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The Role of Essential Fatty Acids on Adrenocortical Function

—A Collective Review—

by

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I. INTRODUCTION

HIKASA et al.^{1)~31)} demonstrated that fat administration to surgical patients had a very favorable effect on protein, carbohydrate and fluid metabolism. It may be said that the significance of fat as a “*variable element*” (as a caloric source) has been almost completely clarified by their works.^{1)~31)}

However, since the classical discovery of Burr and Burr³⁶⁾ that certain polyunsaturated fatty acids are essential for growth and, in fact, for survival, it has been generally recognized that fat has a much more important physiological significances as a “*constant element*” than as a “*variable element*”. And these polyunsaturated fatty acids are called essential fatty acids (EFA). Among the various polyunsaturated fatty acids which are called essential, linoleic and arachidonic acids are especially important. At present, the term “EFA” should be given to fatty acids of linoleic acid family (Table 1).

Although it has been well known that EFA deficient animals are of less resistance to operative insult, fast, cold, X-ray irradiation etc., its mechanism has not yet been elucidated. HIKASA, MATSUDA and NAGASE began the experiments on this point in 1958.

MATSUDA³⁷⁾ observed that EFA deficient animals showed an earlier exhaustion of liver glycogen during fasting and succumbed to starvation sooner than controls and that their adrenal cortex showed a picture of exhaustion histologically.

Table 1 Metabolic pathway of fatty acids of linoleic acid family.

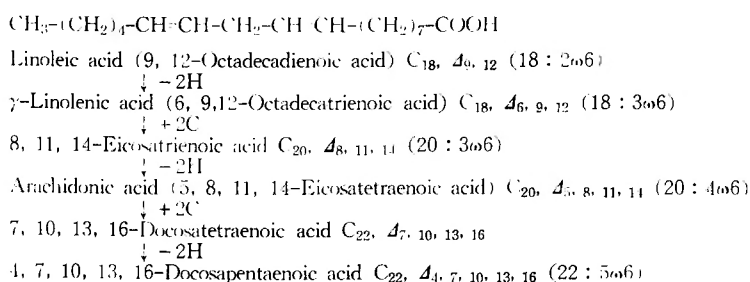


Table 2 Composition of the diets.

	Fat diet	Fat-deficient diet	* Fatty acid composition of sesame oil.	
Starch	60%	80%	16 : 0	9.5%
Casein	16	16	16 : 1	0.7
Sesame oil*	20	0	18 : 0	5.1
Salt-mixture	3	3	18 : 1	34.6
Vitamin-mixture	0.5	0.5	18 : 2	48.9
Choline chloride	0.5	0.5	18 : 3	0.7
			20 : 0	0.5

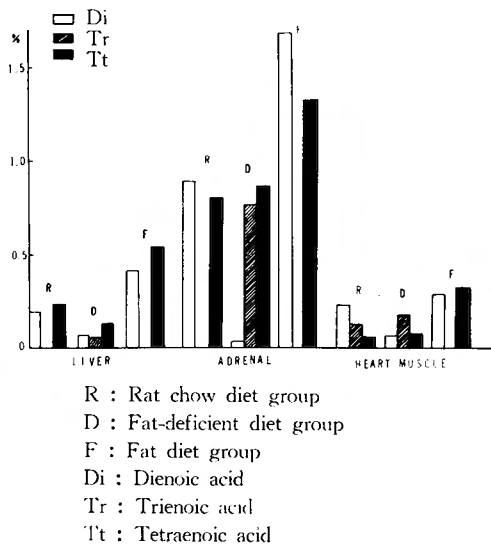


Fig. 1 PUFA content of liver, adrenal and heart muscle.

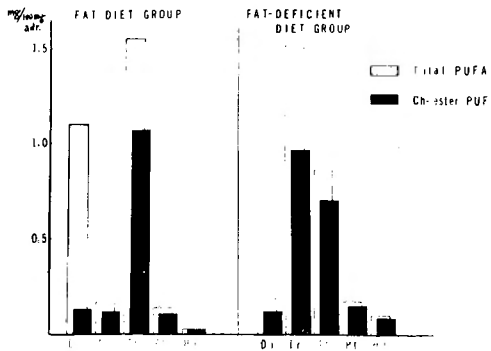


Fig. 2 Adrenal PUFA content of total lipids and cholestol esters of the two diet groups.

ISHIMARU³⁸⁾ observed electron microscopically that the adrenocortical fasciculata cells of EFA deficient animals show a decrease in number of mitochondria and changes in their inner structure.

NAGASE et al.⁹⁾³⁹⁾⁴⁰⁾ observed that EFA deficient animals had increased capillary permeability and were prone to develop the acute postoperative pulmonary edema, and he suggested that the increased capillary permeability was due not only to structural changes in capillary wall but also to decreased adrenocortical capacity.

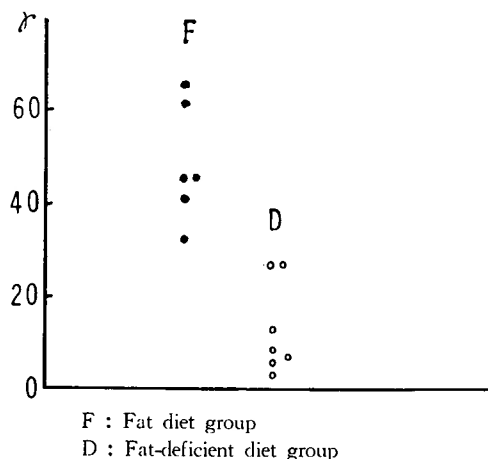
Subsequently, our laboratory began to study the relationship between EFA and adrenocortical function. Some important results obtained by our colleagues will be reviewed briefly.

As experimental animals, male albino rats of Wistar strain were used in all experiments. The weaning rats were fed rat chow until their body weight reached about 40~50 g, then were divided into three groups: The first group was fed a synthetic diet practically devoid of fat, the second a synthetic EFA-rich fat diet and the third rat chow. The composition of each diet is shown in Table 2. The rats were maintained on each diet ad libitum for 12 weeks in all experiments. During the course of the feeding period, all of the rats receiving the fat deficient diet showed the signs of EFA deficiency: Scaly paws and tails, loss of hair on the back, on the neck and around the face and stunted growth.

Table 3 The fatty acid composition of adrenal total lipids and cholesterol esters of the two diet groups.

Fatty acid	Fat diet group		Fat-deficient diet group	
	Total	Ch-ester	Total	Ch-ester
14:0	1.2	1.4	1.6	1.9
16:0	15.5	9.6	17.0	11.7
16:1	1.4	2.5	5.9	5.3
18:0	1.5	1.0	3.1	1.4
18:1	34.3	18.3	31.6	18.8
18:2	15.0	5.3	2.4	1.8
18:3	0.2	0.9		
20:1	1.8	6.2	4.7	7.0
20:3	0.2	3.5	7.7	11.0
20:4	11.2	19.0	4.8	7.1
22:0	0.5	1.6	1.0	1.7
22:3	0.7	1.4	7.7	13.8
22:4	11.1	21.5	7.1	13.1
22:5	1.5	2.3	2.0	1.9
22:6	1.1	1.1	2.2	1.8

5 rats in each group.

**Fig. 3** Resting levels of urinary formaldehydrogenic corticosteroid.

II. DISTRIBUTION OF EFA IN THE BODY AND ITS CHANGE IN EFA DEFICIENT ANIMALS

Using an alkaline isomerization method which is a modification of HOLMAN-HAYES' method⁽⁴¹⁾⁽⁴³⁾, JINDO⁽⁴²⁾⁽⁴³⁾ in our laboratory has demonstrated that the adrenals contain the largest amount of EFA in the whole body and that the rats fed the fat deficient diet show a marked decrease in dienoic and tetraenoic acids and an increase in trienoic acid (Fig. 1).

MURAOKA⁽⁴⁴⁾, after gaining the almost same results by the same method, has analyzed the fatty acid composition of adrenal total lipids and cholesterol esters of the two diet groups by gas liquid chromatography and obtained the results shown in Fig. 2 and Table 3.

III. ADRENOCORTICAL FUNCTION OF EFA DEFICIENT ANIMALS

TAMAKI⁽⁴⁵⁾ has measured the urinary formaldehydrogenic corticoids (UFC) and the plasma fluorometric corticoids (PFC) of the rats both in the resting state and under various stresses. And as shown in Figs. 3~9 and Table 4, he has demonstrated that the EFA deficient rats show lower levels of UFC and PFC even in the resting state than the controls and that this difference is augmented by stress.

FUKUDA⁽⁴⁶⁾ has measured the corticosterone in the serum and the adrenal gland and obtained the same results as those of TAMAKI.

And they have concluded that the ad-

renocortical capacity was greatly reduced in the EFA deficient animals.

IV. THE ADRENAL GLAND OF THE RATS FED FATS OTHER THAN SESAME OIL AND OF THE RATS FED CHOLESTEROL

As mentioned above, the resting levels of plasma, urinary and adrenal corticoids of the rats fed sesame oil are higher than those of the fat deficient group. This fact may be interpreted as, that the administration of sesame oil by itself acted as a stressor upon

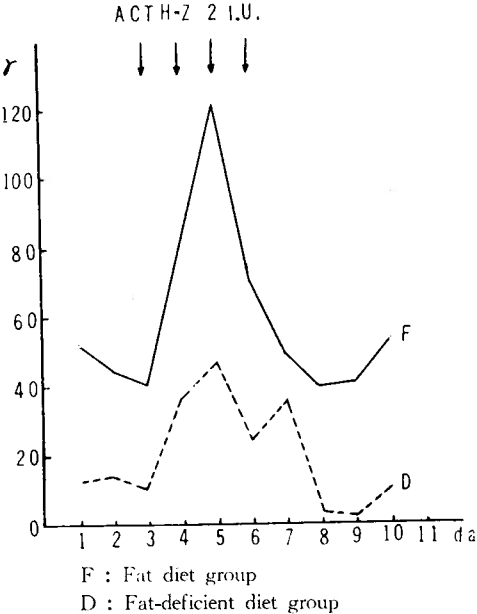


Fig. 4 Effect of ACTH-Z on urinary formaldehydogenic corticosteroid levels.

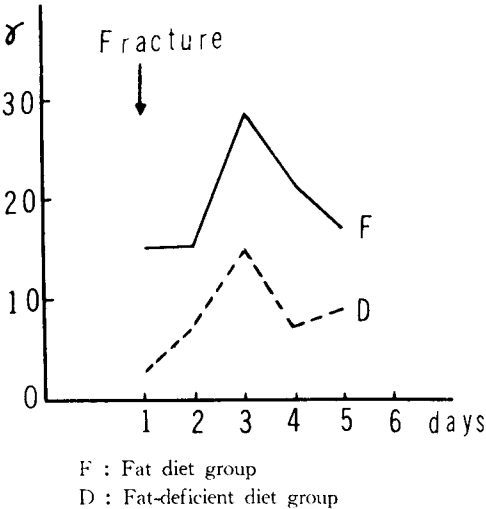


Fig. 6 Changes in urinary formaldehydogenic corticosteroid levels following bilateral fracture of ulna and radius.

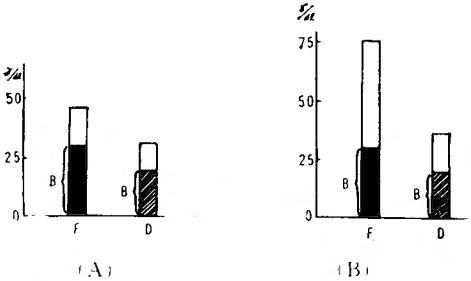


Fig. 5 Effect of ACTH on plasma fluorometric corticoids.

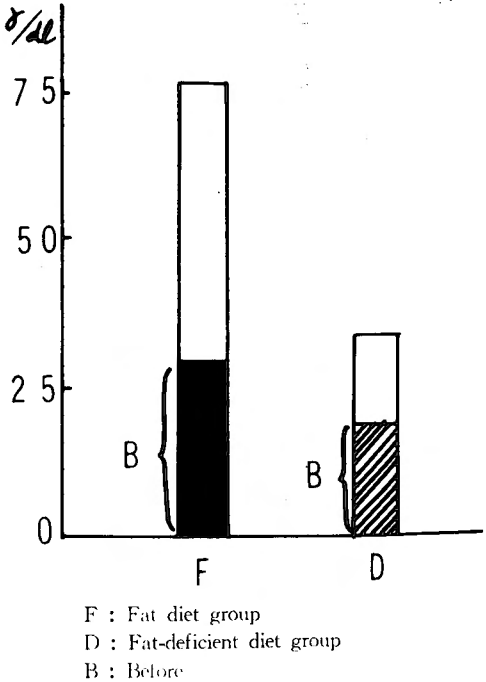
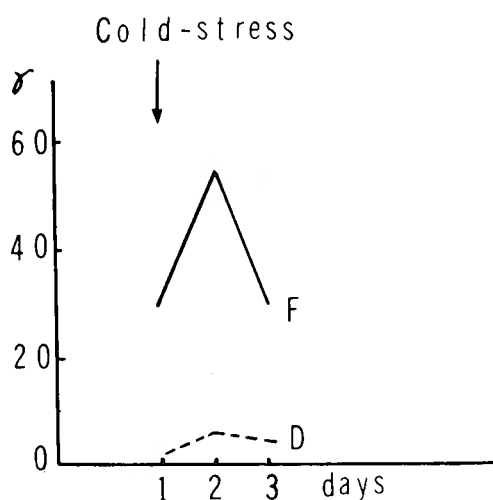


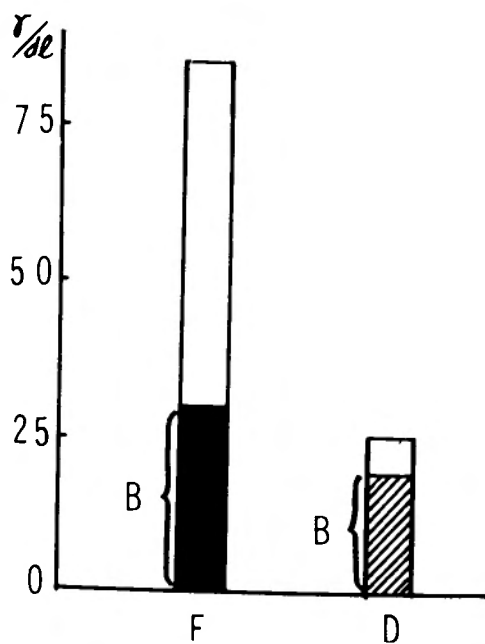
Fig. 7 Changes in plasma fluorometric corticoid levels following bilateral fracture of ulna and radius.



F : Fat diet group

D : Fat-deficient diet group

Fig. 8 Changes in formaldehydogenic corticosteroid levels in rats exposed to 7°C for 1 hour.



(4 rats in each group)

F : Fat diet group

D : Fat-deficient diet group

B : Before

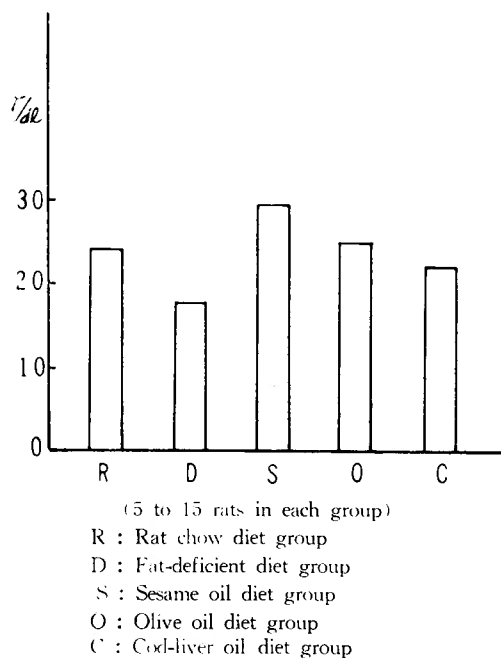
Fig. 9 Changes in plasma fluorimetric corticoid levels in rats exposed to 7°C for 1 hour.

Table 4 Resting levels of plasma fluorimetric corticoids.

No.	Fat diet group		Fat-deficient diet group	
	B. W.	γ/dl	B. W.	γ/dl
1	170 g	24.2	285 g	21.3
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

the organism. But, TAMAKI⁴³⁾ and KUMANO⁴⁷⁾ has gained the results as shown in Fig. 10 and Table 5. From these results, it follows not only that the administration of sesame oil by itself does not act as a stressor but also that adrenal weight is of no significance in adrenocortical function.



(5 to 15 rats in each group)

R : Rat chow diet group

D : Fat-deficient diet group

S : Sesame oil diet group

O : Olive oil diet group

C : Cod-liver oil diet group

Fig. 10 Resting levels of plasma fluorimetric corticoids in each group.

Table 5 Adrenal weight of rats fed each diet for 2 months (6 rats in each group).

Group	Adrenal Weight (mg/100g body weight)
Fat-deficient diet	11.3
Sesame oil diet	13.9
Olive oil diet	17.1
Cod liver oil diet	27.3
Cholesterol plus fat-deficient diet	13.7
Cholesterol plus sesame oil diet	16.2

V. ROLE OF EFA IN THE SYNTHESIS OF ADRENAL STEROIDS

From all of the observations in our laboratory and the reports of HAYASHIDA and PORTMAN⁽⁴⁸⁾ that the adrenals of EFA deficient rats secrete smaller quantities of steroid hormones in vitro under stimulation of ACTH and of SKOVSTEAD et al⁽⁴⁹⁾., who obtained the same result in vivo, it is obvious that EFA in the adrenals is one of the most important factors influencing adrenocortical function.

Nevertheless, the functional roles of EFA in the adrenals have not yet been clarified.

From the fact that cholesterol can serve as a precursor of adrenal steroid hormones and that administration of ACTH to rats produces a marked drop in the cholesterol ester fraction of the adrenals, it has been considered that adrenal cholesterol esters may be involved in steroid hormone synthesis.

MURAOKA⁽⁴⁴⁾ intended to clarify the role of EFA in the synthesis of adrenal steroids. His work will be reviewed briefly. As mentioned before, he analysed adrenal PUFA (polyunsaturated fatty acids) content, fatty acid composition of the rats fed either an EFA rich diet or an EFA deficient diet using an alkaline isomerization method and gas-liquid chromatography. As shown in Fig. 2, in both groups, the greater part of PUFA except dienoic acid occurred in their cholesterol ester fraction. Table 3 shows that adrenal total lipids of the fat diet (EFA sufficient) group exhibit the high proportions of 18 : 2 (mostly linoleic) and 20 : 4 (mostly arachidonic) acids and a low of 20 : 3 and 22 : 3 acids. On the contrary, the EFA deficient group shows the high proportions of 20 : 3 and 22 : 3 acids and a low of 18 : 2, 20 : 4 and 22 : 4 acids. And the fatty acid composition of the adrenal cholesterol esters of both groups shows differences similar to those of the total lipid, but C₁₆ and C₁₈ acid proportions are smaller and C₂₀ and C₂₂ acids are greater than that of the total lipids. From these results, it is obvious that EFA, especially arachidonic acid, is contained largely in cholesterol ester fraction.

Subsequently, MURAOKA⁽⁴⁴⁾ studied the changes in adrenal PUFA content and fatty acid composition of adrenal lipids following ACTH injection. As shown in Table 6, all PUFA in total lipids and in cholesterol esters decreased following injection of ACTH in both groups. And as shown in Table 7, area per cent of 20 : 4 acid in gaschromatogram decreased in cholesterol esters following ACTH injection in both groups.

Table 8 shows that 2 hours after injection, 20 : 4 acid in cholesterol esters decreased significantly ($p < 0.01$), but the other fatty acids did not show any significant change.

FUKUDA⁽⁴⁶⁾ has, in the same animals, demonstrated that serum corticosterone levels rise and reach their maximum after 1~2 hours following ACTH injection and that the corticosterone response in serum is markedly reduced in the EFA deficient rats (Fig. 11).

Tables 9 and 10, and Fig. 12 are the results of the experiments of successive administration of ACTH for 4 days. The adrenals of the fat diet group contained arachidonic acid abundantly both in total lipids and in cholesterol esters from the first day

Table 6 Changes in adrenal PUFA content of the two diet groups following a single administration of ACTH-Z 3 I.U. (mg/100mg adrenal.)

Fat diet group

	No. of rats	Total PUEA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	1.10	0.17	1.55	0.14	0.03	0.13	0.12	1.07	0.11	0.03
1	5	0.72	0.15	0.99	0.12	0.03	0.11	0.08	0.68	0.10	0.02
2	5	0.73	0.15	0.94	0.11	0.03	0.11	0.08	0.75	0.09	0.02
4	5	0.78	0.14	1.15	0.10	0.02	0.12	0.08	0.80	0.07	0.03
6	5	0.69	0.10	1.02	0.10	0.02	0.08	0.05	0.55	0.07	0.02
12 hrs.	5	0.80	0.18	1.44	0.12	0.02	0.12	0.11	1.05	0.09	0.02

Fat-deficient diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.19	1.51	0.86	0.17	0.10	0.12	0.96	0.70	0.15	0.09
1	5	0.18	1.55	0.84	0.18	0.08	0.09	0.97	0.68	0.15	0.07
2	5	0.16	1.02	0.75	0.14	0.11	0.07	0.95	0.63	0.13	0.10
4	5	0.12	1.09	0.45	0.12	0.06	0.08	0.59	0.33	0.10	0.06
6	5	0.16	1.05	0.70	0.15	0.06	0.09	0.77	0.52	0.13	0.05
12 hrs.	5	0.09	0.42	0.29	0.06	0.02	0.05	0.28	0.13	0.04	0.02

Table 7 Changes in the fatty acid composition of adrenal cholesterol esters following a single administration of ACTH-Z 3 I.U..

Fatty acid	Fat diet group No. 1				Fat diet group No. 2				Fat-deficient diet group No. 1				Fat-deficient diet group No. 2			
	Be-fore	2	4	6	Be-fore	2	4	6	Be-fore	2	4	6	Be-fore	2	4	6
14:0	1.4	1.6	1.6	1.1	1.3	1.5	1.6	1.1	1.9	1.4	1.7	2.0	2.2	1.7	2.1	1.7
16:0	9.6	11.5	11.0	11.3	9.5	9.9	10.8	9.5	11.7	15.7	12.6	21.5	11.2	10.9	10.6	10.2
16:1	2.5	2.6	2.5	2.4	2.6	2.4	2.6	2.2	5.3	5.0	4.8	5.8	6.0	5.5	6.2	5.0
18:0	1.0	1.1	0.8	0.9	1.1	1.0	1.2	1.1	1.4	1.3	1.3	2.0	1.5	1.4	1.4	1.3
18:1	18.3	18.7	14.6	14.8	18.4	14.9	15.3	14.4	18.8	18.5	11.7	17.1	18.1	16.8	15.3	15.8
18:2	5.3	5.7	4.8	5.1	4.4	3.7	3.8	3.2	1.8	1.7	1.8	1.8	2.2	1.9	2.1	2.0
18:3	0.9	1.6	2.7	2.5	0.3	0.2	0.2	0.1								
20:1	6.2	7.8	7.6	5.9	4.3	4.9	5.6	5.5	7.0	6.4	5.7	5.1	8.1	8.0	8.8	7.8
20:3	3.5	3.0	2.8	3.0	3.0	2.8	2.2	3.8	11.0	10.8	12.0	6.2	9.6	12.0	7.1	10.0
20:4	19.0	12.8	13.6	13.8	20.0	15.8	11.7	11.6	7.1	5.4	6.2	3.5	1.7	4.1	3.7	4.4
22:0	1.6	2.8	3.5	3.2	1.4	1.9	2.2	1.7	1.7	1.1	1.5	2.1	2.3	1.9	3.3	2.1
22:3	1.1	1.2	1.6	1.1	1.2	1.5	2.2	4.5	13.8	15.7	25.0	16.7	13.9	16.5	14.8	17.7
22:4	24.5	25.1	27.2	29.3	21.9	29.7	30.6	28.4	13.1	13.4	10.8	12.5	13.4	13.3	16.4	14.4
22:5	2.3	2.6	2.5	2.7	5.2	6.3	4.4	5.6	1.9	2.5	2.4	1.7	3.1	2.9	4.4	3.6
22:6	1.1	0.7	1.3	1.2	2.2	3.2	3.9	4.0	1.8	2.0	2.0	1.5	3.1	2.5	2.5	3.5

5 rats in each group.

Table 8 Ratio changes in cholesterol ester fatty acids after a single administration of ACTH-Z 3 I. U.

Fatty acid	Before	2 hrs. after ACTH injection	Significant change $p=0.05$
14:0	1.00	$1.07 \pm 0.16^*$	—
16:0	1.00	1.16 ± 0.08	—
16:1	1.00	1.11 ± 0.17	—
18:0	1.00	0.94 ± 0.11	—
18:1	1.00	0.92 ± 0.06	—
18:2	1.00	0.95 ± 0.05	—
20:1	1.00	1.02 ± 0.09	—
20:3	1.00	1.02 ± 0.08	—
20:4	1.00	0.77 ± 0.04	+ ($p < 0.01$)
22:0	1.00	1.09 ± 0.23	—
22:3	1.00	1.06 ± 0.09	—
22:4	1.00	1.07 ± 0.05	—
22:5	1.00	1.19 ± 0.11	—
22:6	1.00	0.98 ± 0.16	—

* Standard error of the mean.

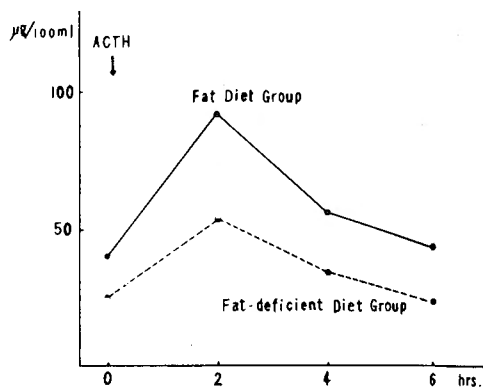


Fig. 11 Changes in serum corticosterone levels of the two diet groups following a single administration of ACTH-Z 3 I. U..

Table 9 Changes in adrenal PUFA content of the two diet groups during daily administration of ACTH-Z 3 I. U. for 4 days (mg/100mg adrenal)

Fat diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.87	0.13	1.43	0.13	0.04	0.14	0.08	0.95	0.10	0.03
2	5	0.61	0.09	1.04	0.10	0.03	0.10	0.05	0.50	0.06	0.02
4 days	5	0.52	0.10	1.00	0.09	0.04	0.08	0.07	0.59	0.06	0.03

Fat-deficient diet group

	No. of rats	Total PUFA					Ch-ester PUFA				
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx
Before	5	0.13	0.75	0.81	0.13	0.09	0.08	0.62	0.57	0.10	0.08
2	5	0.11	0.73	0.76	0.12	0.08	0.07	0.56	0.47	0.10	0.07
4 days	5	0.37	0.56	0.44	0.07	0.06	0.03	0.23	0.20	0.06	0.04

Table 10 Changes in the fatty acid composition of adrenal cholesterol esters of the two diet groups during daily administration of ACTH-Z 3 I. U. for 4 days

Fatty acid	Fat diet group			Fat-deficient diet group		
	Before	2	4	Before	2	4
14:0	0.9	0.9	1.0	1.7	2.0	2.3
16:0	9.6	12.1	10.5	11.6	11.8	15.2
16:1	0.9	0.7	0.8	3.7	1.2	1.2
18:0	1.4	1.5	1.2	1.6	1.3	1.4
18:1	14.4	17.1	14.7	18.8	15.5	15.7
18:2	6.5	6.8	6.3	tr	tr	tr
20:1	1.9	5.6	6.3	7.2	7.1	7.4
20:3	3.5	1.6	2.5	10.6	10.2	8.4
20:1	23.1	19.0	18.7	7.6	6.3	5.4
22:0	1.0	2.1	1.9	1.8	2.0	1.8
22:3	0.7	0.8	1.0	14.3	16.7	15.2
22:4	26.4	26.0	30.2	14.7	15.6	14.9
22:5	2.0	1.9	1.4	1.7	2.1	2.3
22:6	3.6	3.4	3.6	2.7	2.6	3.3

5 rats in each group.

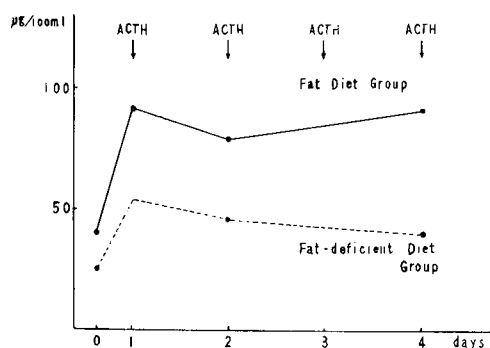


Fig. 12 Changes in serum corticosterone levels of the two diet groups during daily administration of ACTH-Z 3 I. U. for 4 days.

malignant tumors (Table 11).

FUKUDA⁴⁶⁾ investigated the relationship between serum EFA content and urinary 17-OHCS in surgical patients with various diseases. The results shown in Fig. 14 and Table 12 indicate the intimate relationship between serum EFA and adrenocortical function.

Moreover, FUKUDA⁴⁶⁾ investigated the relationship between serum EFA content and adrenocortical capacity in gallstones, especially cholesterol stone patients. As shown in Fig. 15, the serum EFA levels, especially tetraenoic acid levels of patients with gallstones without distinct liver malfunction are lower than controls. And their adrenocortical capacity is greatly suppressed and the degree of suppression is greater than that of the

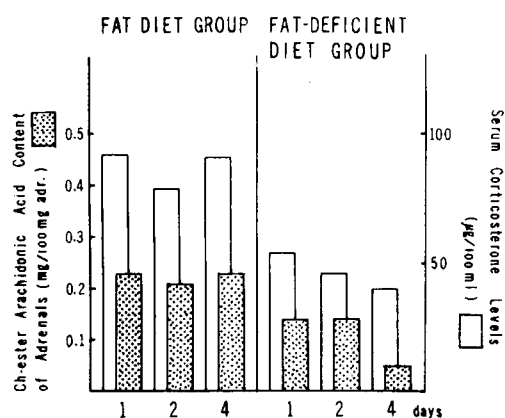


Fig. 13 Serum corticosterone levels and arachidonic acid content in adrenal cholesterol esters of the two diet groups during daily administration of ACTH-Z 3 I. U. for 4 days.

through the fourth day, but in the adrenals of the fat deficient group, arachidonic acid which had already been low while in the resting state, decreased markedly, especially in cholesterol esters during the 4 days. Fig. 13 shows that serum corticosterone levels change in parallel with adrenal arachidonic acid content in cholesterol esters during successive ACTH administration. Therefore, it is no doubt that cholesteryl arachidonate plays a very important role in the synthesis of glucocorticoids.

VI. RELATIONSHIP BETWEEN SERUM EFA CONTENT AND THE EXCRETION OF URINARY 17-OHCS IN SURGICAL PATIENTS

JINDO⁴²⁾ demonstrated that an EFA deficient state was found in patients with ma-

Table 11 Levels of PUFA in various diseases.

Sample	Diseases	Cares	Di	Tr	Tt	Pt	Hx
Serum	Healthy	5	60.0	9.5	17.6	3.5	9.4
	Ulcus ventr.	9	51.0	6.3	13.7	3.8	6.3
	Ulcus duod.	4	47.8	3.3	9.8	3.4	7.3
	Carc. ventr.	23	41.9	4.1	9.3	2.5	4.5
	Carc. bronch.	3	42.1	4.3	9.6	1.9	4.0
	Carc. panc.	2	41.7	6.2	8.7	5.8	13.2
	Carc. oesoph.	6	41.0	4.7	9.1	3.8	5.4

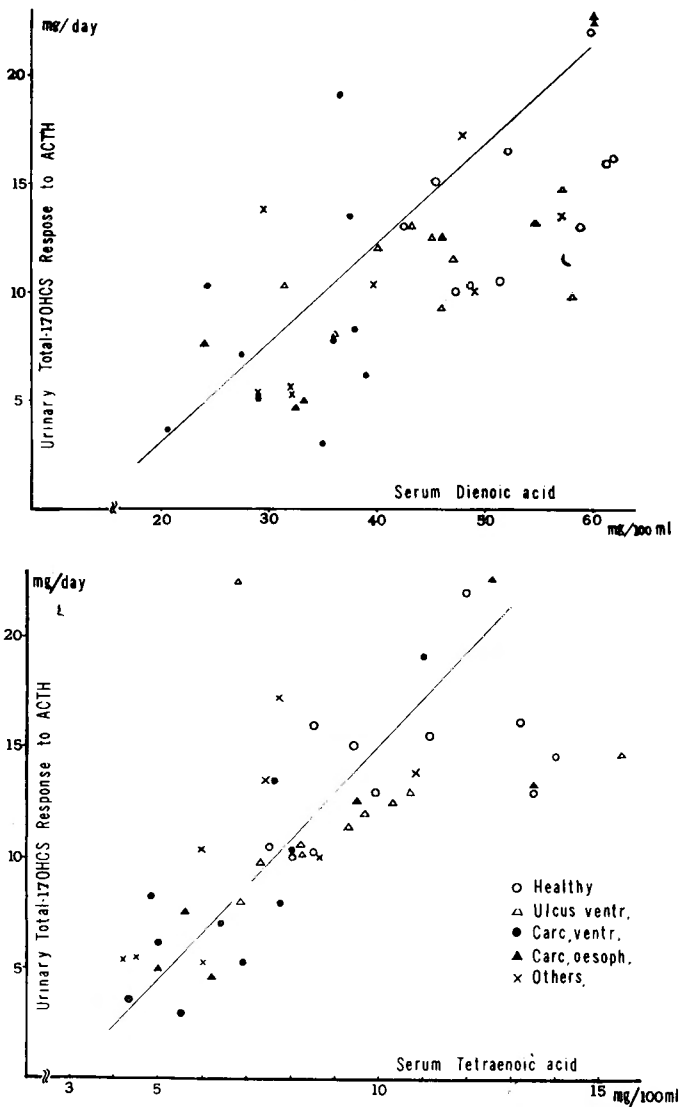
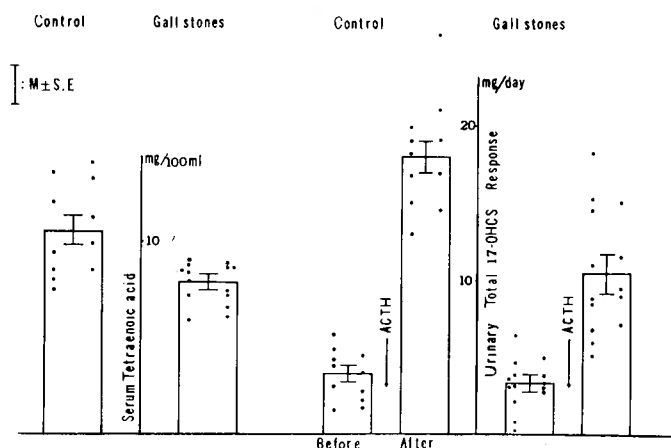


Fig. 14 Relationship between serum PUFA levels and adrenocortical capacity in surgical patients.

Table 12 Relationship between serum PUFA deficiency and reduced adrenocortical capacity.

Group	Serum EFA	Cases	Cases of Reduced Adrenocortical Capacity (%)
I	Di-sufficient Tt-sufficient	24	0 (0)
II	Di-deficient Tt-sufficient	11	2 (18.1)
III	Di-deficient Tt-deficient	10	7 (70)

**Fig. 15** Serum tetraenoic acid level and adrenocortical capacity in patients with gallstones.

gastric cancer cases. These facts suggest that cholelithiasis is a disease induced under peculiar metabolic disturbances in EFA. Further investigation on this problem has been done by our colleagues.^{50)~58)}

VII. DISCUSSION AND CONCLUSION

We have shown that EFA deficient animals contain a smaller amount of linoleic and arachidonic acids in the adrenals and they have a decreased adrenocortical function.

Moreover, we have obtained the results which suggest that cholesteryl arachidonate is one of the main precursors of glucocorticoids.

DAILEY et al⁵⁹⁾ have demonstrated that cholesteryl linoleate added to hog adrenal homogenates is not converted to steroids. This result may be interpreted as showing that the adrenal homogenates can not convert linoleate to arachidonate. Since the liver is the sole organ which can convert linoleate to arachidonate in the body.

From these results, it is quite plausible that in EFA deficient animals, the formation of cholesteryl arachidonate in the adrenals or the supply of materials necessary for it from plasma, might be disturbed. The precise role of arachidonic acid is still obscure. However, at present we consider that arachidonic acid probably plays a role as activator of enzyme systems from the view-point of mitochondrial membrane structure.

We know that further precise investigation on the mechanism of synthesis of adrenal

steroids in the body is beyond our ability. Some one may consider it rather curious that we, not biochemists but surgeons, have done these rather basic experimental works. But, as mentioned in the introduction, we began these works from our clinical experience that the administration of fat rich in EFA has favorable effects on surgical patients. We are content that we could clarify some mechanisms of the favorable effects of fat administration and that we could relieve many patients (both general and cardiovascular^{60)~63)}) in our clinic on the basis of the results of our experiments.

We sincerely hope that many full-fledged biochemists will investigate further the precise mechanism of synthesis of adrenal steroids in the body and will criticize our works.

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