

EXPERIMENTAL STUDY ON THE EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON ADRENOCORTICAL FUNCTION

by

YASUTSUGU TAMAKI

From the 2nd Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. YASUMASA AOYAGI)

Received for publication May 15, 1961

I. INTRODUCTION

The process of fat metabolism in the body, in contrast to that of carbohydrate and protein metabolism, has not yet been sufficiently clarified and it may be said that the relationship between fat metabolism and hormones is hardly solved. However, since DRURY's report, it has been well recognized that diabetes mellitus impairs seriously fat synthesis in the liver and CHERNICK proved the impairment of utilization of glucose-C¹⁴ for the lipogenesis in the disease. MASRI and BRADY recently proved that insulin promoted the synthesis of long chain fatty acids from acetate in vitro in rat liver tissue cultures. MASORO, in starved rats, and BRADY, in pancreatectomized rats, found that the rate of fat synthesis from glucose or acetate decreased markedly. HAUGAARD and TEPPERMAN found that there was a marked increase in the fatty acid synthesis from acetate in livers in which the glycogen content was increased. These findings suggest that there is an intimate relation between fat and carbohydrate metabolism, and also that glucocorticoids have some relation with fat metabolism. BRADY revealed the fact that cortisone and growth hormone suppressed lipogenesis in the liver, and insulin promoted it. LI and INGLE proved histologically and chemically in rats that ACTH mobilized lipids to the liver.

Using a fat emulsion, "Fatgen", prepared in our laboratory, HIKASA et al. investigated the process of fat metabolism. The effect of fat on the liver glycogen content as reported by our colleague MATSUDA and the significance of a deficiency of essential fatty acids (EFA) in the development of acute postoperative pulmonary edema as reported by NAGASE, led to the assumption that there might be an intimate relation between EFA and the synthesis of adrenocortical hormones. In the meanwhile, many investigators have recognized that cortisone and ACTH prevent the development of experimental arteriosclerosis which is closely concerned with impaired fat metabolism. SEIFTER et al. observed that cortisone decreased capillary permeability, and CHAMBER et al. reported that capillary permeability was increased in adrenalectomized rats, and MENKIN proved that the adrenocortical extracts suppressed the increase of capillary permeability in various stresses such as trauma, secondary shock, etc.

The present investigation was designed to clarify the effect of fat deficiency,

especially of EFA deficiency, on adrenocortical function by measuring the glucocorticoids in the urine and blood of rats under various stresses.

II. EXPERIMENTAL ANIMALS AND METHODS

1. Experimental Animals

Male albino rats of the Wistar strain supplied by the Animal Center of Kyoto University were used. The weanling rats were fed a standard diet (rat chow, produced by ORIENTAL Yeast Ind. Co. Ltd., Japan) until their body weight reached 50~60 g and then were divided into three groups: the first group was fed a standard diet, the second, a diet containing 30% sesame oil and the third, a fat-free diet. These animals were carefully kept for two or three months in a room maintained at a constant temperature of 20°C and then used for the experiment.

AS NAGASE reported, each gram of the casein used in this study contained about 1.4 mg of total lipids, 0.2 mg of trienoic acid and no other unsaturated fatty

Table 1 Composition of the diets

| | 30% sesame oil diet | Fat-free diet |
|------------------------|---------------------|---------------|
| Starch | 50% | 80% |
| Casein | 16 | 16 |
| Sesame oil | 30 | 0 |
| Salt-mixture | 3 | 3 |
| Vitamin-mixture | 0.5 | 0.5 |
| Choline chloride | 0.5 | 0.5 |
| Standard diet | | |
| Carbohydrate | 51.4% | |
| Protein | 24.8 | |
| Fat | 5.6 | |
| Mineral | 5.7 | |
| Water | 7.0 | |
| etc | 5.5 | |
| Vitamin-mixture per 1g | | |
| V. A | 1250 | I. U. |
| B ₁ | 0.5 | mg |
| B ₂ | 0.75 | “ |
| B ₆ | 0.05 | “ |
| B ₁₂ | 0.5 | “ |
| C | 3.75 | “ |
| D | 100 | I. U. |
| E | 0.5 | mg |
| K | 0.1 | “ |
| Niacin | 5.0 | “ |
| Pantothenic acid | 0.25 | “ |
| Folic acid | 0.25 | “ |

acids. Therefore, the fat-free diet used in the present study was not completely free from fat, but was a relatively fat-deficient diet and the author called it a fat-free diet for convenience's sake. The lipid content of the starch was less than 0.01% and the EFA content was not in the range of measurement. The source of EFA used was a purified and peroxide-free sesame oil with a linoleic acid content of 40.0%. The content of unsaturated fatty acid in the rat chow was: dienoic acid 0.41%, trienoic acid 0.124%, tetraenoic acid 0.06%, pentaenoic acid 0.09% and hexaenoic acid 0.03%. The total weight of each diet was 10 to 20 g daily, but the number of calories was the same in each diet, which was prepared daily.

2. Method of Measurement of Corticoids

The urinary formaldehydogenic corticoids (UFC) and the plasma fluorometric corticoids (PFC) were measured in rats under various stresses.

i) Method for the Measurement of Urinary Formaldehydogenic Corti-

coids

Rats were kept in false-bottomed cages for several days prior to the experiments and then 24-hour urines were collected. The cages had bottoms with double filters to prevent contamination of urine with food and feces. Urine adhering to the apparatus was washed off with 5~10 ml of distilled water and added to the collected urine. The urine was used as quickly as possible, but, if necessary, it was frozen in an electric refrigerator for about five days and then used. There are various methods for the measurement of urinary corticosteroids. That used in the present study is presented in Table 2 and is the one described by HEGI in "Chemical Method for the Measurement of Hormones".

The chief adrenocortical steroid in rat is known to be corticosterone. Therefore, corticosterone, generously supplied by TEIKOKUZOKI Pharmaceutical Co. Ltd., was used as a standard reagent.

The extinction coefficients of corticosterone and hydrocortisone were 205 and 242 and corticosterone was recovered in the range of 93.7% on the average of three times by this method. This method was excellent for the measurement of urinary corticosteroids (Fig. 1 and Table 2).

Reagents:

- 1) Chloroform (C. P. grade)

Fig. 1 Relationship between absorption factor and amount of formaldehydogenic corticosteroids

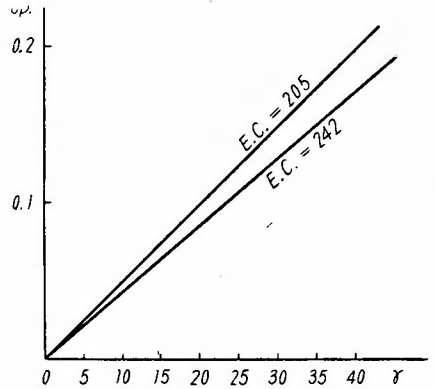
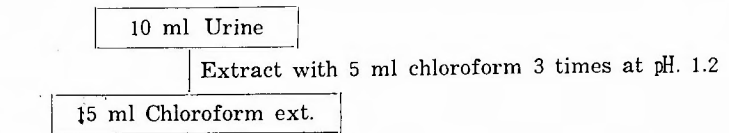


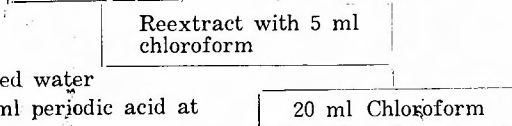
Table 2 Method of measurement of urinary formaldehydogenic corticosteroids

I. EXTRACTION



II. OXIDATION

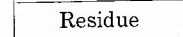
Addition of 4 ml distilled water to the residue and 0.5 ml periodic acid at $25 \pm 1^\circ\text{C}$ for 30 min. Then 0.5 ml stannous chloride is added.



1. Wash with 3 ml water
2. Absorption of the water with sodium sulfate
3. Evaporate to residue in vacuum at less than 50°C

III. DISTILLATION AND COLOR FORMATION

Distill 4 ml to 3 ml chromotropic acid reagent. Heat to 100°C for 30 min. and read at $570 \text{ m}\mu$ in SHIMAZU-QB 50 photoelectric colorimeter.



- 2) $\frac{1}{10}$ N NaOH
- 3) Sodium Sulfate
- 4) Periodic Acid Reagent: 690 mg potassium periodate in 0.25 M. sulfuric acid.
- 5) Stannous Chloride Reagent: Dissolve 280 mg of stannous chloride in 2 ml of concentrated hydrochloric acid and dilute with water to a volume of 8 ml. Make up fresh for every run.
- 6) Chromotropic Acid Reagent: Dissolve 150 mg of chromotropic acid in 2 ml of distilled water and add 48 ml of concentrated hydrochloric acid. Make up fresh for every run.
- 7) 3 M. Sulfuric Acid

Procedure:

A 24-hour urine sample was collected and 10 ml of it was used. The pH was adjusted to 1~1.2 with 3 M. sulfuric acid using an indicator paper. Immediately thereafter, extraction with 5 ml of chloroform was carried out by shaking for 3 minutes. The emulsion thus formed was separated by centrifugation and the chloroform layer was drawn off by suction. The remainder was reextracted twice more with 5 ml of chloroform. The chloroform was washed with 3 ml of 1/10 N NaOH 3 times, and the sodium hydroxide was extracted once with 5 ml of chloroform. The chloroform extracts were all combined and washed with 3 ml of water 3 times and then dried with anhydrous sodium sulfate. The total extract was evaporated to a residue under reduced pressure at a temperature of about 50°C. The residue was redissolved with 4 ml of distilled water, and 0.5 ml of periodic acid was added to this aqueous fraction.

Oxidation was allowed to proceed at a temperature of 25°C for 30 minutes. Excess oxidant was removed by the addition of 0.5 ml of stannous chloride solution, and 4 ml of distillate from the oxidized sample was added in 3 ml of chromotropic acid reagent being in the test tube. The tube was placed in a boiling water bath for 30 minutes. And then it was rapidly cooled with water to room temperature and the color of the sample was measured by a photoelectric colorimeter, SHIMAZU QB-50, at a wave length 570 m μ using a blank made from 4 ml of distilled water through the oxidative procedure mentioned above.

ii) Method for the Measurement of the Plasma Fluorometric Corticoids

This method is a modification of SILBER's and ZENKEL's method and is shown in Table 3. Rats were given nembutal (0.1 ml/100g intraperitoneally) prior to the collection of blood. Blood was taken from the abdominal aorta and used immediately. SILBER reported that the mean level of PFC was 24.3 ± 10.20 γ /dl in male rats of 200 g. The author also obtained 24.0 ± 7.19 γ /dl at rest by this method. The withdrawal of corticosterone was 104.3% on the average of 3 times. All reagents were chemically pure grade, but petroleum ether and alcohol were additionally treated by the following method before use. Petroleum ether: Add 1/10 volume sulfuric acid and shake. Repeat until colored layer of sulfuric acid disappears and wash with water 2 or 3 times. Distill in an all glass distillator and then collect the vapor at 40°~60°C. Alcohol: Add 5 ml of 50% NaOH and 5g of powdered zinc in 1000 ml of

Table 3 Method of measurement of plasma fluorometric corticoids

| | |
|-----------------------|---------------------------------------------|
| Plasma (1~2 ml) | |
| | Wash with petroleum ether (5~10 ml) |
| Plasma (1~2 ml) | |
| | Extract with chloroform (10~20 ml) |
| Chloroform (10~20 ml) | |
| | Wash with 1/10 N NaOH (1~2 ml) |
| | Absorption of the water with sodium sulfate |
| Chloroform (10 ml) | |
| | Extract with fluorescent reagent (4.5 ml) |
| | Measurement of fluorescence after 2 hours |

alcohol. Then shake and boil for about 30 minutes and distill (Table 3).

Reagents:

- 1) Chloroform (C. P. grade)
- 2) 1/10 N NaOH
- 3) 50% Ethyl Alcohol
- 4) Sulfuric Acid (C. P. grade)
- 5) Fluorescent Reagent: Add 24 volumes of sulfuric acid to one volume of 50% alcohol cooling in ice-water. Make up fresh for every run.
- 6) Standard Reagent: Dissolve 1 γ of corticosterone in 1 ml of distilled water.

Procedure:

Rat plasma (1 or 2 ml) was washed with 5 volumes of petroleum ether by shaking for 30 seconds. After centrifugation at 2500 r. p. m. for 5 minutes, the bulk of the aqueous phase was carefully removed by aspiration and discarded. Extraction with 5 volumes of chloroform was carried out immediately by shaking for 30 seconds and then centrifugation was done for 5 minutes at 2500 r. p. m. The plasma layer was carefully removed. One or two ml of 1/10 N NaOH was added to the solvent extract. The solvent extract was quickly shaken for 15 seconds and centrifuged for 3 minutes at 2500 r. p. m. and then alkaline wash was discarded. Sodium sulfate was added to the solvent extract and 4.5 ml of the fluorescent reagent was added to 10 ml of the solvent extract. After shaking for 30 seconds and centrifuging for 5 minutes at 2500 r. p. m. the chloroform layer was removed. After 90 minutes, the fluorescence of the sample was measured by a photoelectric colorimeter, SHIMAZU QB-50, at an exciting wave length of 450 $m\mu$ (Filter: K-7) and at an emitted wave-length of 520 $m\mu$ (Filter: YA-3). As a standard reagent, 1 γ of corticosterone dissolved in 1 ml of distilled water was used.

III. RESULTS

1. Resting Levels of Corticoids in Rats

The rats were kept in individual cages for several days prior to the experiment

Fig. 2 Resting levels of urinary formaldehydogenic corticosteroids in the standard diet group

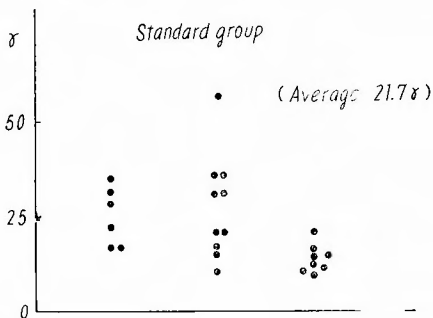


Fig. 3 Resting levels of urinary formaldehydogenic corticosteroids

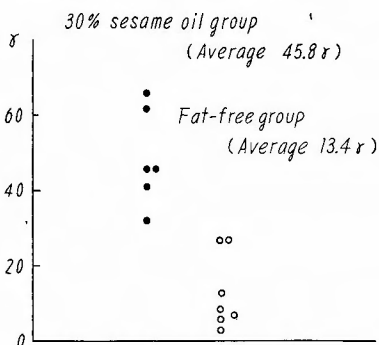


Table 4 Resting level of plasma fluorometric corticoids in the standard diet group

| No. | B. W. | γ/dl |
|-----|----------------|------|
| 1 | 145 g | 32.5 |
| 2 | 160 | 17.1 |
| 3 | 185 | 17.7 |
| 4 | 235 | 18.2 |
| 5 | 140 | 23.0 |
| 6 | 210 | 23.4 |
| 7 | 240 | 28.0 |
| 8 | 240 | 25.2 |
| 9 | 260 | 18.3 |
| 10 | 220 | 40.1 |
| 11 | 195 | 20.9 |
| 12 | 110 | 31.3 |
| 13 | 100 | 25.5 |
| 14 | 115 | 12.3 |
| 15 | 85 | 26.5 |
| | Mean 24.0±7.19 | γ/dl |

Fig. 4 Resting levels of plasma fluorometric corticoids in each group

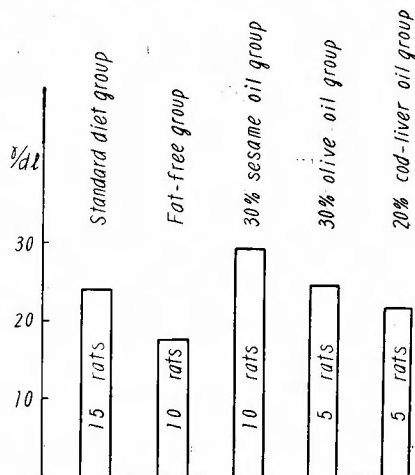


Fig. 5 Changes in urinary formaldehydogenic corticosteroid levels during the course of experimental feedings

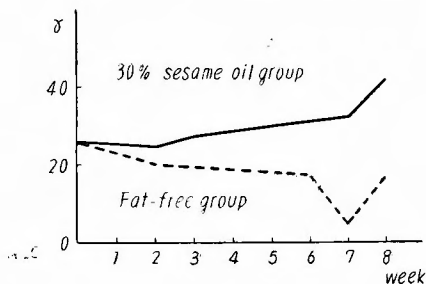


Table 5 Resting levels of plasma fluorometric corticoids

| No. | 30% sesame oil group | | Fat-free group | |
|-----|----------------------|------|----------------|------|
| | B.W. | γ/dl | B.W. | γ/dl |
| 1 | 170 g | 21.2 | 285g | 24.3 |
| 2 | 160 | 16.3 | 175 | 21.4 |
| 3 | 212 | 36.3 | 200 | 22.4 |
| 4 | 230 | 39.9 | 170 | 18.1 |
| 5 | 250 | 24.2 | 150 | 10.8 |
| 6 | 154 | 32.3 | 185 | 16.5 |
| 7 | 190 | 43.8 | 185 | 33.4 |
| 8 | 180 | 15.9 | 195 | 6.2 |
| 9 | 245 | 33.5 | 235 | 11.3 |
| 10 | 235 | 26.2 | 240 | 20.2 |
| | Mean 29.3±8.99 | γ/dl | Mean 18.5±7.30 | γ/dl |

in order to avoid various stresses. As is shown in Figs. 2 and 3, the resting level of UFC is 21.7 γ in the standard diet group, 13.4 γ in the fat-free diet group, 45.8 γ in the 30% sesame oil treated group, 38.2 γ in the 20% sesame oil treated group, 31.7 γ in the 30% olive oil treated group and 23.7 γ in the 20% cod-liver oil treated group. The resting level of PFC is 24.0 γ /dl in the standard diet group, 18.5 γ /dl in the fat-free diet group, 29.3 γ /dl in the 30% sesame oil treated group, 25.2 γ /dl in the 30% olive oil treated group and 21.8 γ /dl in the 20% cod-liver oil treated group. Thus, the resting levels of UFC and PFC are higher in the high fat diet groups than in the fat-free diet group. Moreover, in the high fat diet groups glucocorticoid secretion is more abundant in the sesame oil treated group than in the olive oil or cod-liver oil treated groups. It is believed that this phenomenon may be due to the fact that sesame oil contains much more linoleic acid than olive oil or cod-liver oil. This problem will be discussed later in detail (Figs. 2, 3, 4, and 5, and Tables 4 and 5).

2. Results in Rats under Various Stresses

i) Administration of ACTH

The levels of UFC were measured 24 hours after the intraperitoneal injection of ACTH. The 30% sesame oil treated group showed a higher level than the fat-free diet group. Moreover, the same result was obtained after the daily subcutaneous administration of ACTH-Z for 4 days. The level of PFC measured 2 hours after the subcutaneous administration of ACTH-Z was also much higher in the high fat diet group. These results suggest that in the high fat diet group the adrenocortical function is better maintained and the adrenal cortex can more successfully respond to the increased demand for glucocorticoids after ACTH administration than in the fat-free diet group and that adrenocortical function may be relatively reduced in the fat-free diet group. Next, the following experiments were

Fig. 6 Effect of ACTH on urinary formaldehydogenic corticosteroid levels

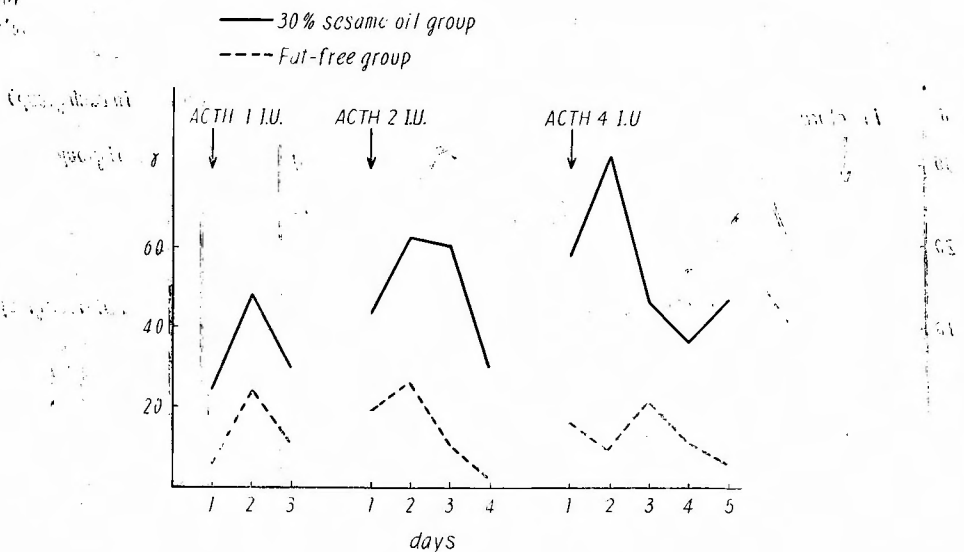


Fig. 7 Effect of ACTH-Z on urinary formaldehydogenic corticosteroid levels

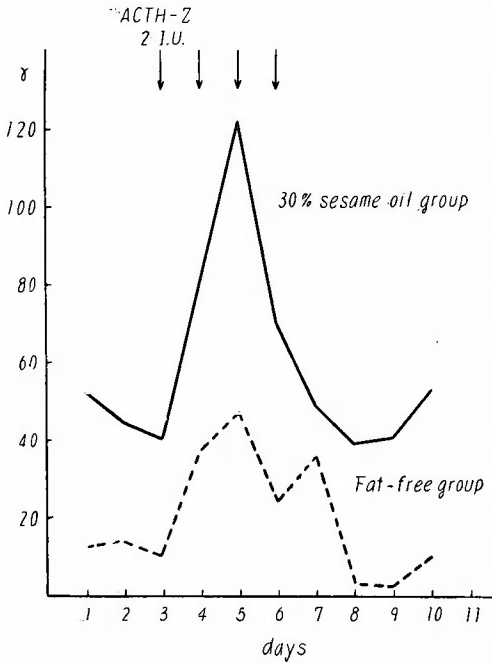


Fig. 8 Effect of ACTH on plasma fluorometric corticoids

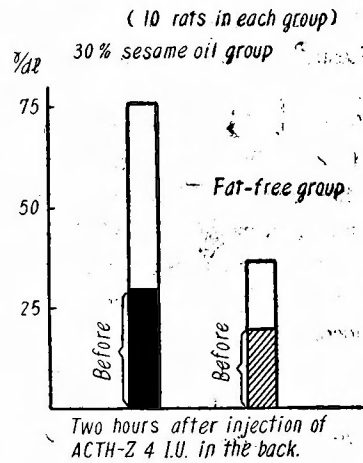
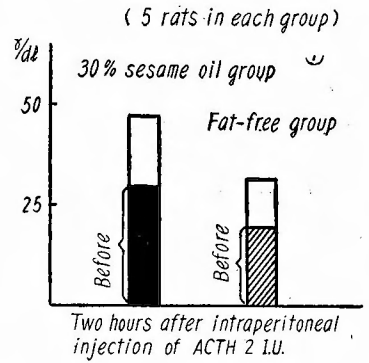


Fig. 9 Changes in urinary formaldehydogenic corticosteroid levels following bilateral fracture of ulna and radius

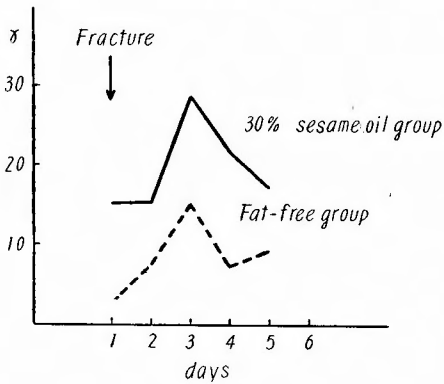
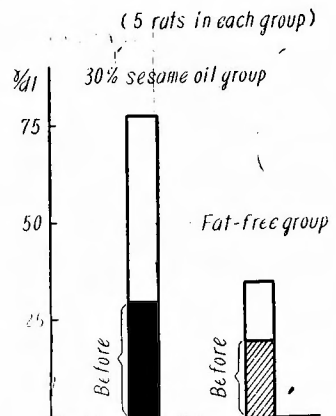


Fig. 10 Changes in plasma fluorometric corticoid levels following bilateral fracture of ulna and radius



carried out in order to test this assumption (Figs. 6, 7 and 8).

ii) Fracture

The level of UFC 24 hours after and the level of PFC 1 hour after bilateral fracture of the ulna and radius were measured (Figs. 9 and 10).

iii) Exposure to cold

The rats were immersed in ice-water for a moment and then were kept in an ice-box at a temperature of 7°C for 1 hour and the level of UFC was measured after 24 hours, and that of PFC at once (Figs. 11 and 12).

Fig. 11 Changes in formaldehydogenic corticosteroid levels in rats exposed to 7°C for 1 hour

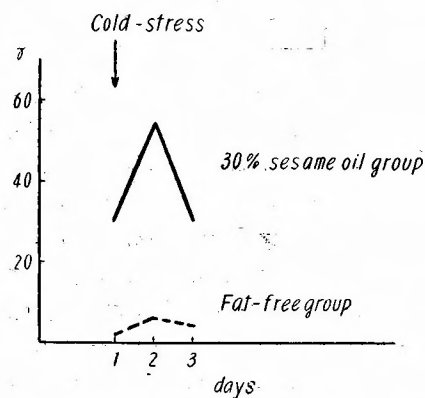
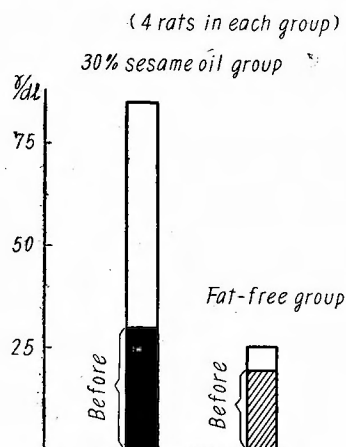


Fig. 12 Changes in plasma fluorometric corticoid levels in rats exposed to 7°C for 1 hour



iv) Formalin injection

The level of PFC was measured 1 hour after the subcutaneous injection of 1 ml of 5% formalin solution. The level of UFC could not be measured by this method because the injected formalin was excreted in the urine (Fig. 13).

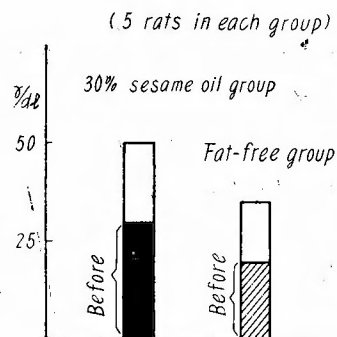
v) Operative insult

The rats were anesthetized with 0.1 ml of nembutal and a 2 cm long laparotomy was performed and the abdominal cavity manipulated with a pincette. The level of PFC was measured 4 hours later. The level of UFC could not be measured because the urine volume was too small after the operation (Fig. 14).

vi) Starvation

Rats were kept separately in metal cages and starved, water being given ad libitum. Only rats weighing about 250 g and of good nutrition were used for this experiment.

Fig. 13 Changes in plasma fluorometric corticoid levels after injection of 5% formalin (1 ml) in the back



Under any of these various stresses, the increase in UFC and PFC were greater in the high fat diet group than in the fat-free diet group. That is, the adrenocortical response is more vigorous and the adrenal cortex can respond better to the increased demand for glucocorticoids in the body in the high fat diet group than in the fat-free diet group under any type of stress. As SANDBERG has revealed, these results show that the adrenocortical function reduced in the fat-free diet group, but is perfectly normal in the high fat

diet group. This had already been suggested by NAGASE's and MATSUDA's experimental results and definitely confirmed by measuring the changes of the levels of UFC and PFC under these various stresses. When the level of UFC and PFC during starvation is considered in connection with the liver glycogen content and the histological changes in the adrenal cortex as reported by MATSUDA, the meaning of these results is more easily understood. As is well known, glycogenolysis progresses first during starvation and there is a marked decrease of liver glycogen. The decrease of the depot glycogen becomes the first stress on the body during starvation and starts the body-defense reaction. Next, an increase in the activity of the pituitary-adrenocortical system is induced and both protein catabolism and gluconeogenesis are stimulated by the mobilized glucocorticoids. The carbohydrate deficiency is compensated by protein catabolism and the threat to homeostasis is checked. If starvation continues longer, deficiency of depot fat and protein in the body gradually progress, and it becomes difficult to maintain homeostasis. Then

the stage of exhaustion, the pathological phase of starvation, begins. However, animals that ordinarily ingest fat containing enough EFA can utilize effectively the storage fat, with a caloric value twice that of carbohydrate and protein, and can economize better on glycogen during starvation than those previously fed mainly carbohydrate, since the former are accustomed to utilize fat. Therefore, in the former the liver glycogen content decreases very slowly even in starvation and the onset of the body-defense reaction is markedly delayed. Moreover, the former

Fig. 14 Changes in plasma fluorometric corticoid levels following surgical insult

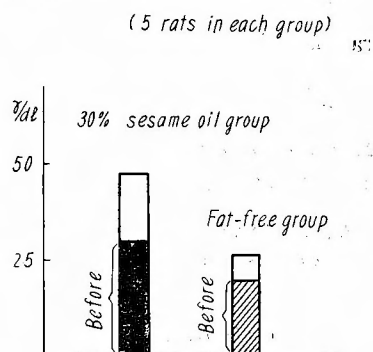


Fig. 15 Effect of fasting on urinary form-aldehydogenic corticosteroid levels



can respond adequately to the increased demand for glucocorticoids, once the pituitary-adrenocortical function is stimulated, and gluconeogenesis progresses satisfactorily. In the latter temporary gluconeogenesis occurs early in starvation, since at an early stage decrease of depot glycogen starts, but the demand for glucocorticoids cannot be supplied for as long as in the former, so relative adrenocortical insufficiency is rapidly induced and the histological changes in the adrenal cortex indicate that exhaustive degeneration develops at an early stage and the latter die sooner than the former. (Figs. 15, 16 and 17).

Fig. 16 Effect of fasting on plasma fluorometric corticoid levels

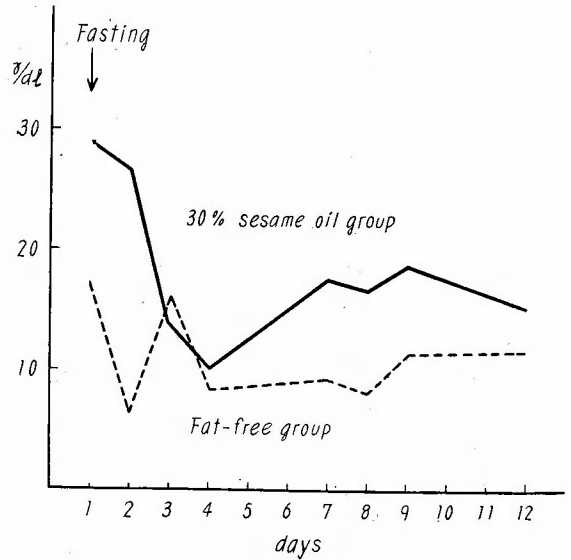
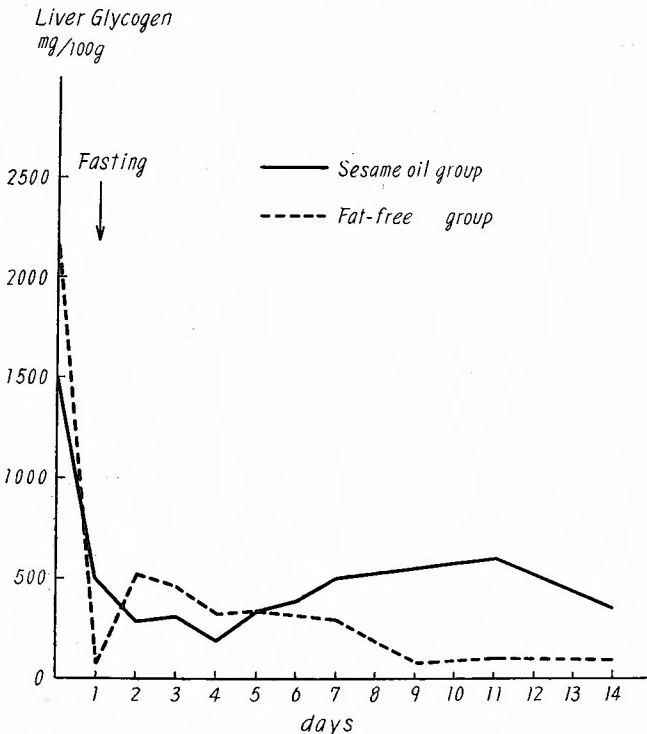


Fig. 17 Changes in liver glycogen content during fasting



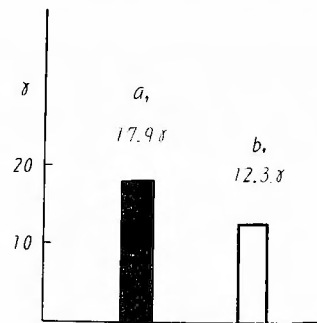
3. Effect of Highly Unsaturated Fatty Acids

Sesame oil, which was used in this study, contains a large amount of polyunsaturated fatty acids, especially EFA. It has been proved that when sesame oil is administered in sufficient quantity, adrenocortical function is maintained. However, certain fats impair the physiological function.

According to our colleague KISHIMOTO, highly unsaturated fatty acids impair the physiological function, and this impairment becomes more significant when highly unsaturated fatty acids contain peroxide which happens easily when fat is in direct contact with air.

Cuttlefish oil contains a large amount of highly unsaturated fatty acid. In order to investigate the effect of highly unsaturated fatty acids and of peroxide on adrenocortical function, cuttlefish oil containing no peroxide or containing a large amount of peroxide were added to each diet in amounts of 20%. These diets were fed to male rats, weighing about 50 g, for about 50 days and the resting level of UFC was measured. As is shown in Fig. 18, in these two groups the resting levels of UFC are lower than in normal rats, especially in the rats fed the peroxide-containing diet. That is to say, the administration of highly unsaturated fatty acid causes a sharp fall in the level of UFC. In order to maintain adrenocortical function, it is obvious that attention should be paid to the quality of administered fat and that fat containing peroxide should be carefully avoided. Since the resting levels of UFC and PFC are usually lower in rats fed cod-liver oil, cuttlefish oil and olive oil than in those fed sesame oil, it is apparent that the administration of sesame oil does not stimulate the pituitary-adrenocortical system and that only when fat containing enough EFA is given can adrenocortical function be well maintained (Fig. 18).

Fig. 18 Resting levels of urinary formaldehydogenic corticosteroids in two rat groups : a) 20% cuttlefish oil containing no peroxide and b) 20% cuttlefish oil containing a large amount of peroxide



4. Effect of Vitamin B₆ Deficiency

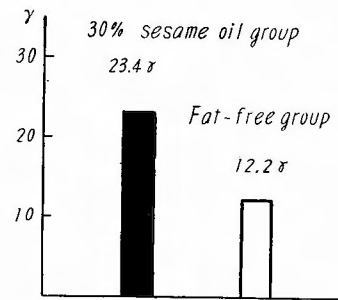
Vitamin B₆ has an intimate relation with fat metabolism, especially with EFA metabolism, and it has recently been shown that this vitamin plays an important role in body-fat synthesis. YAMADA reported that vitamin B₆ deficiency causes coenzyme A deficiency in the liver and severely impairs fat metabolism. Moreover, it has been shown by the measurement of 17-KS level and by THORN'S test that adrenocortical function is depressed by vitamin B₆ deficiency. ISHIHARA has also obtained the same results in rats by measuring plasma corticoid level and by observing the histological changes in the adrenal cortex. MATSUBARA reported that in vitamin B₆ deficiency the hypophyseal tissue degenerated and the hypophyseal function was impaired. In the present study, UFC was measured in order to

determine whether or not adrenocortical function is depressed by the impairment of arachidonic acid synthesis due to vitamin B₃ deficiency, even when linoleic acid is fully administered. Male rats weighing about 50 g were divided into two groups and fed the diets shown in Table 6. In each group, the resting levels of UFC were measured. A commercial casein which had been steeped in 99% alcohol for 5 days in order to extract vitamin B₃ and dried after washing with water several times was used (Table 6).

Table 6 Composition of the vitamin B₃ deficient diet

| | 30% sesame oil diet | Fat-free diet |
|---------------------------------------------------|---------------------|---------------|
| Saccharose | 50 % | 80 % |
| Casein | 16 | 16 |
| Sesame oil | 30 | 0 |
| Choline chloride | 0.5 | 0.5 |
| Salt-mixture | 3 | 3 |
| Vitamin-mixture (V. B ₃ Deficiency) | 0.5 | 0.5 |

Fig. 19 Resting levels of urinary formaldehydogenic corticosteroids in vitamin B₃ deficiency



The resting level of UFC of rats fed a diet containing 30% sesame oil and deficient in vitamin B₃ for one month is much lower than that of rats fed a diet containing 30% sesame oil plus vitamin B₃. On the other hand, the resting level of UFC of rats fed a fat-free diet deficient in vitamin B₃ is almost equal to that of rats fed a fat-free diet containing enough vitamin B₃. Therefore, it is concluded that in vitamin B₃ deficiency impairment of the synthesis of arachidonic acid from linoleic acid occurs first and then the depression of adrenocortical function follows. That is, the adrenocortical function depends mostly on the arachidonic acid content in the body (Fig. 19).

IV. SUMMARY AND DISCUSSION

Up to the present, in the investigation of the relation between fat and adrenocortical hormones, all workers have kept their eyes only on the effect of adrenocortical hormones on fat metabolism and have hardly directed their attention to the effect of fat on the synthesis of adrenocortical hormones. And in the investigation of the nutritional effect of fat, the kind and quality of the administered fat were often not sufficiently considered. Recently we have demonstrated repeatedly that a fat of good quality and containing a large amount of EFA should be administered daily even to patients suffering from various diseases and that only fat of good quality has many important nutritional effects, not only as a caloric source but also as a constant element in the body. In the study on the relation between fat metabolism and adrenocortical function, MAKI, NAGASE and MATSUDA, in our laboratory, have made it definite that in EFA deficiency the volume of EFA, which is contained in the adrenal gland in a larger amount than in any other organs,

decreases and the adrenocortical capacity is greatly reduced. In the present study the author has tried to prove more exactly the fact that adrenocortical capacity decreases in EFA deficiency by measuring the levels of UFC and PFC of rats under various conditions. It was well recognized that the resting levels of UFC and PFC in the high fat diet group were higher than in both the standard and the fat-free diet groups and that the adrenocortical capacity in the high fat diet group was maintained at a normal level, but at a lower level in the fat-free diet group. Moreover, it was well ascertained that these phenomena were due to the EFA content in diet.

The level of UFC and PFC of the sesame oil administered group were higher than those of the olive oil administered group even at a resting stage. And, even if a large amount of linoleic acid was administered, the glucocorticoid secreting ability decreased markedly in vitamin B₃ deficiency, since vitamin B₃ plays an important role in the synthesis of arachidonic acid from linoleic acid. BLUMENFELD has also already demonstrated these facts and also the fact that the adrenal cortex atrophied in rats fed a fat-free diet.

The results obtained by measuring the levels of UFC and PFC in two rat groups under various stresses (ACTH administration, fracture, exposure to cold, formalin injection, operative insult and fasting) showed also that the adrenocortical capacity of the EFA deficient rats decreased. The animal fed a high fat diet rich in EFA has a large adrenocortical capacity and its adrenal cortex can respond sufficiently to increased demand for glucocorticoids in the body at any emergency. On the other hand, the adrenal cortex of the EFA deficient animal contains a small amount of EFA and can not respond to an increased demand for glucocorticoids in the body under various stresses. These facts were clearly understood when the changes of the levels of UFC and PFC were compared with the changes of the liver glycogen content and with the histological changes in the adrenal cortex during starvation.

It was clearly demonstrated here that in EFA deficient animals, the glucocorticoid secreting ability of the adrenal cortex always decreased, and that when the animals were exposed to any severe stresses, the decrease of that ability became greater and the adrenocortical capacity was greatly reduced too.

As mentioned above, we have always paid attention to the specific effect of EFA contained in the fat administration. By what mechanism do EFA affect the adrenal cortex? The relationship of EFA to the synthesis of steroid hormones has not yet been completely solved.

According to SINCLAIR, however, EFA may be concerned with the synthesis of steroid hormones from cholesterol or with the metabolism of steroid hormones such as the esterification of the hydroxyl on C₂₁ in cortisone or hydrocortisone or both. As we have already demonstrated, there is a large amount of EFA not only in the adrenal cortex, but also in the testes and ovary, in which steroid hormones are synthesized, so that it seems probable that EFA play an important role in the transformation of cholesterol, which is one of precursors of steroid hormones, as

an enzyme promoting the process of glucocorticoid synthesis from cholesterol. It is expected that this problem will be solved in the near future by detailed studies on the process of the synthesis of steroid hormones by means of radioisotope C^{14} . Even though administration of fat is necessary for maintenance of adrenocortical function, highly unsaturated fatty acids and peroxides should never be administered, because these acids, especially the latter, impair physiological function as shown both by our colleague KISHIMOTO and by the present study. We must always be careful to administer fat of good quality containing a large amount of EFA. The preservation and cooking of fat should be undertaken with care. We should use only fats which contain a large amount of linoleic acid, which is a precursor of arachidonic acid, and give sufficient amount of vitamin B₆.

It is evident that the higher levels of UFC and PFC in the high fat diet group is not due to the rise of fat-utilization in the body or to the promotion of fat-oxidation, because rats fed sesame oil, which contains a much larger amount of EFA than olive oil or cod-liver oil show the highest level of UFC and PFC. Therefore, the adrenocortical function is kept in a healthy condition only when EFA is administered in sufficient quantities.

V. CONCLUSION

The effect of fat deficiency, especially of EFA deficiency, on adrenocortical function was studied by measuring the levels of UFC and PFC under various conditions.

(1) The resting levels of UFC and PFC in the sesame oil treated group were clearly higher than those in the fat-free group. The ability of the adrenal cortex to secrete glucocorticoids was completely normal in the former, but severely reduced in the latter. That was, adrenocortical function was reduced in the latter.

(2) The adrenocortical capacity in the sesame oil treated group was greater than in the fat-free group and the adrenal cortex of the former group could easily respond to the increased demand for glucocorticoids under any type of stress, but the adrenal cortex of the latter group could not.

(3) These effects of fat administration on the adrenocortical function are due to the presence of EFA in the fat.

(4) The following two facts were also proved, though indirectly.

a) The most potent fatty acid having the same physiological effect as EFA in the body is arachidonic acid.

b) Arachidonic acid can be synthesized from linoleic acid in the presence of vitamin B₆ in the body. Therefore, a sufficient supply of a fat of good quality rich in linoleic acid and of vitamin B₆ is necessary for the wholesome maintenance of adrenocortical function.

The author wishes to express his sincere gratitude to Dr. Y. HIKASA, the lecturer of our clinic, for his helpful suggestions and kind guidance in the course of the work and to H. MURANAKA and H. IMURA, in our second medical department, for their advice in regard to the measurement of glucocorticoids.

REFERENCES

- 1) Blumenfeld, C. M. : Weight changes in the suprarenal glands of albino rats on vitamin E deficient and fat deficient diets. *Endocrinology*, **18**, 367, 1934.
- 2) Brady, R. O. et al. : Synthesis of radioactive fatty acids in vitro and its hormonal control. *J. Biol. Chem.*, **193**, 459, 1951.
- 3) Brady, R. O. and Gurin, S. : Biosynthesis of labeled fatty acids and cholesterol in experimental diabetes. *Ibid*, **187**, 589, 1950.
- 4) Burstein, S. : The guinea pig as a laboratory animal for corticoid excretion studies: Modification of the formaldehydogenic method. *Endocrinology*, **50**, 412, 1952.
- 5) Chamber, R. and Zweifach, B. W. : Intercellular cement and capillary permeability. *Physiol. Reviews.*, **27**, 436, 1947.
- 6) Chernick, S. S. and Chaikoff, I. L. : Insulin and hepatic utilization of glucose for lipogenesis. *J. Biol. Chem.*, **186**, 535, 1950.
- 7) Drury, D. R. : Effect of cortisone on lipid metabolism of plasma, liver and aorta on retrogression of atherosclerosis in the rabbit. *Am. J. Physiol.*, **187**, 66, 1956.
- 8) Li, C. H. et al. : Effect of adrenocorticotrophic hormone upon liver and urinary phosphorus in normal force-fed rat. *Proc. Soc. Exp. Biol. and Med.*, **70**, 753, 1945.
- 9) Eik-nes, K. et al. : Change in plasma level of 17-hydroxycorticosteroids during the intravenous administration of ACTH. *J. Clin. Invest.*, **33**, 1502, 1954.
- 10) Le Femine, A. A. et al. : The adrenocortical response in surgical patients. *Ann. Surgery*, **146**, 26, 1957.
- 11) Fukui, S. et al. : Measurement of the chemocorticoids and clinical significance. "Rinsho Byori" in *Jap.*, **7**, 19, 1954.
- 12) Haugaard, E. S. and Stadic, W. C. : Relation between glycogen content and synthesis of fatty acids by rat liver. *J. Biol. Chem.*, **199**, 741, 1952.
- 13) Hegi, M. : Chemical method for the measurement of hormones (Horomon Kagaku Kensa Ho). *Nankodo in Jap.*, 1951.
- 14) Hikasa, Y. et al. : Parenteral administration of fats. I. Fat metabolism in vivo, studied with fat emulsion. *Arch. Jap. Chir.*, **27**, 396, 1958.
- 15) Hikasa, Y. et al. : Parenteral administration of fats. II. Clinical application of fat emulsion. *Ibid*, **27**, 736, 1958.
- 16) Hikasa, Y. et al. : Parenteral administration of fats. III. Qualitital investigation on nutritional effects of fats and recent studies on fat metabolism in vivo. *Ibid*, **28**, 835, 1959.
- 17) Inaba, M. and Kawamura, M. : A supplement of fundamental experiment about the quantitative examination of urine formaldehydogenic steroids. "Tokyo Gikeikai Ika Daigaku Sashi" in *Jap.*, **70**, 3, 1950.
- 18) Ishihara, N. : Corticoid content in blood of the vitamin B₆ deficiency rat and biohistology of the adrenal gland. *Vitamin*, **18**, 34, 1954.
- 19) Kinsell, L. W. and Michaelis, G. D. : Essential fatty acid, lipid metabolism and atherosclerosis. *Lancet*, **1**, 334, 1958.
- 20) Kishimoto, A. : Experimental studies on the toxicity of rancid fats. *Arch. Jap. Chir.*, **29**, 948, 1960.
- 21) Kaneda, Z. et al. : Examination about the measurement of formaldehydogenic corticoids. "Nishin Igaku" in *Jap.* **41**, 11, 1954.
- 22) Masoro, E. J. et al. : Previous nutritional state and glucose conversion to fatty acids in liver slices. *J. Biol. Chem.*, **185**, 845, 1950.
- 23) Matsuda, S. : Experimental study on the nutritional significance of fat from the view-point of effects on the liver glycogen content. *Arch. Jap. Chir.*, **8**, 3008, 1959.
- 24) Menkin, V. : Effect of adrenal cortex extract on capillary permeability. *Am. J. Physiol.*, **129**, 691, 1940.
- 25) Miyake, G. : Measurement of the adrenocortical hormones and application. (Fukusinhisitu Horomon No Sokutei To Rinsho). "Saishin Igaku" in *Jap.*, **6**, 9, 1951.
- 26) Peron, M. F. F. G. and Dorfman, R. I. : The fluorometric determination of corticosterone

- in rat adrenal tissue and plasma : Effect of administering ACTH subcutaneously. *Endocrinology*, **65**, 717, 1959.
- 27) Nagase, M. : Experimental study on pathogenesis of acute postoperative pulmonary edema. *Arch. Jap. Chir.*, **29**, 69, 1960.
 - 28) Nelson, D. H. and Samuels, L. T. : The level of free 17-hydroxycorticosteroid in blood. *J. Clin. Endocri. and Metabo.*, **12**, 519, 1952.
 - 29) Nishikawa, M. et al. : Chemical assay of the urinary adrenocortical hormone. "Naibunpitu No Tudo" in *Jap.*, **2**, 461, 1955.
 - 30) Okaniwa, H. : Fat metabolism and glucose. "Saishin Igaku" in *Jap.*, **14**, 8, 1959.
 - 31) Pincus, G. : The hormones, Academic press in C. Publisher, 135, 1950.
 - 32) Sandberg, A. A. et al. : The effects of surgery on the blood levels and metabolism and 17-hydroxycorticosteroids in man. *J. Clin. Invest.*, **33**, 1509, 1954.
 - 33) Silber, R. H. et al. : Practical procedure for estimation of corticosterone or hydrocortisone. *Clin. Chemistry*, **4**, 278, 1958.
 - 34) Sinclair, H. M. : *Essential Fatty Acid*, Butterworths Scientific Publication, 250, 1958.
 - 35) Tanaka, H. and Idemura, K. : Fatty acid and skin. "Saishin Igaku" in *Jap.*, **14**, 8, 1958.
 - 36) Tepperman, J. and Tepperman, H. M. : Effects of antecedent food intake pattern on hepatic lipogenesis. *Am. J. Physiol.*, **193**, 55, 1958.
 - 37) Yamamoto, T. : Studies on the cardiovascular lesion in vitamin B₆ deficient rats. *Vitamin*, **19**, 117, 1960.
 - 38) Yamada, K. et al. : Experimental study of the fat metabolism in vitamin B₆ deficient rats. *J. J. G. E. in Jap.*, **52**, 439, 1955.
 - 39) Yamada, K. : Clinical study on Vitamin B₆. "Nihon Rinsho" in *Jap.*, **15**, 4, 1957,
 - 40) Zenker, N. and Berstein, D. E. : The estimation of small amount of corticosterone in rat plasma. *J. Biol. Chem.*, **231**, 695, 1958.

和文抄録

不可欠脂酸の欠乏が副腎皮質機能に及ぼす
影響に関する実験的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

玉 木 泰 嗣

生体内に於ける脂質の中間代謝過程は、糖質及び蛋白質のそれに比較して、遙かに不明の点が多く、殊に脂質代謝と副腎皮質ホルモンとの関係は、未だ多くの未知の分野を賅している。われわれの教室に於ては、経静脈性注入可能なゴマ油乳剤“Fatgen”の作製に成功して以来、これを用いて脂質の中間代謝過程の究明に努めて来たが、この間に、松田が行つた脂質の投与が肝グリコーゲン量に及ぼす影響についての研究成績、更に長瀬が行つた術後急性性肺水腫の発生素因としての不可欠脂酸欠乏の意義についての研究成績は、何れも不可欠脂酸と副腎皮質ホルモンの生合成機能との間に、何等か密接な関係のあるらしいことを臆測させた。そこで、脂質殊に不可欠脂酸の欠乏の有無が、副腎皮質の Glucocorticoids 分泌能に対して、どのような影響を及ぼすものであるかを、尿中及び血中 Glucocorticoids 量の測定によつて検討した。

即ち、ウイスター系雄性ラツテを2群に分け、一群は合成無脂質飼料で、他の一群は30%ゴマ油含有合成飼料で夫々2~3ヵ月間飼育して、実験に供した。そして、次の結果をえた。

(1) 30%ゴマ油含有合成飼料で飼育したラツテでは、無脂質飼料で飼育したラツテに比べて、安静時に於ても尿中及び血中 Corticoids 量は高値を示し、且

つ副腎の Glucocorticoids 分泌能は完全な正常状態を示すが、後者に於ては、分泌能は著しく低下している。即ち、副腎皮質機能の低下していることが認められる。

(2) ACTH 投与、骨折、寒冷刺激、ホルマリン投与、外科侵襲及び饑餓等の各種ストレス負荷時に於て、前者は、その個体の要求する Glucocorticoids 需要量に充分応じ得るのに反して、後者では、その需要量を充足し得ない。即ち、副腎皮質機能予備力では、前者がはるかに後者に勝つていることが認められる。

(3) このような、脂質投与の有無が、副腎皮質機能に及ぼす作用の本態は、脂質中に含有される不可欠脂酸によるものである。

(4) 生体内で、終極的に不可欠脂酸としての特有の作用を発揮するものは、不可欠脂酸中のアラキドン酸であつて、而もそれは、生体内でビタミンB₆の作用下にリノール酸から合成されうることが、間接的に立証された。従つて、日常食物として摂取される油脂中には、アラキドン酸は殆ど含有されていないのが通常であるから、吾人は、リノール酸を充分に含有した脂質をビタミンB₆と共に、充分に摂取することが副腎皮質機能を健常に保持してゆくために必要である。