EXPERIMENTAL STUDIES ON THE EFFECT OF ADMINISTRATION OF ESSENTIAL FATTY ACIDS UPON ADRENOCORTICAL CAPACITY FROM THE VIEW-POINT OF CHOLESTEROL METABOLISM

by

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I. INTRODUCTION

Recently, as studies on fat metabolism have advanced, the significance of fat not only as a variable element but also as a constant element has become clear, and the presence of essential fatty acids (abbreviated as EFA) which are indispensable for maintaining physiological functions of organisms has come to be recognized as very important. Furthermore, it has been demonstrated recently that EFA are very intimately concerned with adrenocortical capacity. It has also been clarified that adrenocortical capacity decreases when excessive cholesterol is administered to rabbits, and that the feeding of cholesterol to fat-deficient animals promotes the symptoms of EFA-deficiency.

Thus the physiological significance of the EFA involved in cholesterol metabolism in vivo, and the effect of the administration of fat on adrenocortical cholesterol content or on adrenocortical capacity have been only partially clarified.

The present study was designed to make clear the effect of administration of fats, especially EFA, on adrenal cholesterol, which is considered to be one of the precursors of adrenocortical hormones, and an attempt was made to demonstrate the relationship between adrenal EFA content and adrenocortical capacity in rats fed a fat-free diet or fat diet, with or without added cholesterol.

II. EXPERIMENTAL ANIMALS AND METHODS

A) Experimental Animals

Male weanling albino rats of the WISTAR strain supplied by the Animal Center in Kyoto University were used for this study. They were fed a rat chow (a product of ORIENTAL Yeast Ind. Co. Ltd. Japan) until their body weight reached about 40～50g, then were divided into the following six groups; fat-free diet group, sesame oil diet group (20% sesame oil), olive oil diet group (20% olive oil), cod liver oil diet group (20% cod liver oil), cholesterol plus sesame oil diet group (adding cholesterol in a concentration of 1.0% to the sesame oil diet) and cholesterol plus fat-free diet group (adding cholesterol in a concentration of 1.0% to the fat-free diet). These rats were placed in a room maintained at a constant temperature of 20°C and were fed at libitum the experimental diets for a period of about two months prior to the experiments to be described. As the relative adrenal weight or adrenal cholesterol content tends to vary with such factors as age, body weight, duration of feeding or condition of feeding etc, an attempt was made to keep these factors as constant as possible.

The weight composition of each diet is as follows. Fat-free diet: starch 80%, casein 16%, mixed salts 3%, vitamin mixture 0.5% and choline chloride 0.5%. Fat diet: fat (sesame oil, olive oil or cod liver oil) 20%, starch 60%, the other components being just the same as in the fat-free diet (Table 1, 2).

Table 1 Composition of the diets.

	Fat-free diet	Fat diet
Starch	80 %	60 %
Casein	16 〃	16 〃
Fat (sesame oil, olive oil, or cod liver oil)	0 〃	20 〃
Salt-mixture	3 〃	3 〃
Choline chloride	0.5 〃	0.5 〃
Vitamin-mixture	0.5 〃	0.5 〃

Table 2 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 〃
Niacine	5.0 〃
Pantothenic acid	0.25 〃
Folic acid	0.25 〃

As the source of EFA a purified and peroxide-free sesame oil was used; its linoleic acid content was 40.4%. Therefore, a rat eating 10g of the sesame oil diet daily takes in about 800mg of linoleic acid per day. According to our colleague NAGASE, each gram of the casein used in this study contained about 1.39mg of total lipids and 0.20mg of trienoic acid (fatty acids having three double bonds in a molecule, linolenic acid being one of these acids), and no other unsaturated fatty acids. Therefore, a rat eating 10g of the fat-free diet daily takes in less than 0.32mg of EFA per day. In this respect, the fat-free diet used in this study is not absolutely but comparatively fat-free.

As previously mentioned, the feeding of cholesterol to EFA-deficient animals promotes symptoms of deficiency, so 1% cholesterol was added to the diet in order to accelerate the development of EFA-deficiency.

B) Experimental Methods

1) Determination of Cholesterol

After animals were anesthetized by an intraperitoneal injection of nembutal and sacrificed by bleeding from the aorta, both adrenal glands were excised and weighed exactly by torsion balance. Then the excised samples were homogenized and lipids were extracted in hot ethanol-ethyl ether (3 : 1) solution. Using these extracts, total cholesterol (abbreviated as T-chol) and esterified cholesterol (abbreviated as E-chol) were determined by YANAGISAWA's method, which is a modification of ZUCKERMANN's and KINGSLEY's method. The esterified cholesterol ratio (abbreviated as EQ) was expressed by the E-chol. to T-chol. ratio.

The values reported in this paper are mean-values of three to six samples.

2) Determination of Unsaturated Fatty acids

The unsaturated fatty acids contained in the adrenals of rats were determined by

JINDO's micromethod, which is a modification of the method of HOLMAN and HAYES.

The values reported are mean-values of three samples.

III. EXPERIMENTAL RESULTS

A) Effect of Feeding the Fat-free Diet or Fat Diet on the Adrenal Cholesterol Content in the Resting State

Six diet groups of rats (fat-free diet, sesame oil diet, olive oil diet, cod liver oil diet, cholesterol plus sesame oil diet and cholesterol plus fat-free diet) were used, and their adrenal weights and adrenal T-chol. and E-chol. contents were measured. Each group contained six rats.

During the course of the feeding, no symptoms of EFA-deficiency were observed in the simple fat-free diet group, but marked retardation in growth and loss of hair on the back were observed in the cholesterol plus fat-free diet group.

As seen from the results shown in Table 3, the adrenal T-chol. and E-chol. contents were greater in general in the fat diet groups (sesame oil diet, olive oil diet and cod liver oil diet groups) than in the fat-free diet group. Moreover, the kind of fat administered greatly influenced the adrenal cholesterol content. That is, the adrenal T-chol. content increased slightly in rats on the olive oil diet and increased greatly in those on the cod liver oil diet, in comparison with the level in those on the sesame oil diet. No significant difference was observed in the adrenal weights among rats on the sesame oil diet group, the olive oil diet group and the fat-free diet group, although the adrenal weights of those on the cod liver oil diet increased markedly.

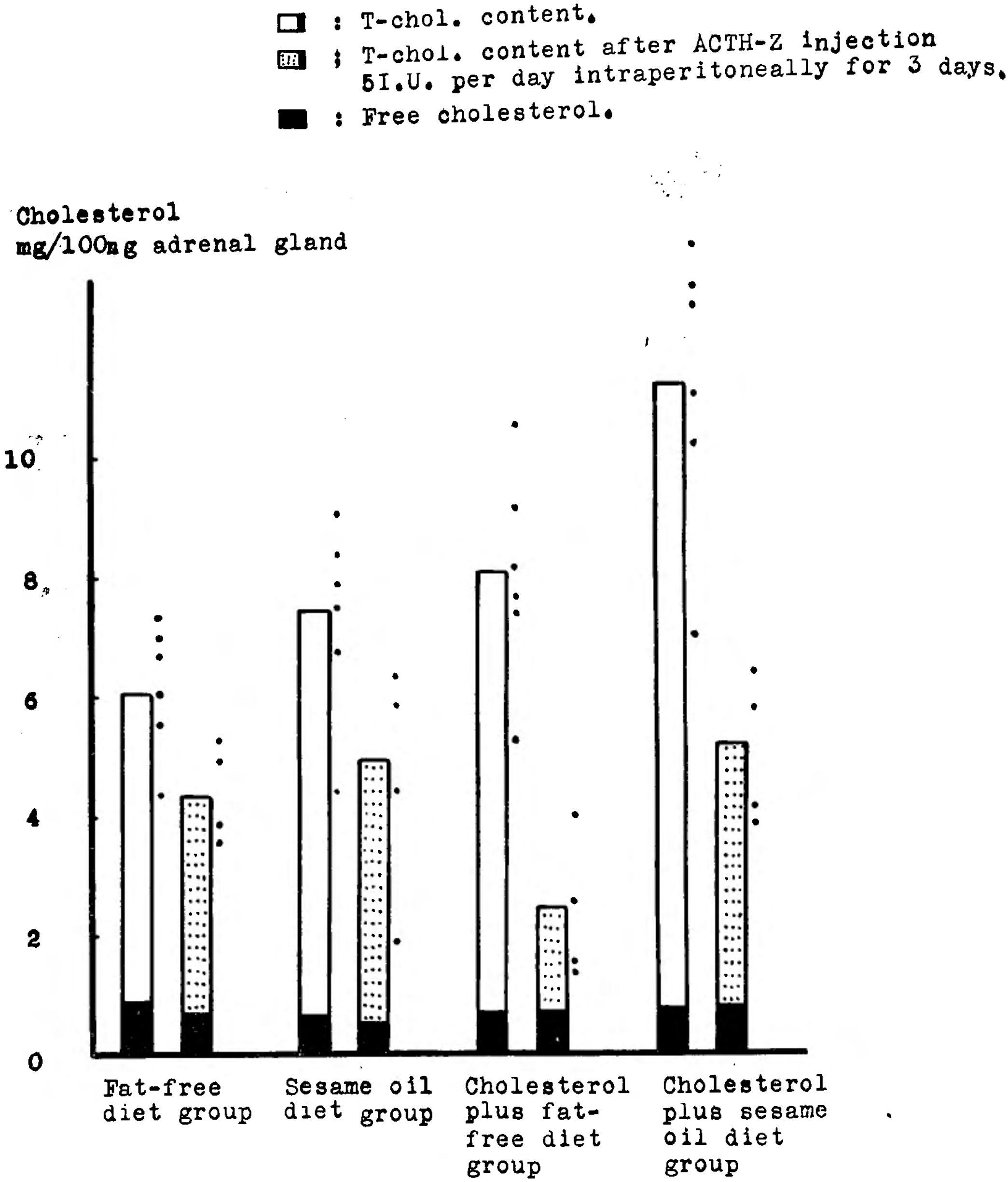
Addition of cholesterol to the diet caused the adrenal weight and the adrenal T-chol. and E-chol. contents to increase greatly both in the cholesterol plus fat-free diet group and in the cholesterol plus sesame oil diet group.

Table 3 Adrenal cholesterol content of rats fed each diet for 2 months. Each group contains 6 rats.

Group	Adrenal weight	T-chol. content	EQ
	mg/100g body weight	mg/100mg adrenal gland	%
Fat-free diet	11.3	6.0	85.0
Sesame oil diet	13.9	7.3	90.1
Olive oil diet	17.1	7.8	91.2
Cod liver oil diet	27.3	14.2	94.5
Cholesterol plus fat-free diet	13.7	8.2	90.8
Cholesterol plus sesame oil diet	16.2	11.3	93.9

The unsaturated fatty acid content in some organs of rats was studied by our colleagues JINDO and NAGASE, who showed that the organs of rats fed a fat-free diet showed a marked decrease of dienoic acid and tetraenoic acid and a great increase of trienoic acid (Fig. 7). In the present study, the adrenal unsaturated fatty acid content of rats fed a fat-free diet or a sesame oil diet containing one percent cholesterol was measured. The results are shown in Table 5 and Fig. 8. The adrenals of rats in the cholesterol plus sesame oil diet group contained a large amount of EFA, while those of rats in the

Fig. 1 Effect of ACTH-Z injection on adrenal cholesterol content. Dot represents an individual rat.



cholesterol plus fat-free diet group showed a more typical pattern of EFA-deficiency than those of rats in the simple fat-free and cholesterol-free diet groups. Therefore, it is postulated that the administration of cholesterol to EFA-deficient animals accelerates EFA-deficiency.

The urinary formaldehydogenic corticoid (abbreviated as UFC) concentration of rats fed a fat-free diet, a rat chow or some kind of fat diets was investigated by our colleague

TAMAKI. As seen from the results shown in Table 4, the UFC concentration of the rats fed the fat diet was higher than that of those on a fat-free or chow diet. Among the fat diet groups the UFC concentration was the highest in the sesame oil diet group, it was lower in rats fed the cholesterol plus sesame oil or the cholesterol plus fat-free diet than in those fed the sesame oil or the fat-free diet. The author noted signs of marked EFA-deficiency in the adrenals of the cholesterol plus fat-free diet group, as already described in this paper.

It can therefore be assumed that adrenal weight and adrenal cholesterol content are not proportional to the adrenocortical function to secrete glucocorticoids, and that it is rather the kind of fat administered or the adrenal EFA content that is concerned with adrenocortical capacity. Consequently, when rats are fed the olive oil diet containing less EFA or the cod liver oil diet containing a large amount of highly unsaturated fatty acids, docosenoic acid and eicosenoic acid which are harmful to organisms, their adrenocortical capacity decreases, although their adrenal weight and adrenal cholesterol content increase, in comparison with rats fed the sesame oil diet rich in EFA. Moreover, the addition of cholesterol to fat-free diet promotes adrenal EFA-deficiency and decreases adrenocortical capacity, although the adrenal cholesterol content increases.

Table 4 Resting levels of urinary formaldehydogenic corticoids in each group.

Fat-free diet	av. 13.4 γ
Rat chow	〃 21.7〃
20% sesame oil diet	〃 38.2〃
30% sesame oil diet	〃 45.8〃
30% olive oil diet	〃 31.7〃
20% cod liver oil diet	〃 23.7〃
1% cholesterol plus 20% sesame oil diet	〃 31.3〃
1% cholesterol plus fat-free diet	〃 10.8〃

B) Changes in Adrenal Cholesterol Content during Stress, and Relationship between Fat-feeding and Adrenocortical Capacity

a) Fasting

Nineteen rats of the sesame oil diet group and seventeen of the fat-free diet group were fasted for a period. Water was supplied at libitum. On the 2nd, 6th and 10th or

Table 5 EFA content in adrenals of rats fed cholesterol plus fat-free diet or cholesterol plus sesame oil diet. (percentage of organ weight)

	Cholesterol plus fat-free diet group	Cholesterol plus sesame oil diet group
Dienoic acids	0.20	0.92
Trienoic acids	0.68	0.56
Tetraenoic acids	0.54	1.50

Table 6 EFA content in adrenals of rats after ACTH-Z injection (5 I.U. per day intraperitoneally for 3 days). (percentage of organ weight)

	Cholesterol plus fat-free diet group	Cholesterol plus sesame oil diet group
Dienoic acids	0.04	0.25
Trienoic acids	0.82	0.22
Tetraenoic acids	0.06	0.39

11th days three to five rats of each group were sacrificed and their adrenal weights, adrenal T-chol. content and E-chol. content were measured.

The changes in adrenals of the above two groups during the period of fasting were as follows. First of all, at an early stage of fasting, E-chol. content and EQ increased. Subsequently, T-chol. content increased, and at the same time adrenal weight increased also. However, at the end stage of fasting, adrenal T-chol. content and EQ diminished extremely (Table 7, Figs. 2, 3, 4).

Table 7 Changes in adrenal cholesterol content during fasting.

	Duration of fasting	Day	Control	2	6	10	11
Fat-free diet group	Number of rat		4	4	4	3	4
	Adrenal weight	mg/100g body weight	18.7	19.8	25.1	30.3	39.9
	〃	mg/100g body weight before fasting	18.7	17.2	19.1	18.2	22.0
	T-chol. content	mg/100mg adrenal gland	5.73	5.81	8.52	7.39	1.67
	EQ	%	87.8	92.8	92.0	88.5	74.2
Sesame oil diet group	Number of rat		4	4	4		5
	Adrenal weight	mg/100g body weight	17.9	18.9	17.1		32.2
	〃	mg/100g body weight before fasting	17.9	16.0	15.1		19.1
	T-chol. content	mg/100mg adrenal gland	7.12	6.05	8.10		8.30
	EQ	%	89.6	89.7	92.1		87.3

Fig. 2 Effect of fasting on adrenal cholesterol content.

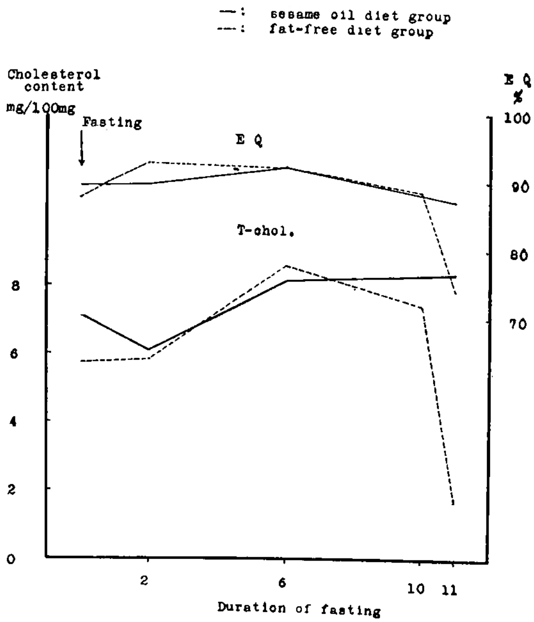
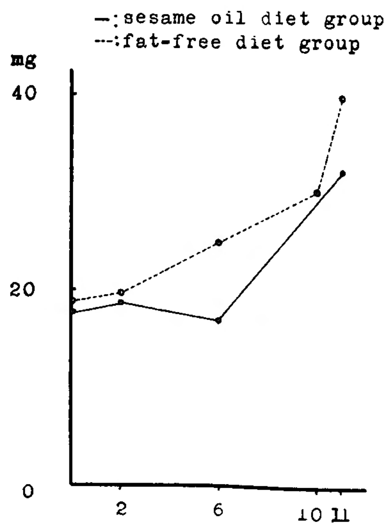


Fig. 3 Effect of fasting on adrenal weight. (mg/100g body weight)



These changes progressed more slowly and mildly in the sesame oil diet group than in the fat-free diet group. In the fat-free diet group EQ had already increased greatly on the second day, then both adrenal T-chol. content and adrenal weight increased markedly on the 6th day. However, in the sesame oil diet group EQ was still not increased on the 2nd day; EQ and T-chol. content increased on the 6th day and adrenal weight increased on the 11th day. The survival period of fasting rats was markedly prolonged by previous feeding with the sesame oil diet, as our colleague MATSUDA has demonstrated.

The above observations were compared with the experimental results of MATSUDA on the changes in the liver glycogen content and the histological findings of the adrenal cortex, and with the results of TAMAKI on the changes in the UFC concentration in response to fasting of rats fed the fat-free diet or the sesame oil diet (Figs. 5, 6). The increase of EQ in the early stage of fasting corresponds to the situation in which decrease of liver glycogen due to glycogenolysis acts as a stressor to the organism and stimulates the hypophyseal-adrenocortical system to secrete glucocorticoids, which in turn accelerate gluconeogenesis. The subsequent increase of adrenal T-chol. content and of adrenal weight correspond to the

Fig. 4 Effect of fasting on adrenal weight. (mg/100g body weight before fasting)

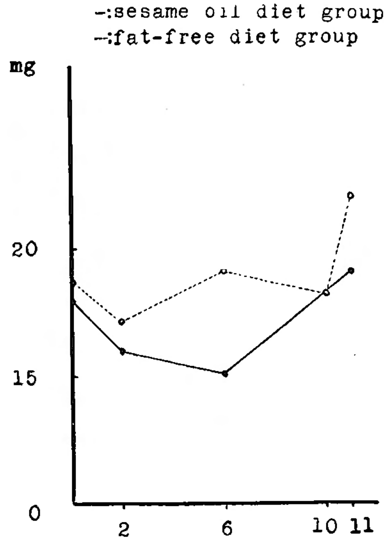


Fig. 5 Changes in liver glycogen content during fasting (by MATSUDA).

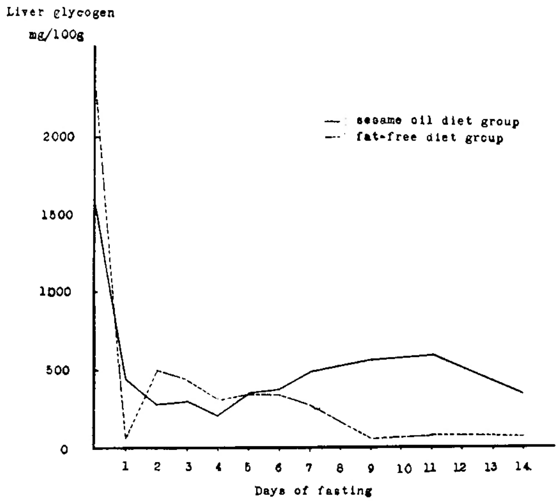
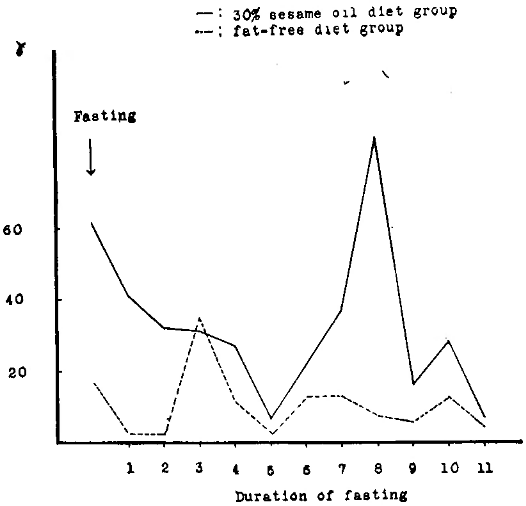


Fig. 6 Effect of fasting on urinary formaldehydogenic corticoid levels (by TAMAKI).



stage in which gluconeogenesis continues to progress and the organism can respond adequately to fasting. And the extreme depletion of T-chol. and EQ corresponds to the stage in which the adrenal cortex begins to be exhausted and becomes unable to maintain homeostasis, and the destruction of the various organs and tissues advances irreversibly until death.

b) ACTH-Injection

Four groups of rats which were fed the fat-free diet or the sesame oil diet, with or without the addition of 1% cholesterol, were used in this study.

ACTH-Z was injected in a dose of 5 I. U./day intraperitoneally into four rats of each group for three days repeatedly. Twenty four hours after the last injection all rats were sacrificed and adrenal weight, adrenal T-chol. and E-chol. contents were measured. At the same time histological investigations of the adrenal cortex were made by means of Sudan Ⅲ and Hematoxylin-Eosin staining. In addition the adrenal unsaturated fatty acid content was determined both in the cholesterol plus fat-free diet group and the cholesterol plus sesame oil diet group under exactly the same experimental conditions.

Following ACTH-Z injection, adrenal weight increased and adrenal T-chol. content diminished in all rats, but what decreased in amount was the E-chol.. However, no significant differences were observed in the degree of depletion of adrenal T-chol. content between the fat-free diet group and the sesame oil diet group. On the contrary, a marked difference was observed between the cholesterol plus fat-free diet group and the other three diet groups. That is, the degree of depletion of adrenal T-chol. content as well as of E-chol. content was more marked in the cholesterol plus fat-free diet group than in the other three diet groups (Table 8, Fig. 1).

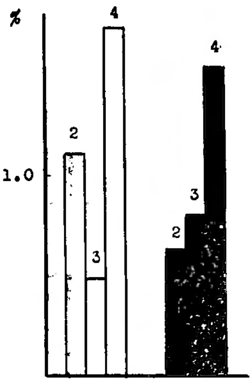
Table 8 Effect of ACTH-Z injection on adrenal cholesterol content (4 rats fed each diet for 2 months) after injection of ACTH-Z intraperitoneally, 5 I.U. per day for 3 days.

Group	Adrenal weight	T-chol. content	EQ
	mg/100g body weight	mg/100mg adrenal gland	%
Sesame oil diet	22.5	4.71	91.7
Fat-free diet	21.3	4.29	87.8
Cholesterol plus sesame oil diet	24.5	5.08	85.1
Cholesterol plus fat-free diet	27.8	2.40	72.0

Moreover as shown in Table 6 and Fig. 9, although the adrenal EFA content both in the cholesterol plus fat-free diet group and in the cholesterol plus sesame oil diet group decreased markedly, in the latter a fairly large amount of dienoic and tetraenoic acids was still contained, while in the former a marked decrease of these fatty acids and an increase of trienoic acid (this acid is considered to be not linolenic acid but 5, 8, 11-eicosatrienoic acid) were observed.

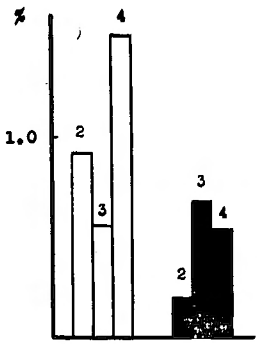
Among the histological findings, a depletion of lipid granules was noted, and cell-columns became irregular following ACTH-Z injection. Furthermore, in many cases cytolysis and formation of vacuoles, mainly in the zona fasciculata, were clearly observed and generally believed to be exhaustive changes. These exhaustive changes were most marked in the cholesterol plus fat-free diet group.

Fig. 7 EFA content in adrenal glands of rats fed sesame oil diet or fat-free diet (by NAGASE).



□ : Sesame oil diet group
■ : Fat-free diet group
2 ; Dienoic acids
3 · Trienoic acids
4 : Tetraenoic acids

Fig. 8 EFA content in adrenal glands of rats fed cholesterol plus sesame oil diet or cholesterol plus fat-free diet.



□ : Cholesterol plus sesame oil diet group
■ : Cholesterol plus fat-free diet group
2 : Dienoic acids
3 : Trienoic acids
4 : Tetraenoic acids

Fig. 9 EFA content of adrenal grands of rats fed cholesterol plus sesame oil diet or cholesterol plus fat-free diet after ACTH-Z injection (5I. U. per day intraperitoneally for 3 days).

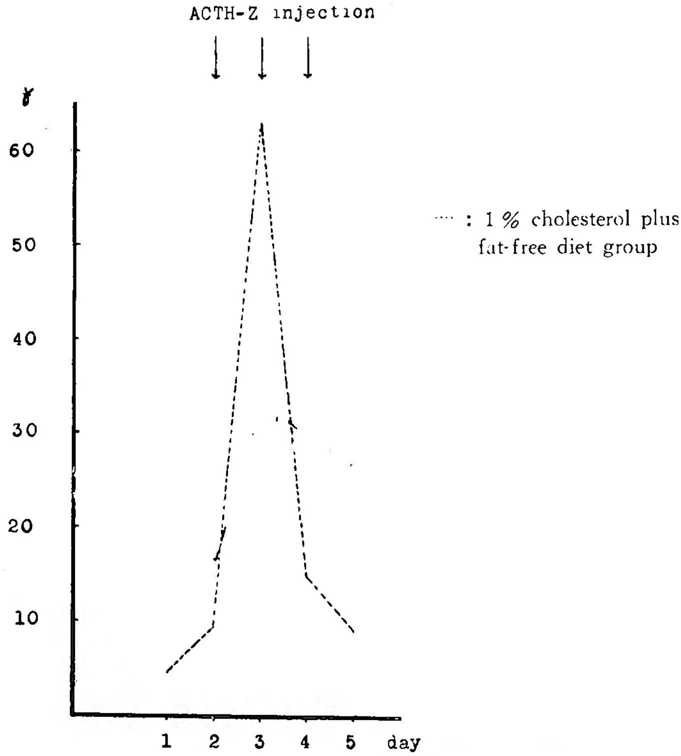
□ : Cholesterol plus sesame oil diet group
■ : Cholesterol plus fat-free diet group
2 : Dienoic acids
3 · Trienoic acids
4 · Tetraenoic acids

Simultaneously the changes in UFC concentration following ACTH-Z injection were also investigated under exactly the same experimental conditions as mentioned above by our colleague TAMAKI (Fig. 10). When ACTH-Z (5I. U. per day) was injected repeatedly for three days, UFC concentration in the cholesterol plus sesame oil diet group was maintained at an increased level during the ACTH-Z injections, while in the cholesterol plus fat-free diet group, although UFC concentration showed an abnormal increase during the initial period of injections of ACTH-Z, it later diminished markedly and no longer responded to subsequent ACTH-Z injections. These results agree completely with the changes in adrenal T-chol. content and E-chol. content and with the histological findings in the adrenal cortex following ACTH-Z injection.

It is postulated from these observations that the cholesterol plus sesame oil diet group has the adrenocortical capacity to respond to repeated ACTH injections, but that the cholesterol plus fat-free diet group has not. And the fact that there is no significant difference between the simple fat-free diet group and the sesame oil diet group in regard to the adrenocortical response to ACTH injection leads us to the assumption that the simple fat-free diet group still has a fairly large amount of EFA in the body which enables it to respond to the ACTH injections. It is evident that EFA-deficiency is promoted by feeding of the cholesterol plus fat-free diet, as previously demonstrated.

Since adrenal cholesterol is more abundant in the cholesterol plus fat-free diet group than in the simple fat-free diet group, precursors of steroid hormones are present in sufficient amounts in the cholesterol plus fat-free diet group. Therefore, in regard to the above mentioned difference in adrenocortical response to ACTH injection between the

Fig. 10 Effect of ACTH-Z injection (5I. U. per day intraperitoneally for 3 days) on urinary formaldehydogenic corticoid levels.



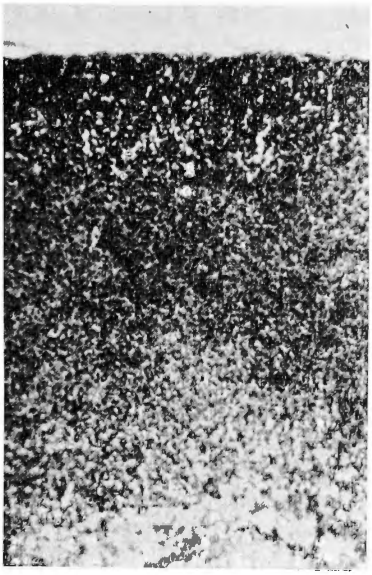


Fig. 11 Adrenal gland of cholesterol plus sesame oil diet group, after administered ACTH-Z 5I. U. per day intraperitoneally for 3 days. Sud. III.

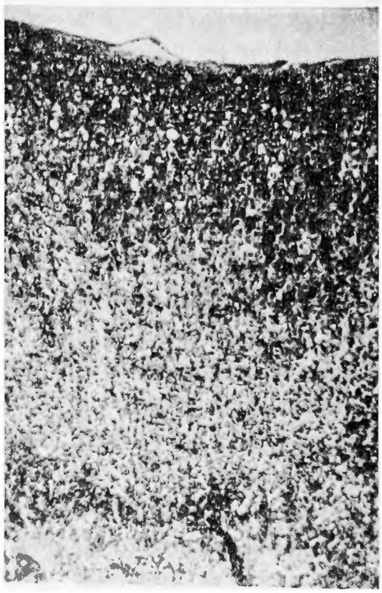


Fig. 12 Adrenal gland of cholesterol plus fat-free diet group, after administered ACTH-Z 5I. U. per day intraperitoneally for 3 days. Sud. III.

cholesterol plus fat-free diet group and the cholesterol plus sesame oil diet group, it is believed that adrenal EFA play an important role. And it is postulated that when there is EFA-deficiency induced by feeding the cholesterol plus fat-free diet, adrenal EFA are deficient, too; consequently the biosynthesis of steroid hormones from E-chol. or the metabolism of steroid hormones, or both in which EFA may be involved is disturbed and exhaustion of the adrenal cortex is induced already by these amounts of ACTH. On the other hand, when EFA are supplied in adequate amounts, the ability of the adrenal cortex to synthesize or to metabolize steroid hormones, namely the adrenocortical capacity, in response to ACTH injections is well sustained.

IV. DISCUSSION

The effect of fat-feeding on adrenal weight or adrenal lipid content has been studied by many investigators, who have reported that the administration of fat causes adrenal enlargement and that rats fed diets containing oils have more sterol in their adrenals than control rats in which fat is substituted for oils.

It has been demonstrated in many mammals that changes in adrenal weight depend mainly on changes in the cortex, although there is also a considerable range in the size of the medulla. Furthermore, it is well known that stress of various kinds increases the weight of the adrenals in general as well as activating the hypophyseal-adrenocortical system to secrete adrenocortical hormones. So it seems reasonable to consider that the adrenal weight may indicate the adrenocortical capacity for sustained secretion of steroid hormones. The author has demonstrated in this paper that no significant differences are observed in adrenal weights between rats fed a sesame oil diet and those fed a fat-free diet, but the administration of cod liver oil containing a large amount of highly unsaturated fatty acids, docosenoic acid and eicosenoic acid causes a marked increase of adrenal weight. These observations may also indicate that the administration of sesame oil never acts as a stressor to the organism. The administration of cod liver oil has a toxic effect on the organism especially on the parenchymatous organs such as the liver, kidney, etc., as our colleague KISHIMOTO has demonstrated.

We have also observed that as a result of the administration of cod liver oil or of excessive cholesterol orally, the adrenocortical capacity for sustained secretion of glucocorticoids decreases, although the adrenal weight and the adrenal cholesterol content increase. Therefore, it seems probable that adrenocortical capacity changes in parallel with neither the increase of adrenal weight nor the increase in adrenal cholesterol content. So neither adrenal weight nor adrenal cholesterol content by itself is an index of adrenocortical capacity. It has been pointed out that the quality of administered fat causes marked differences in adrenal weight, adrenal cholesterol content or secretion of glucocorticoids. From these observations it has been postulated that the quality of administered fat plays a very important role in maintaining normal adrenocortical capacity.

BURR and BURR demonstrated in 1929 that rats fed a fat-free diet showed a marked retardation in growth and specific dermal signs, and that these were cured by feeding a small amount of fat. Since then, the specific nutritional significance of fat has been investigated by numerous workers, and it has been demonstrated more clearly that this effect of fat depends on the presence of EFA, linoleic, linolenic, and arachidonic acids. These three fatty acids have the structure -CH=CH-CH_2-CH=CH- in common, are indispensable for maintaining physiological functions, cannot be synthesized in animal bodies, and should be included in the diet.

Although these three fatty acids are classed as EFA, the principal unsaturated fatty acid required by the animal organism is arachidonic acid, and it has been demonstrated by numerous workers that arachidonic acid can be derived from linoleic acid in the animal organism in the presence of vitamin B_6. MEAD et al. have demonstrated that the trienoic acid found in large amounts in the organs of fat-deficient rats is not linolenic acid but 5, 8, 11- eicosatrienoic acid. This acid, though having the structure -CH=CH-CH_2-CH=CH-, has no function as EFA. MONTAG et al. have postulated that this acid comes from the oleic acid family rather than from the linoleic or linolenic acid families.

Recent studies have confirmed the fact that EFA are closely related to cholesterol metabolism in the organism, and the esterification of cholesterol with EFA is a necessary process in the transportation of cholesterol from the blood to various tissues. KELSEY et al. concluded that 85% of fatty acids in beef plasma are unsaturated, and as much as 62% is linoleic acid. Moreover it has been pointed out that a deficiency of unsaturated fatty acids may result in an elevation of serum cholesterol and in so-called idiopathic hyperlipemia or hypercholesteremia. These facts have led some clinicians to use preparations of EFA for the purpose of preventing or improving hyperlipemia or hypercholesteremia.

It has been generally accepted that EFA are mainly concerned with the esterification of cholesterol, and cholesterol esterified with EFA is metabolically more active than that esterified with fatty acids other than EFA. Since in EFA-deficient animals cholesterol cannot help combining with fatty acids other than EFA, their cholesterol becomes less active and accumulates in various organs.

Although recent advances in endocrinology have fairly well clarified the effect of adrenocortical hormones on fat metabolism, the effect of fat-feeding on the adrenocortical capacity has been studied only a little. ROBERTS et al. postulated that feeding animals with a high carbohydrate diet or a high fat diet adapted the specific enzyme systems to the administered nutrients in order to utilize them most effectively. It has also been clarified by WHITNEY et al. that animals which are well fortified by fat-feeding can utilize more economically their storage-fat during starvation and can protect themselves from the rapid depletion of their liver glycogen reserve. Furthermore, comparative studies by MATSUDA and TAMAKI in our laboratory on the changes of the liver glycogen content, UFC concentration and histological changes in the adrenal cortex in starving animals indicate that an adequate supply of EFA can be expected to have the above-mentioned favourable effects on the subsequent state of undernutrition or stress due to fasting. In other words, it is considered that the previous supply of EFA has a functional relationship to the adrenal cortex and strengthens the ability of the organism to resist stress.

Our colleagues MAKI, JINDO and NAKASHIO have demonstrated that the adrenals of rats and dogs contain a large amount of EFA. HOLMAN and GREENBERG previously demonstrated that the testes and ovaries contained a relatively large amount of EFA. From these observations it has been postulated that EFA have a very important role in the synthesis and metabolism of steroid hormones. Our impression is that EFA combine with cholesterol to form an active esterified cholesterol, from which adrenocortical hormones are biosynthesized, and that in EFA-deficiency it is impossible to form active esterified cholesterol, and consequently the ability to secrete adrenocortical hormones decreases markedly. Therefore it is postulated that adrenocortical capacity may be determined by the kind of fatty acids with which adrenal cholesterol is esterified ; even if a large amount of cholesterol is contained in the adrenal gland, adrenocortical capacity decreases when that cholesterol is esterified with fatty acids other than EFA, and if adrenal cholesterol is sufficiently esterified with EFA, adrenocortical capacity is maintained at a normal level.

V. SUMMARY

Recent studies have clarified that lipid is not only stored or mobilized as a calorigenic substance, namely as a variable element, but also exists as a constant element in various organs and plays an important role in the specific function of these organs.

In this study, the changes in adrenal T-chol. content and E-chol. content and in the level of unsaturated fatty acids in the adrenals were measured under various conditions in rats fed a fat-free diet or a fat diet with or without added cholesterol, and the results were compared with the changes in the liver glycogen content and in the UFC concentration. It has been clarified that the administered fat has a marked influence on the adrenal cholesterol content, and that changes in the adrenal cholesterol content do not represent faithfully the adrenocortical capacity to secrete steroid hormones in response to stress ; on the contrary, adrenal EFA are rather intimately related with this adrenocortical capacity. Moreover it has been revealed that the administration of excessive amounts of cholesterol to EFA-deficient rats accelerates the development of EFA-deficiency in animals and that even if the adrenal cholesterol content is increased, the adrenal EFA content diminishes and adrenocortical capacity decreases markedly.

It has been postulated that EFA in the adrenal cortex form a metabolically active cholesterol ester which is considered to be as a main precursor of adrenocortical hormones, and that EFA are concerned with the biosynthesis or metabolism of steroid hormones, or both. And it has been concluded that in EFA-deficiency, the adrenal EFA content also decreases, inducing a reduction in adrenocortical capacity. Furthermore it has been considered that the administration of fats which contain less EFA or a large amount of harmful highly unsaturated fatty acids, causes a reduction in adrenocortical capacity even if the adrenal weight or adrenal cholesterol content increases.

From this view-point, the quality of administered fat, especially a proper supply of EFA should be considered in order to maintain normal adrenocortical capacity.

VI. CONCLUSION

In rats fed a fat-free diet or a fat diet with or without added cholesterol, the effect of the administration of fat on adrenal cholesterol content and the relationship between adrenal EFA content and adrenocortical capacity were studied.

1) Adrenal T-chol. content and E-chol. content were greater in the fat diet groups than in the fat free diet group. However, the quality of administered fat had a marked influence on the adrenal cholesterol content; the adrenal T-chol. content increased slightly in rats fed an olive oil diet and greatly in those on a cod liver oil diet, in comparison with those fed a sesame oil diet.

The administration of excessive cholesterol to EFA-deficient rats aggravated their EFA-deficiency, though the adrenal T-chol. and E-chol. contents increased.

2) In the fasting state the adrenal cholesterol content and adrenal weight changed as follows: in the early stage of fasting EQ increased, subsequently T-chol. content increased and at the same time adrenal weight increased, then during the course of fasting both T-chol. content and EQ diminished greatly until death occurred. These changes progressed more slowly and mildly in the sesame oil diet group than in the fat-free diet group.

3) Following ACTH-Z injection, a great difference was observed in the degree of depletion of adrenal T-chol. and E-chol. contents between the cholesterol plus fat-free diet group and the simple fat-free diet group, the sesame oil diet group or the cholesterol plus sesame oil diet group. At the same time dienoic and tetraenoic acids diminished greatly and trienoic acid increased in the adrenals of the cholesterol plus fat-free diet group in contrast to those of the cholesterol plus sesame oil diet group. Also typical findings of exhaustion were observed histologically in the adrenals of the cholesterol plus fat-free diet group.

4) A comparative study on the changes in adrenal cholesterol content, in adrenal EFA content, in UFC concentration and in liver glycogen content was done in the resting state and in response to fasting or to ACTH-Z injection. And it was postulated that the EFA-status of the organism, especially of the adrenals, was very intimately concerned with adrenocortical capacity.

5) From the results of our experiments it is concluded that the changes in the adrenal cholesterol content or the adrenal weight do not represent adrenocortical capacity faithfully, but that adrenocortical capacity is rather reflected on the kind of fatty acids with which adrenal cholesterol esterifies; and that even if much cholesterol is contained in the adrenals, the adrenocortical capacity decreases if that cholesterol is esterified with fatty acids other than EFA, while if the cholesterol is sufficiently esterified with EFA, normal adrenocortical capacity is maintained.

Therefore, an adequate supply of EFA is essential to maintain the physiological function of the organism.

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和文抄録

コレステロール代謝よりみた不可欠脂酸投与の副腎皮質機能予備能力に及ぼす影響についての実験的研究

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京都大学動物センターから供与された雄性 Wistar 系白鼠を使用し，これを無脂質食及び脂質食あるいは更にそれに夫々 Cholesterol の一定量を添加した食餌で飼育した各試獣群を作つて，その各々についてステロイドホルモンの Precursor としての意義を持つ副腎 Cholesterolの示す態度を検討し，併せて副腎中の不可欠脂酸（EFA）含有量と副腎皮質機能予備能力との関連性をも検討した．その結果次の事実が判明した．

1) 脂質食で飼育しつつある試獣の副腎中の総 Cholesterol 量及びEster型 Cholesterol 量は，無脂質食群のそれに較べて何れも一般に大である．併し，投与される脂質の質的組成の如何によつてそれら副腎内の含有量は著しい差異を示し，多量の EFA を含有するゴマ油を生成分とした食餌を投与した際に較べると，多量の高度不飽和脂酸を含有する肝油を主成分とした食餌を投与した際には，副腎重量，総Cholesterol 量及び Ester 型 Cholesterol 量の増加が遙かに著しい．

また，EFAの摂取が不充分な際には，過剰の Cholesterol の投与は益々生体内 EFA，ひいては副腎中の EFAの欠乏状態の発生を助長する．

2) 飢餓時に於ける副腎重量並に副腎に含有される総Cholesterol 量，Ester型 Cholesterol 量の消長を観察すると，まず初期にはエステル比の増加がみられ，次いで総Cholesterol量の増加，更に副腎重量の増大という一定の経日的変化を示し，遂には著明な総Cholesterol 量の減少及びエステル比の低下を招き，試獣は斃死するに至る．而してこのような経日的変化はゴマ油食群に於ては無脂質食群に較べて明らかに遷延し，緩除に進行する傾向がある．

3) 反覆して行う ACTH-Z 注射に際しては，副腎の総 Cholesterol 量及び Ester 型 Cholesterol 量は減少し，一方その重量は増大するが，この際無脂質食群とゴマ油食群の間には有意の差異を認め得なかつた．しかし Cholesterol を添加した無脂質食で飼育した試獣に於ては，単なる無脂質食群あるいは Cholesterol を添加したゴマ油食群よりも遙かに著明に総 Cholesterol 量及び Ester 型 Cholesterol 量が減少した．而して同時に Cholesterol を添加した無脂質食群に於ては副腎中に含有される EFA の枯渇がみられ，そして斯る試獣の副腎皮質は組織学的にも完全な疲憊状態に陥つていることが判明した．

4) そして，正常時，飢餓時あるいは ACTH-Z 注射時に於ける副腎総 Cholesterol 量の消長，副腎 EFA 含有量の消長，副腎の Glucocorticoids分泌能力及び肝 Glycogen 含有量の消長等を互に相対比して眺めると，生体内 EFA ひいては副腎中のEFA保有量と副腎皮質機能予備能力とは極めて密接な関係を有することが理解される．そして副腎重量や副腎中の Cholesterol 含有量は副腎皮質機能の状態を忠実に表現するものではなくて，副腎皮質機能予備能力の如何は結局副腎中の Cholesterol とエステル結合をしている相手の脂酸の種類の如何によつて決定されるものであつて，副腎中に如何に多量の Cholesterol が含まれていても，それが EFA 以外の脂酸と結合して居ればその機能は低下するし，それと反対に副腎中の Cholesterol が充分に EFA と結合しているならばその副腎皮質機能予備能力も亦健常に保持され得ることが判明した．

5) 従つて，かかる意味からしても個体が日々常に一定量の EFA を摂取しなければ，その個体の正常な機能は遂行され得ないものであると言い得る．