week, compared with those before total panrceatectomy, and gradually tended to decrease thereafter. The prolonged release of Cpd-F after insulin administration was observed after the operation, and in the 4th postoperative week and thereafter, the adrenal cortex was in a state to become readily exhausted and the animals were apt to fall into shock, accompanied with the lowering of the level of both Cpd-F and blood sugar.

2) Blood sugar level;

The blood sugar level was elevated after total pancreatectomy, showing also a marked elevation by the administration of ACTH until about the 4 th week after the operation, while this response of blood sugar to ACTH was weakened when the adrenocortical reserve was declined. Insulin shock occurred sometimes after the administration of inlusin, showing a marked decrease of blood sugar level.

3) Eosinophil counts;

The number of circulating eosinophils decreased gradually after total pancreatectomy and it became almost impossible to find eosinophil cells in blood stream. The Thorn's test showed about 88% in normal dogs, and maintained about 80% after total pancreatectomy.

4) Adrenal weight;

After total pancreatectomy the ratio of the adrenal weight to the preoperative body weight showed a decrease, if compared with the ratio of dogs that did not undergo total pancreatectomy.

5) Histological findings of the adrenal cortex;

Sudanophile granules in the adrenal cortex decreased in almost all cases after total pancreatectomy. The cells in the fascicular zone, especially in its outer area, showed the finding of fine granular atrophy and devastation in many cases. About 2 weeks after the operation, the adrenal cortex showed a transitory hyperactivity in one case.

From these results, it is suggested that after total pancreatectomy the adrenal cortex shows temporary hyperactivity corresponding to the striking changes of metabolism induced by the operation, and then gradually its function is lowered to keep some sort of endocrinological balance, which is so unstable as to be easily broken.

(The results of this experiment were reported briefly at the 33rd and 34th annual meeting of the Japan Endocrinological Society).

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REFERENCES

1) Abe, M., Kozaka, T. and Yoshimura, H.. Pancreas and Hormone. 1. Kyodo-isho, Tokyo, 1958.
2) von Mehring, J. and Minkowski, O.. Diabetes mellitus nach Pankreasexstirpation. Arch. exper. Path. Pharmak., **26**, 371, 1889.
3) Bailey, O. T., Bailey, C. C. and Hagan, W. H.: Alloxandiabetes in the rabbit. Amer. J. Med. Sc., **208**, 450, 1944.
4) Dragstedt, L. R.: Some physiologic problems in surgery of the pancreas. Ann. Surg., **118**, 576, 1943.
5) Rockey, E. W.: Total pancreatectomy for carcinoma, case report. Ann. Surg., **118**, 603, 1943.
6) Priestly, V. T., Comfot, M. W. and Radcliffe, Jr.. Total pancreatectomy for hyperinsulinism due to islet-cell adenoma. Ann. Surg., **119**, 211, 1944.
7) Jaumann, R.: Die Rolle der Nebenniererinde in der Insulinregulation beim Diabetes mellitus. Schweiz. Med. Wschr., **87**, 1069, 1957.
8) Wallace, E. Z., Christy, N. P. and Jailer, J. W.. Clinical application of the simplified Silber-Porter method for determing plasma 17-OhCSs. J. Clin. Endocrinol. & Met., **15**, 1073, 1955.
9) Friskey, R. W. etal.. Adrenocortical function in diabetes. J. Clin. Endocrinol. & Met., **16**, 986, 1956.
10) Bliss, E. L., Nelson, D. H. and Samuel, L. T.: Effects of intravenous ACTH on blood level of 17-OHCS and circulating leucocytes. J. Clin. Endocrinol. & Met., **14**, 423, 1954.
11) Nelson, D. H. et al.. The levels of 17-OHCSs in peripheral blood of human subjects. J. Clin. Endocrinol. & Met., **11**, 1021, 1951.
12) Persky, H. and Health, H. A.. Effect of intravenous corticotropin on the level of plasma hydrocortisone in man. J. Clin. Endocrinol., **17**, 632, 1957.
13) Eik-Nes, K. et al.. Changes in plasma levels of 17-OHGSs during the intravenous administration of ACTH. J. Clin. Endocrinol. & Met., **15**, 13, 1955.
14) Wakizaka, T., Kimura, M. and Nagamori, M.. The influence of various hormones on the levels of fluorocorticoids and P. B. I. in plasma. Zyuzen Igakukai Zassi., **58**, 494, 1956.
15) McQuarrie, I. et al.. Effects of pituitary adrenocorticotropic hormone in children with non-addisonian hypoglycemia. Proc. Soc. exper. Biol. Med., **71**, 555, 1949.
16) Markowitz, J. et al.: Experimental surgery. 4th Edit. 351, 1959, Williams & Wilkins.
17) Yao, E.: Studies on relations between insulin sensitivity and liver glycogen in totally depancreatized dog. Arch. Jap. Chir., **28**, 2992, 1959.
18) Sweat, M. L. et al.: Adrenocortical steroids in human peripheral and adrenal venous blood as determined by fluorescence. Fed. Proc., **12**, 141, 1953.
19) Takeda, R.: A critism of the Sweat method for the estimation of cortical hormones in Plasma. Fol. Endocrinol. Jap., **3**, 73, 1955.

20) Somogyi, M.. A photometric adaptation of the Somogy method for the determination of glucose. J. Biol. Chem., **153**, 375, 1944.
21) Bloor, W. R.: The determination of cholesterol in Blood. J. Biol. Chem., **24**, 227, 1916.
22) Ashbel, R. and Seligmann, A. M.: A new reagent for the histochemical demonstration of active carbonyl groups, a new method for staining ketonic steroids. Endocrinol., **44**, 565, 1949.
23) Yoshioka, H.: Pancreatectomy. J. J. S. S., **55**, 766, 1954.
24) Takeda, M.. The relationship between anterior-pituito-adrenocortical hormones and carbohydrate metabolism. Clin. Endocrinol. (in Japan), **2**, 3, 1954.
25) Renold, A. E. et al.: Intravenously administered ACTH, a preliminary report. N. England. J. Med., **244**, 796, 1951.
26) Foa, P. P. et al.: Influence of prolactin on blood sugar in normal and depancreatized dog. Amer. J. Physiol., **180**, 313, 1955.
27) Thorn, G. W. et al.: A test for adrenal cortical insufficiency. J. A. M. A., **137**, 1005, 1948.
28) Thorn, G. W. et al.. Use of the intravenous ACTH test clinical practice. J. Clin. Endocrinol. & Met., **13**, 604, 1953.
29) Bliss, E. L., et al.: The effects of insulin, histamine, bacterial pyrogen and the antabuse-alcohol reaction upon the levels of 17-OHCS in the peripheral blood in man. Metabolism, **3**, 493, 1953.
30) Marks, L. H. et al.: The adrenocortical response to insulin coma. J. Clin. Endocrinol. & Met., **18**, 235, 1958.
31) Honjo, I.: Total pancreatectomy. J. J. S. S., **55**, 795, 1954.
32) Honjo, I., Hasegawa, M., and Kawamura, K.: Total pancreatectomy and carbohydrate metabolism. Clin. Endocrinol. (in Japan), **2**, 1667, 1954.
33) Yoshimura, H.: Morphology of the adrenal gland for clinical study. Nihon-Rinsho, **14**, 163, 1956.
34) Mori, S. and Ando, T.: Adrenal cortex. Nihon-Rinsho, **14**, 306, 1956.
35) Akasu, H.: Adrenal cortex and Placenta. 13, Igaku-Shoin, 1955.
36) Hirai, M.: A critism of the histochemistry for adrenal cortex. Fol. Endocrinol. Jap., **37**, 504, 1961.
37) Kinsell, L. W. et al.: The case for cortical steroid hormone acceleration of gluconeogenesis from fat in diabetic subjects. J. Clin. Endocrinol. & Met., **14**, 161, 1954.
38) Renold, A. E.. The use of intravenous ACTH, a study in quantitative adrenocortical stimulation. J. Clin. Endocrinol. & Met., **12**, 763, 1952.
39) Miller, S. and Mason, H. L.. The excretion of 17-KS by diabetes. J. Clin. Endocrinol., **5**, 220, 1945.
40) Oji, T.: The excretion of urinary steroids by diabetics. Fol. Endocrinol Jap., **34**, 1281, 1959.
41) Yamagata, S.: Diabetes mellitus and Hormone-balance. Fol. Endocrinol. Jap., **36**, 698, 1960.
42) Venning, E. H. and Browne, J. S. L.: Excretion of glycogenic corticoids and of 17-KS in various endocrine and other disorder. J. Clin. Endocrinol., **7**, 79, 1947.
43) Matsui, N.: Adrenocortical activity by diabetes. Fol. Endocrinol. Jap., **35**, 1464, 1960.
44) Martin, M. M. and Pond, M. H.: Pituitary insucficiency associated with diabetes mellitus. J. Clin. Endocrinol. & Met., **14**, 1046, 1954.
45) Kraus, E. J.: Pankreas und Hypophyse. Beitr. path. Anat., **68**, 258, 1921.
46) Hasegawa, M.: Histologic changes of the anterior Hypophysis after total pancreatectomy, experiment indog. Arch. Jap. Chir., **24**, 154, 1955.
47) Houssay, B. A. and Biasotti, A.. Le diábète pancréatique des Chiens hypophysectomies. Compt. rend. Soc. Biol., **105**, 121, 1930.
48) Houssay, B. A.: Diabetes as a disturbance of endocrine regulation. Amer. J. Med. Sc., **193**, 581, 1937.
49) Houssay, B. A. et al.: The hypophysis and secretion of insulin. J. exper. Med., **75**, 547, 1942.
50) Tsuchiya, N.: The effects of pancreatectomy in rabbit upon the parotic gland, genital gland and the abdominal organs. Fukuoka act. Med., **17**, 257, 1924.
51) Ōno, Y.: Pancreatectomy. J. J. S. S., **55**, 815, 1954.
52) McAuthur, J. W. et al.. Studies concerning the role of the adrenal cortex in the pathologic physiology of diabetic acidosis. J. Clin. Invest., **33**, 420, 1954.
53) Long, C. N. and Lukens, F. D.: The effects of adrenalectomy and hypophysectomy upon the experimental diabetes in the cat. J. exper. Med., **63**, 465, 1936.
54) Yamamoto, Y.: Studies on the later type of fatty liver of the insulin treated completely depanc-

reatized dog. Arch. jap. Chir., **28**, 1613, 1959.
55) Nomura, T.: The experimental histological studies of the adrenal cortex in the totally depancreatized dog. Tokyo-Zyoshiigakkai-Zassi, **27**, 33, 1957.
56) Perlmutter, M. and Mufson, M.: The hypoglycemic and eosinopenic response to insulin. J. Clin. Endocrinol., **11**, 277, 1951.
57) Goldner, M. G.: Stress, corticoids and diabetes. Diabetes, **7**, 410, 1958.
58) Sakai, E. and Nasu, S.,: The organ weight of the experimental diabetic rabbit. Shindai-Kiyo, in Japan, **6**, 124, 1956.

和文抄録

「インシュリン」投与下に於ける膵全剔犬の副腎皮質機能

金沢大学医学部第2外科学教室（主任 本庄一夫教授）

相川公太郎

犬の膵全切除後，インシュリンにより血糖値をコントロールし，血中 Cpd-F，血糖値，好酸球数，副腎重量を測定し，副腎皮質の組織像を検し，副腎皮質機能を検索した．

1) 血中 Cpd-F は膵全切除後4週位迄は術前より高いものが多く，ACTH に対する反応も4週位は高く，以後，次第に低下の傾向を示す様である．インシュリンに対しては術後は Cpd-F の放出が持続し，4週以後では皮質は疲労し易く，屢々ショックを来し，その際 Cpd-F は血糖値と共に低値を示す．

2) 血糖値は術後上昇し，ACTH に対しては術後4週位は非常に著明な上昇を示すに反し，皮質機能の減退している時には血糖値も ACTH に反応しなくなる様である．血糖はインシュリンにより著明な低下を示しショック症状を起すことがある．

3) 術後好酸球数は次第に減少するが，ソーンテストでは術後も大体80%前後の減少率を維持している．

4) 術後，副腎の比体重重量は正常犬に比し減少している様である．

5) 術後，副腎皮質の脂肪顆粒は一般に減少し，束状層細胞の物に外側部に於いて細顆粒状の組織崩壊像即ち機能低下の傾を見ることが多いが，2週前後では一時的に機能亢進像を見ることがある．

以上より膵全切除後，副腎皮質は代謝の著明な変動に対応して，一時的に皮質機能亢進を示すが，やがて次第に機能低下の状態で一応内分泌平衡を保つが，極めて不安定な状態である様に思われる．

（尚本論文の要旨は第33回及び34回日本内分泌学会総会に於いて発表した．）

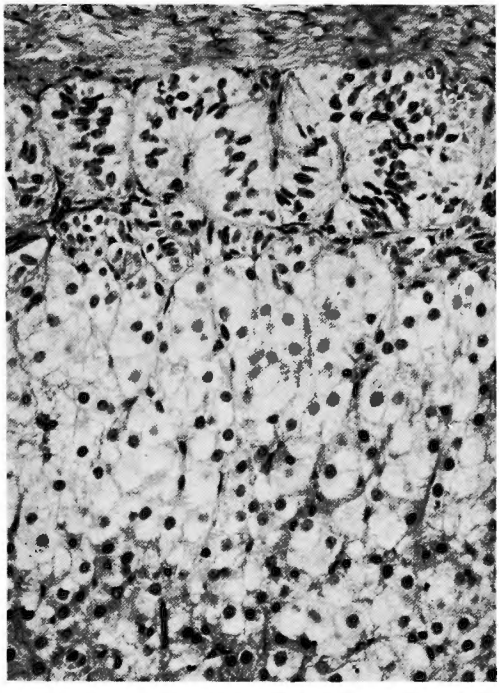

Fig. 6 No. 40 ♂ (H-E) ×100
Surved for 77 days.

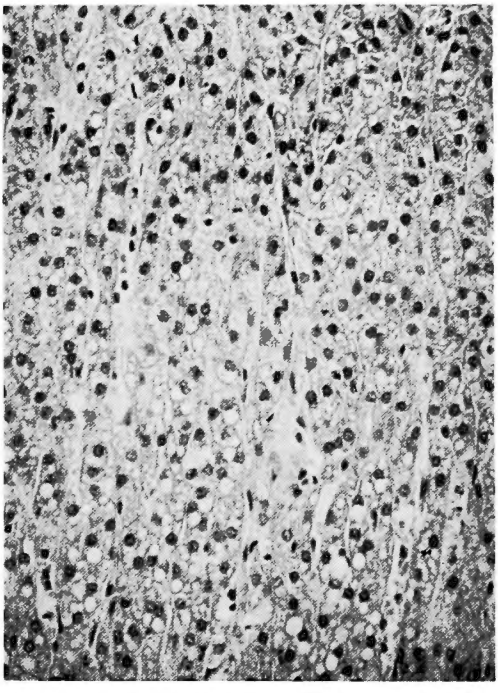

Fig. 7 No. 51 ♀ (H-E) ×100
Survived for 16 days.

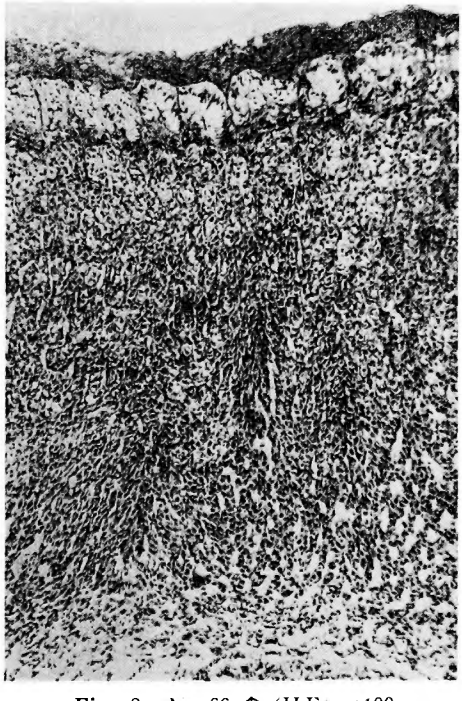

Fig. 8 No. 66 ♂ (H-E) ×100
Survied for 47 days.

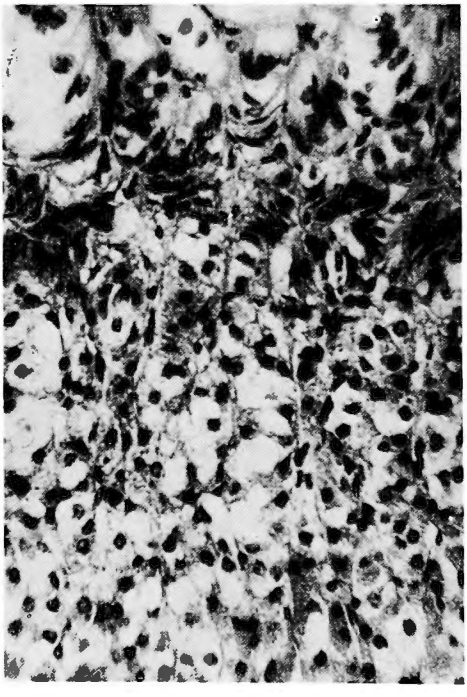

Fig. 9 No. 66 ♂ (H-E) ×400
High-power micrograph of Fig. 8

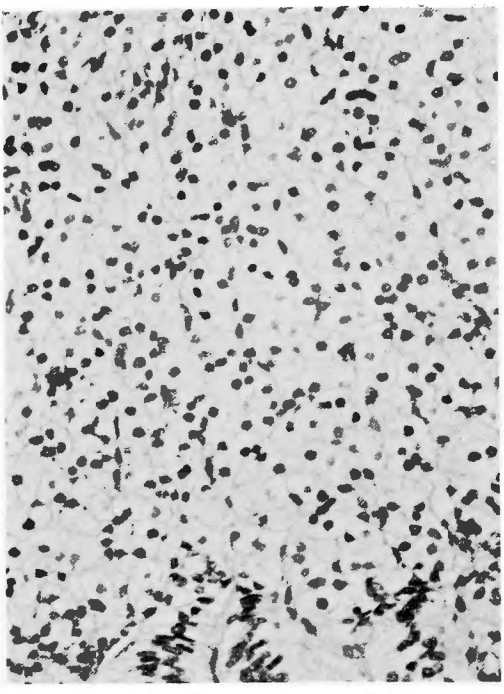

Fig. 10 No. 82 ♁ (H-E) ×100
19th day after total pancreatectomy

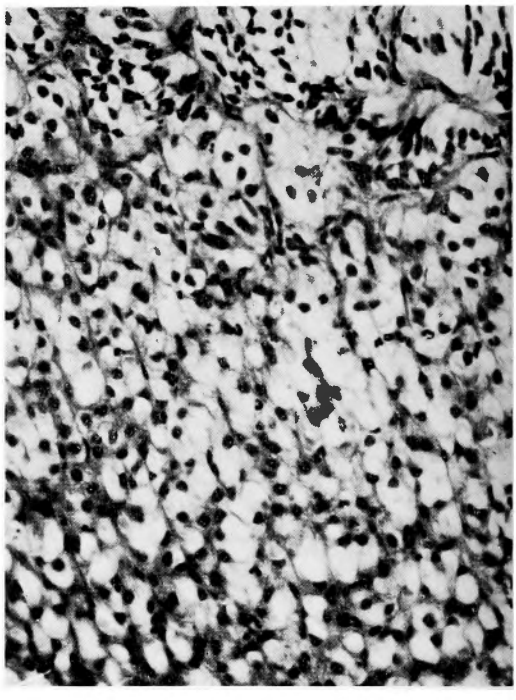

Fig. 11 No. 82 ♁ (H-E) ×400
82nd day after total pancreatectomy

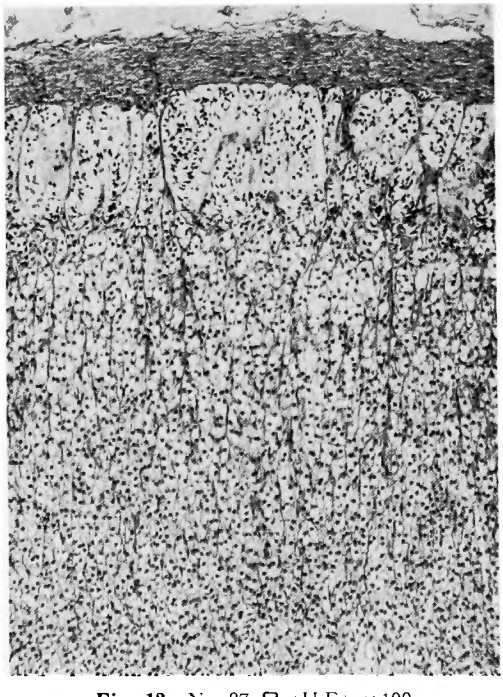

Fig. 12 No. 87 우 (H-E) ×100
Survived for 34 days.

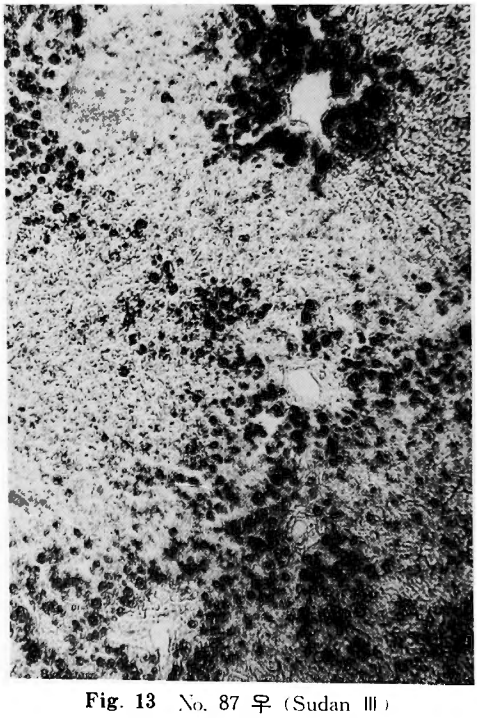

Fig. 13 No. 87 우 (Sudan III)
Fatty infiltration in the liver. ×100

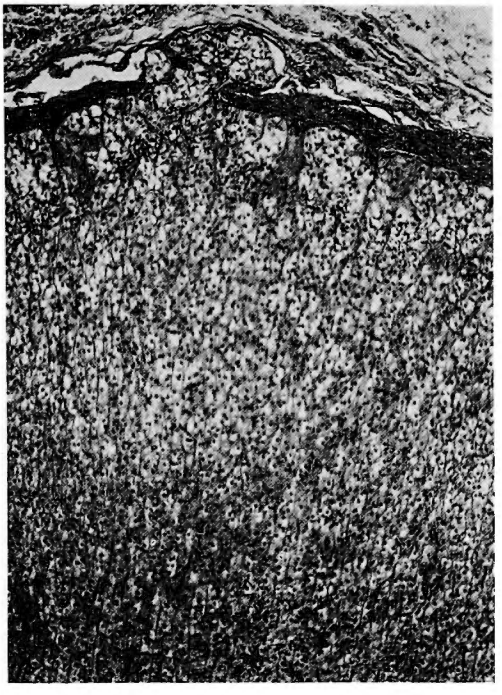

Fig. 14 No. 88 ♂ (H-E) ×100
Survived for 33 weeks.

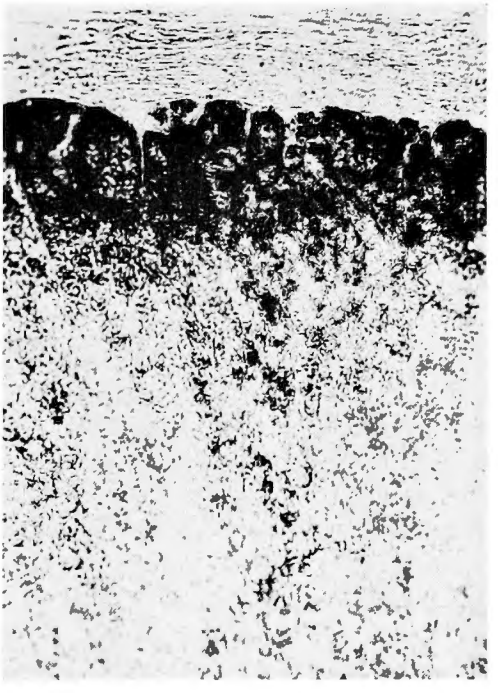

Fig. 15 No. 80 ♀ (Suda Ⅲ)
Survived for 36 days. ×100

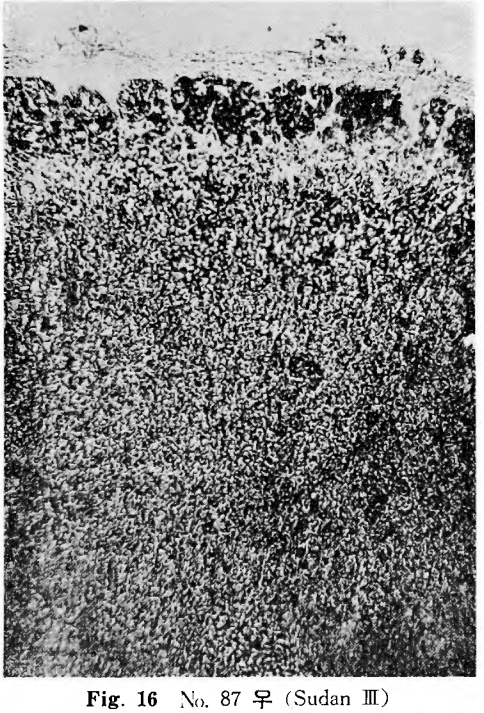

Fig. 16 No. 87 ♀ (Sudan Ⅲ)
Survived for 34 days. ×100

Fig. 17 No. 45 ♀ (Ketosteroid)
Survived for 78 days. ×400