Essential fatty acid deficiency and adrenal cortical function in vitro

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Abstract Adrenocortical cells were prepared from rats maintained on essential fatty acid-deficient diets and control litter mates. Cells from control rats had high concentrations of essential fatty acids in the cholesteryl ester fraction of which approximately 22% was arachidonate. In contrast, cells from EFA-deficient rats had only 2.5% arachidonate in the cholesteryl esters, even though the total esterified cholesterol level was comparable to that of controls. In place of the essential fatty acids, the cholesteryl esters of these cells were rich in 20:3 ($n - 9$) and 22:3 ($n - 9$). When cells from EFA-deficient rats were incubated with ACTH or dibutyryl cyclic AMP, the output of corticosterone was the same as in controls. Also sterol esters were hydrolyzed to the same extent as in controls despite the unusual composition of the fatty acid esters. The phospholipids in both control and EFA-deficient cells contained high levels of arachidonate but were not hydrolyzed in either type of cell during incubation with ACTH or dibutyryl cyclic AMP. The results indicate that high levels of the prostaglandin precursors, namely linoleate and arachidonate, are not a sine qua non for the steroidogenic action of ACTH or cyclic AMP.—**Vahouny, G. V., V. A. Hodges, and C. R. Treadwell.** Essential fatty acid deficiency and adrenal cortical function in vitro. *J. Lipid Res.* 1979. **20:** 154–161.

Supplementary key words cholesteryl ester hydrolysis · cyclic AMP · corticosterone · adrenal phospholipids · arachidonic acid · prostaglandins · ACTH

Rat adrenal cortex contains a high concentration of cholesteryl esters which are contained in numerous cytoplasmic inclusion droplets (1–3). These esters represent a major metabolic precursor of the cholesterol required for steroidogenesis, and are extensively hydrolyzed in response to stress or ACTH administration in vivo (1–3) or to ACTH in vitro (4). There is substantial evidence that the hydrolysis of adrenal sterol esters is a direct response to ACTH or cyclic AMP. It has been shown that ACTH will induce hydrolysis of cholesteryl esters even though further utilization of the released free cholesterol is blocked by cyclohexamide (5). Furthermore, it has recently been demonstrated that the enzyme responsible for this hydrolysis, sterol ester hydrolase (EC 3.1.1.13), is activated by a cyclic AMP-dependent protein kinase via a mechanism involving enzyme phosphorylation (6–8).

The cholesteryl esters of rat adrenal cortex have a high content of polyunsaturated acids, particularly arachidonate (9). Since these esters are extensively hydrolyzed during tropic hormone stimulation of the cortex (1–4), it has been suggested that this lipid fraction may also serve as a reserve for the arachidonate substrate for prostaglandin synthesis (1–3, 10). We have recently reported that exposure of rat adrenocortical cells to ACTH results in the specific formation of prostaglandin E_2, and that the major source of the arachidonate substrate for this synthesis is cholesterol arachidonate (11). Thus, the cholesteryl esters serve a dual role in providing a source of cholesterol for steroidogenesis and as precursor of a possible modulator of cyclic AMP formation and/or level (10).

The levels of essential fatty acids, such as arachidonate, are substantially lower in adrenals of rats maintained on EFA-deficient diets (12, 13). These are largely replaced by unusual fatty acids, such as 20:3 ($n - 9$) in the cholesteryl ester fraction (12, 13). Thus, it appears likely that, during stress or ACTH-induced hydrolysis of the cholesteryl esters in the adrenal cortex of EFA-deficient rats, there would be a lower concentration of free linoleate and arachidonate produced and a subsequent defect in prostaglandin formation. This might allow an assessment of the role of prostaglandins in the "steroidogenic cascade" of rat adrenal.

In the present study, adrenocortical cells were prepared from control rats and animals maintained on EFA-deficient diets for 16 weeks. Comparative data were obtained on lipid compositions, changes in cyclic AMP levels in response to ACTH, and the effects of

Abbreviations: EFA, essential fatty acids; ACTH, adrenocorticotropic hormone; dibutyryl cyclic AMP, $N^{6'}$, $O^{2'}$-dibutyryl cyclic adenosine-3′:5′-monophosphate; CEFA, cholesteryl ester fatty acids; PLFA, phospholipid fatty acids; TGFA, triglyceride fatty acids.

ACTH and dibutyryl cyclic AMP on steroidogenesis and on hydrolysis of adrenal cholesteryl esters.

EXPERIMENTAL PROCEDURES

Materials

Trypsin (Type TLR, 180–250 units/mg), collagenase (Type I, CLS, 150–200 units/mg), and lima bean trypsin inhibitor were obtained from Worthington Biochemical Corp. Bovine serum albumin (fatty acid-poor) was from Sigma Chemical Co. ACTH was purchased from the U.S. Pharmacopeia. Dibutyryl derivatives of cyclic AMP and cyclic GMP were obtained from Calbiochem. Fatty acids, methyl esters, and lipid standards were purchased from Applied Science Corp. and Supelco. Reagents for the assay of cyclic AMP were from Amersham-Searle Corp.

Animals and diets

Weanling male albino rats (Wistar strain) weighing approximately 50 g were obtained from Carworth Farms. Animals were divided into two groups, housed in individual cages, and maintained on control or essential fatty acid-deficient diets for 16 weeks. The control animals received standard laboratory chow (Wayne Lab-blox). The pelleted experimental diet was obtained from Teklad Mills (Teklad Test, Diet #170295, Madison, WI). The fat content and fatty acid compositions of the control and test diets are given in Table 1. All animals were allowed their respective diets and water ad libitum, and weight changes were determined at the indicated intervals (**Fig. 1**) throughout the feeding period.

Preparation of adrenal cortical cells

The technique for preparation of rat adrenal cortical cells by collagenase dissociation of tissue minces was as described previously (4). Each animal was killed by decapitation with a minimum of handling and in isolation from all other animals to be used in the study (4). This has been referred to as the quiescent sacrifice technique (14) and has been found to be critical to prevent factitious hydrolysis of cellular sterol esters during control incubation of adrenocortical cells (4). For each experiment adrenals from 10 rats in each group were pooled in 25 ml of cold Krebs–Ringer bicarbonate buffer, pH 7.4, containing 0.2% glucose, 0.5% albumin, and 7.65 mM Ca^{2+} (KRBGA buffer). These were minced and incubated successively in 10 ml of 0.25% trypsin and 10 ml of 0.5% trypsin for 20 min. Incubations were carried out under 95% O_2–5% CO_2 at 90 oscillations/min. The

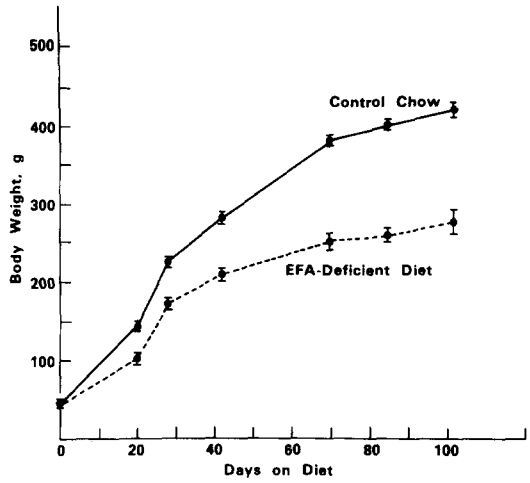

Fig. 1. Changes in body weights of weanling rats maintained on a diet deficient in essential fatty acids, and control litter mates fed commercial rat chow.

tissue fragments remaining after trypsin treatment were incubated five times for 20-min periods in fresh aliquots of KRBGA buffer containing 0.04% collagenase. The supernatants from each incubation were maintained in ice, pooled, and centrifuged at 150 *g* for 30 min. The supernatant was discarded and cells were gently resuspended in KRBGA buffer containing 0.1% lima bean trypsin inhibitor. Cell counts were made using a hemocytometer and the preparations were diluted to give a suspension containing approximately 1.25×10^5 cells/ml.

Aliquots of the cell suspension (1.9 ml) were incubated in 10-ml Teflon vessels after addition of 0.1 ml of Krebs–Ringer buffer or buffer containing the test substances. Incubations were carried out for periods up to 2 hr at 37°C with shaking and under 95% O_2–5% CO_2.

Lipid extraction and analysis

For lipid analysis, aliquots (0.5 ml) of the cell suspension were extracted in 20 volumes of chloroform–methanol according to the method of Folch, Lees, and Sloane Stanley (15). Prior to extraction, 0.25 μCi of [1-^{14}C]palmitic acid was added to correct for losses during extraction, derivatization, and chromatography. These techniques have been described in detail previously (16).

Major lipid classes were separated by thin-layer silicic acid chromatography with hexane–ethyl ether–acetic acid 80:16:4 (by vol). These were visualized under ultraviolet light after treatment with Rhodamine 6G and identified by comparison with authentic standards. Areas corresponding to phospholipids, triglycerides, free fatty acids, and cholesteryl esters were individually scraped into screw-cap

TABLE 1. Lipid compositions of commercial test diets

Measurement	Control Diet	EFA-Deficient Diet
Crude fat, %	4.1	
Medium chain triglycerides, %		5.0
Fatty acids[a], % of total fat		
6:0		1.0
8:0		75.0
10:0		23.0
12:0		1.0
14:0	1.5	Trace
16:0	8.9	
18:0	3.2	
18:1	36.5	
18:2	36.7	
18:3	2.5	
20:4	4.2	
22:4	0.5	
Others	2.6	

[a] Fatty acids are designated by their carbon number and degree of unsaturation.

tubes, extracted (except phospholipids), and the fatty acids were derivatized as previously described (16, 17). The methyl ester derivatives were determined on a Beckman-GC-65 gas–liquid chromatograph equipped with flame ionization detectors and interfaced with a Hewlett-Packard 3380A computing integrator. (Conditions: 6-ft. glass columns, 2 mm i.d.; 10% SP-2340 on 100/120 Chromasorb WAW, Supelco; column temp., 180°C; carrier gas flow, 25 ml/min; internal standard, methyl heptadecanoate.)

Other analyses

For corticosterone assays, 0.2-ml aliquots of the incubation mixtures were extracted in 15 ml of methylene chloride and corticosterone was determined fluorimetrically using standards of authentic corticosterone (18). Cyclic AMP was assayed by the protein binding–displacement technique (19).

RESULTS

Diets and animals

As shown in **Table 1,** the essential fatty acid-deficient diet contained 5% fat of which 98% was medium-chain triglycerides. Of these triglycerides, octanoate and decanoate made up 98% of the fatty acids, and the diet was devoid of longer-chain polyunsaturated fatty acids. The commercial lab chow fed to controls contained 4.1% fat of which more than 40% was in the form of linoleic and arachidonic acids.

Two groups of 40 weanling animals (av wt 48 ± 1 g) were fed these diets for 16 weeks. This schedule has been reported (12) to result in maximal changes in adrenal cholesteryl ester fatty acids in animals fed EFA-deficient diets. Changes in body weight in the control and experimental groups over the 16-week period are shown in Fig. 1. By 22 days, significant differences in animal weights were apparent between control (145 ± 3 g) and experimental animals (102 ± 5 g), and this difference increased during the remainder of the feeding period. By 112 days of feeding, the average weight of control rats was 422 ± 7 g and animals fed the EFA-deficient diet weighed 280 ± 12 g. All animals fed the EFA-deficient diet exhibited dermatitis of the paws and tail, characteristic of essential fatty acid deficiency. Animals fed the control diet were asymptomatic.

In a trial study with three EFA-deficient animals, it was determined that the procedure for cortical cell preparation by proteolytic dissociation of minced adrenals resulted in cells that were microscopically identical to cells from control animals. In subsequent studies it was determined that $1.0–1.25 \times 10^5$ cells/adrenal were obtained from tissue dissociation of adrenals from both control and EFA-deficient animals. Survey transmission electron micrographs of adrenocortical cells from control and EFA-deficient rats did not reveal any apparent abnormalities in the cells from adrenals of EFA-deficient rats.

EFA deficiency and adrenal cell lipids

The content of individual major lipid fractions (determined on the basis of fatty acid contents) of adrenal cells from control and EFA-deficient rats is summarized in **Table 2.** Overall, the total lipid content was comparable for adrenal cells from control rats (254.9 $\mu g/2.5 \times 10^5$ cells) and EFA-deficient rats (237.3 $\mu g/2.5 \times 10^5$ cells). Phospholipid and triglyceride levels were also similar for the two types of cells. However, the level of cholesteryl esters was significantly higher in adrenal cells from control rats ($P < 0.05$). Also, unesterified fatty acids were signifi-

TABLE 2. Lipid composition of adrenal cortical cells from control and EFA-deficient rats

Dietary Group[a]	Fatty Acid Content, $\mu g/2.5 \times 10^5$ Cells[b]			
	Cholesteryl Esters	Phospholipids	Unesterified Fatty Acids	Triglycerides
Control	86.4 ± 3.7	97.3 ± 2.8	23.7 ± 2.0	47.5 ± 6.8
EFA-deficient	75.4 ± 3.5	98.1 ± 1.9	31.8 ± 0.6	32.0 ± 1.9

[a] Weanling rats were fed for 16 weeks on commercial rat chow or a commercial diet deficient in essential fatty acids.

[b] The figures represent means ± SEM for 4–6 analyses of cholesteryl ester and phospholipid fatty acids, and 3 analyses of triglyceride fatty acids and unesterified fatty acids.

cantly lower ($P < 0.05$) than in cells from EFA-deficient rats.

The fatty acid compositions of the three major esterified lipid classes are shown in **Table 3.** As had been reported earlier (9, 12, 13), the major fatty acids esterified to cholesterol in control adrenal cells are arachidonate [20:4 ($n - 6$)], oleate [18:1 ($n - 9$)], adrenate [22:4 ($n - 6$)], palmitate (16:0), and fatty acids more unsaturated than adrenate. The major fatty acids of the phospholipids from control cells were stearate (18:0), arachidonate, palmitate, and oleate. The major TGFA were linoleate [18:2 ($n - 6$)], oleate, palmitate, and stearate.

Animals maintained on EFA-deficient diets for 16 weeks had marked changes in the fatty acid compositions of the major adrenal lipids. As reported earlier (12, 13), EFA-deficiency resulted in a substantial loss of the arachidonate and a complete loss of the adrenate levels of adrenal cholesteryl esters. In their place, there was the occurrence of significant quantities of 20:3 ($n - 9$) and 22:3 ($n - 9$) in the cholesteryl ester fraction. These acids, which were not detected in the cholesteryl esters from control adrenal cells, comprised over one-half of the fatty acid esters of cholesterol in adrenal cells from EFA-deficient rats.

The effect of EFA-deficiency on adrenal PLFA was not as dramatic as with CEFA. The levels of stearate and arachidonate in this lipid fraction were 61% and 58%, respectively, of the levels in normal cells. In addition to a marked increase in phospholipid oleate (130%), the level of 20:3 ($n - 9$) was increased to 30% of the total fatty acids of this lipid fraction. Docosatrienoic acid [22:3 ($n - 9$)] was not detected in the phospholipids. In general, these alterations in adrenal phospholipid fatty acids resulting from EFA-deficiency are comparable to those reported by Walker (13).

A major result of EFA deficiency on adrenal triglycerides was an almost complete loss of linoleate from this lipid fraction (from 25% of TGFA in control cells to 0.2% of TGFA in cells from EFA-deficient rats). In contrast, oleate levels were markedly elevated, accounting for over 53% of the TGFA. These effects of EFA deficiency are also comparable to those reported by Walker (13).

Cyclic AMP levels and corticosterone output in EFA-deficient adrenal cells

The steroidogenic response of normal and EFA-deficient adrenal cells to various stimuli is shown in **Table 4.** Adrenal cortical cells (2.5×10^5 cells/incubation) prepared from animals in each dietary group were incubated for 2 hr at 37°C in the absence (control) and presence of the additions indicated. Corticosterone levels were determined at 30-min intervals and the steroidogenic response is expressed as net corticosterone synthesis (experimental − control)/2.5×10^5 cells per 2 hr. As is apparent from the data in Table 4, corticosterone synthesis by EFA-deficient cells in response to ACTH (7×10^{-10} M) was comparable to that in normal cells. The steroidogenic response to dibutyryl cyclic AMP (0.5 mM) was 3-fold greater than that seen with ACTH, as has been shown previously (4), but again the level of corticosterone output by normal and EFA-deficient cells was the same. When adrenal cells were incubated with dibutyryl cyclic GMP, corticosterone production was less than 4% of

TABLE 3. Fatty acid compositions of major lipid classes of adrenal cortical cells from control and EFA-deficient rats

Fatty Acid[a]	Cholesteryl Esters		Phospholipids		Triglycerides	
	Control	EFA-Deficient	Control	EFA-Deficient	Control	EFA-Deficient
			% of Total			
14:0	2.2 ± 0.1[b]	1.5 ± 0.2			4.7 ± 0.7	2.3 ± 0.5
16:0	12.7 ± 0.7	11.6 ± 0.4	12.7 ± 0.7	7.0 ± 0.5	23.1 ± 0.7	21.7 ± 0.1
18:0	3.5 ± 0.3	4.7 ± 0.9	34.7 ± 1.9	21.1 ± 1.6	13.3 ± 0.8	16.7 ± 1.1
18:1	14.1 ± 0.6	20.4 ± 0.8	10.3 ± 0.6	23.7 ± 2.0	23.8 ± 1.7	53.8 ± 1.3
18:2	5.8 ± 0.1	0.7 ± 0.2	5.9 ± 0.2	nd	25.8 ± 1.4	0.2 ± 0.2
18:3 ($n - 3$)	nd[c]	3.6 ± 0.2	nd	0.6 ± 0.2	nd	nd
22:0 (18:3, $n - 6$)	3.6 ± 0.2	nd	nd	nd	nd	5.6 ± 2.7
20:3 ($n - 9$)	nd	22.7 ± 1.2	nd	30.7 ± 1.6	nd	nd
20:4 ($n - 6$)	22.3 ± 0.3	2.5 ± 0.7	29.0 ± 1.4	16.7 ± 2.7	7.5 ± 0.7	nd
22:3 ($n - 9$)	nd	28.3 ± 3.0	nd	nd	nd	nd
22:4 ($n - 6$)	13.2 ± 0.4	nd	1.8 ± 0.4	nd	nd	nd
>22:4	21.5 ± 0.2	5.6 ± 0.2	nd	nd	nd	nd

[a] Fatty acids are designated by carbon number, degree of unsaturation, and position of the terminal double bond.
[b] Figures represent means ± SEM for four analyses.
[c] Not detected, levels below 0.5% of total fatty acids.

TABLE 4. Corticosterone production by adrenal cells from normal and EFA-deficient rats

Additions to Incubations[a]	Net Corticosterone Production	
	Normal	EFA-Deficient
	μg/2.5 × 10⁵ cells/2 hr	
None	0.00 + 0.04	0.00 + 0.02
ACTH (7×10^{-10} M)	0.78 + 0.18	0.71 + 0.05
Dibutyryl cyclic AMP (0.5 mM)	3.14 + 0.70	2.70 + 0.14
Dibutyryl cyclic GMP (0.5 mM)[b]	0.05	0.10

[a] Incubations were carried out for 2 hr under 95% O_2–5% CO_2. Each flask contained 2.5×10^5 cells contained in 1.9 ml KRBGA buffer (see text for composition of buffer), pH 7.4. Additions were made in 0.1 ml of Krebs–Ringer buffer. The figures represent means ± SEM for four experiments.

[b] Mean, two experiments.

the levels seen after cyclic AMP incubations. Thus, further studies with cyclic GMP were discontinued.

Temporal changes in cyclic AMP levels and corticosterone production by normal and EFA-deficient cells incubated with ACTH are shown in **Fig. 2.** Incubations of normal cells with ACTH resulted in a rapid and transient increase in cyclic AMP, peaking at 10 min and subsequently returning to levels slightly above those in control incubations. This was accompanied by a marked increase in corticosterone synthesis which, as shown earlier (20), leveled off after 30 min of incubation. With EFA-deficient cells incubated with ACTH, the rise in cellular cyclic AMP levels was slower than in normal cells. These however, continued to increase, reaching a peak at 30 min, before displaying a slower decrease between 30 and 60 min. Despite this less transient response of cyclic AMP than was observed in normal cells, the apparent rate of corticosterone production by EFA-deficient cells was the same as with normal cells.

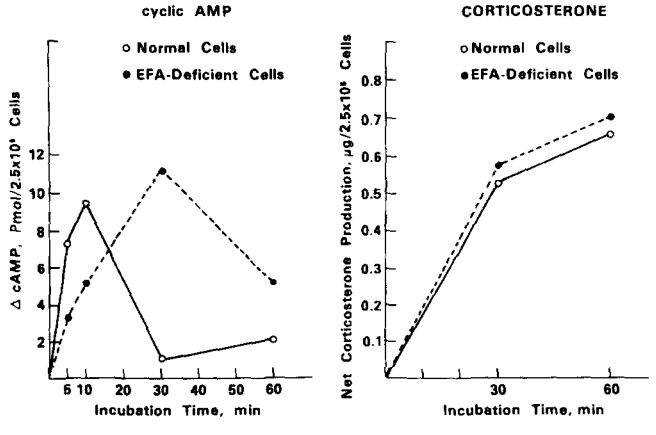

Fig. 2. Effect of ACTH (7×10^{-10} M) on cyclic AMP levels and corticosterone output by adrenocortical cells from control and EFA-deficient rats. Incubation flasks contained 2.5×10^5 cells in a total volume of 2.0 ml of modified Krebs bicarbonate buffer, pH 7.4.

EFA deficiency and ACTH-induced hydrolysis of adrenal cholesteryl esters

As shown in **Fig. 3,** there were no significant changes in the CEFA or PLFA levels in either normal and EFA-deficient cells during 2-hr control incubations at 37°C. Incubations of normal adrenal cells with either ACTH or dibutyryl cyclic AMP resulted in significant hydrolysis of cholesteryl esters, ranging from 31 to 38%. However, as has been shown by us earlier (4), there were no changes in the levels of PLFA in these same cells.

With EFA-deficient cells, incubations with ACTH or the cyclic nucleotide analogue also resulted in substantial hydrolysis of sterol esters with levels ranging from 41 to 48%. Again, as with normal cells, PLFA content was not altered during 2-hr incubations at 37°C.

Changes in the total and individual CEFA in the two types of cells exposed to either ACTH or dibutyryl cyclic AMP are summarized in **Table 5.** With normal adrenal cells exposed to either ACTH or dibutyryl cyclic AMP, the net hydrolysis of cholesteryl esters (26.3 μg and 32.9 μg, respectively) was due largely to hydrolysis of the esters of arachidonate, oleate, linoleate, adrenate, and palmitate. These changes are comparable to those reported earlier in adrenal cells exposed to ACTH (4) and in the remaining adrenals of rats stressed by unilateral adrenalectomy (21). In adrenal cells from EFA-deficient rats, the net hydrolysis of cholesteryl esters due to incubations with ACTH (31.3 μg) or dibutyryl cyclic AMP (33.9 μg) was due largely to hydrolysis of the esters of the unusual polyunsaturated fatty acids. Thus, hydrolysis of the esters of 20:3 (n − 9) and 22:3 (n − 9) accounted for 58–

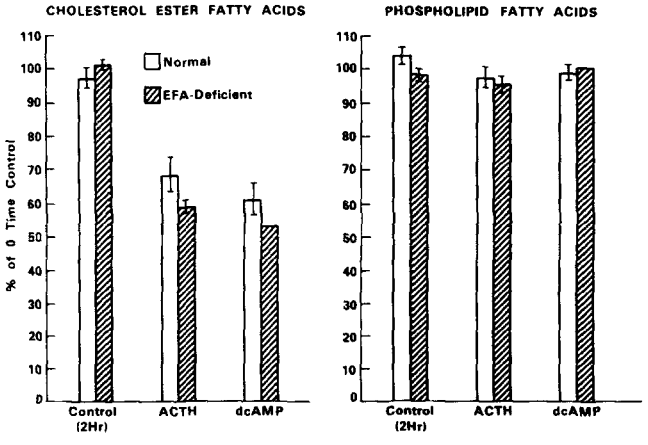

Fig. 3. Effects of ACTH (7×10^{-10} M) and dibutyryl cyclic AMP (0.5 mM) on cholesteryl ester and phospholipid levels in adrenocortical cells from control and EFA-deficient rats. Incubations were for 2 hr at 37°C with 2.5×10^5 cells per flask. Values are expressed as percentages of levels prior to incubations.

TABLE 5. Changes in total and individual fatty acids of cholesteryl esters in adrenocortical cells

	Incubation Conditions[b]					
	Normal Cells			EFA-Deficient Cells		
Measurement[a]	Control[c]	ACTH	dcAMP	Control	ACTH	dcAMP
Total CEFA, μg	85.7 ± 3.3	59.2 ± 4.4	52.8 ± 4.2	75.4 ± 3.5	44.1 ± 2.3	41.5 ± 2.5
Individual CEFA, μg						
14:0	2.1	1.1	1.2	1.4	1.7	1.0
16:0	8.1	5.8	6.5	8.9	4.8	5.8
18:0	4.1	4.1	6.5	3.7	1.8	1.8
18:1	12.7	8.9	6.0	15.5	11.7	11.1
18:2 ($n-6$)	5.7	3.9	2.3			
18:3 ($n-3$?)	1.9			3.6	2.2	2.0
18:3 ($n-6$)	2.4	1.7	0.5			
20:3 ($n-9$)				15.3	10.9	9.6
20:4 ($n-6$)	21.5	15.0	12.1	2.5		
22:3 ($n-9$)				22.8	9.9	7.5
22:4 ($n-6$)	14.9	11.7	9.3			
>22:4	8.0	6.6	7.3	4.1	2.6	1.5

[a] Values are for μg of total or individual cholesteryl ester fatty acids (CEFA)/2.5 × 10^5 cells.
[b] Conditions are the same as in Table 4.
[c] Represents values after 2-hr incubations at 37°C in the absence of ACTH or dibutyryl cyclic AMP.

62% of the total ester depletion. In addition, there was also, as in normal cells, extensive hydrolysis of the palmitate and oleate esters of cholesterol in the EFA-deficient cells.

DISCUSSION

The results of the present study clearly demonstrate that functional adrenocortical cells can be obtained from the adrenals of rats maintained for 16 weeks on an EFA-deficient diet. Compared to cells from adrenals of rats raised on standard laboratory chow, these cells contain a lower level of esterified cholesterol and a higher level of unesterified fatty acid. Others have reported that essential fatty acid deficiency results in an increase in esterified cholesterol and a decrease in phospholipids (13, 22, 23) in whole adrenal glands of rats. However, from the present analysis on adrenal fasciculata cells, it would appear that the lipid changes noted earlier might be a reflection of the effects of EFA-deficiency on the entire adrenal, including the adrenal medulla.

The effect of EFA deficiency on the fatty acid compositions of adrenal cholesteryl esters, phospholipids, and triglycerides were comparable to findings of earlier studies (12, 13). Thus, the normal polyunsaturated fatty acids esterified to cholesterol, namely 18:2 ($n-6$), 22:4 ($n-6$), and >22:4, were reduced from 64% of the CEFA to only 9% in EFA-deficient cells. These were replaced largely by 18:1 ($n-9$), 18:3 ($n-9$), 20:3 ($n-9$), and 22:3 ($n-9$) which comprised 55% of the CEFA in the deficient cells. As had been reported by Walker (13), EFA deficiency did not affect arachidonate levels of adrenal phospholipids to the same extent as those of cholesteryl esters. This is likely due to differences in localization and metabolic turnover of this lipid class in the cell. Overall, phospholipid arachidonate was reduced by 42% and stearate by 40% due to EFA deficiency. These were largely replaced by the increased levels of oleate and 20:3 ($n-9$). EFA deficiency also resulted in a substantial depletion of triglyceride linoleate (normally 26% of TGFA) as had been reported earlier (13), and this was largely replaced by oleate.

Despite these dramatic alterations in the fatty composition of adrenal cell lipids resulting from EFA deficiency, adrenal cells from EFA-deficient rats were comparable to adrenal cells from normal animals with respect to two major functional parameters. First, the steroidogenic response of EFA-deficient cells to either ACTH or dibutyryl cyclic AMP was equivalent to the responses of normal cells to these same challenges. Furthermore, neither type of cell displayed a steroidogenic response to dibutyryl cyclic GMP. Thus it appears that EFA deficiency does not result in a significant effect on cell surface receptors for the tropic hormone, nor on the critical steps subsequent to cyclic AMP production.

A second significant finding in the present studies is that, despite marked alteration in the composition of adrenal CEFA resulting from EFA deficiency, the extent of hydrolysis of cholesteryl esters was comparable to that in control cells during incubations with ACTH or dibutyryl cyclic AMP. Thus, in normal adrenal cells, the major sterol esters hydrolyzed during incubations

with ACTH (or cyclic AMP) were palmitate, oleate, arachidonate, and adrenate. In contrast, the sterol esters of 20:3 ($n - 9$) and 22:3 ($n - 9$) were the primary substrates for sterol ester hydrolase in EFA-deficient cells. Thus, adrenal sterol ester hydrolase shows a broad specificity and will hydrolyze cholesteryl esters based, at least in part, on their relative abundance within the cell.

An apparent difference between cell types was in the rates of cyclic AMP production in response to the ACTH challenge. There was a measurable delay in the accumulation of cyclic AMP in the cells from EFA-deficient rats. The reason for this is not clear, but this lag was not associated with either an alteration in the rate of corticosterone production or the extent of sterol ester hydrolysis.

We have reported earlier (4) that adrenocortical cell phospholipids are not depleted during incubations with ACTH or dibutyryl cyclic AMP. Similar results were obtained in the present study using adrenal cells from control and EFA-deficient rats. Also, current evidence indicates that a major source of arachidonate for prostaglandin synthesis is from hydrolysis of the stored cholesterol arachidonate (rather than from phospholipids) in these cells (11). Thus, in adrenal cells from EFA-deficient rats, it is highly probable that little or no free arachidonic acid would be available for prostaglandin synthesis during the ACTH challenge. It has been suggested that prostaglandins may act as modulators of adenyl cyclase activity in steroidogenic tissue (10). The lack of sufficient concentrations of prostaglandin substrates in EFA-deficient cells may, in part, explain the lag in cyclic AMP production in these cells when exposed to ACTH. However, the rate of corticosterone output and the extent of sterol ester hydrolysis in response to ACTH was not altered by EFA deficiency.

There are several possible explanations for these findings. It is possible that, despite a marked reduction in the levels of prostaglandin synthesis in EFA-deficient cells,[1] these may still be sufficient to satisfy the proposed modulator functions of prostaglandins. Secondly, it is likely that prostaglandins are not obligatory in the hormone cascade but may participate in a modulator role during sustained steroidogenesis. This has been suggested by studies in a variety of model systems (1–3, 20, 24). Thus, during short-term studies with isolated adrenocortical cells exposed to a single challenge with ACTH, variations in prostaglandin synthesis may have little effect on the rapid changes in cyclic AMP levels or on net corticosterone output.

[1] Vahouny, G. V., and V. A. Hodges, unpublished observations.

Finally, and as a less likely explanation for our findings, it is possible that the large amounts of released 20:3 ($n - 9$) in adrenal cells from EFA-deficient rats may represent a potential substrate for the cyclooxygenase system and result in formation of an active "pseudo" prostaglandin. Efforts are currently directed at elucidating the physiological function of prostaglandins in this tissue.

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