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ACYL GROUP COMPOSITION OF LIPIDS AND THE ACTIVITIES OF ($Na^+ + K^+$)-ATPase, 5′-NUCLEOTIDASE AND γ-GLUTAMYLTRANSPEPTIDASE IN SALIVARY GLANDS AND KIDNEYS OF RATS FED DIETS CONTAINING DIFFERENT DIETARY FATS

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Two nutritional models, an essential fatty acid deficiency model and the feeding of saturated versus unsaturated fats, were used in a feeding study in order to assess the relationship between tissue fatty acid composition and the activities of some membrane-associated enzymes. Purified diets containing 7% hydrogenated coconut oil, 7% corn oil, 10% safflower oil or butter were fed to rats for a total of 49 weeks (1 week of pregnancy, 3 weeks of lactation and 45 weeks post-weaning). Tissue homogenates from submandibular salivary glands and kidneys were analyzed for fatty acid composition of total lipids and phospholipids. Changes in fatty acid patterns typical of essential fatty acid deficiency such as an increase in the levels of 16:1 and 18:1, a decrease in 18:2 and 20:4 and an accumulation of 20:3ω9 were observed in salivary glands and kidneys of rats fed the deficient diet. Tissues of rats fed 10% butter also showed fatty acid compositional changes which were somewhat similar to those in essential fatty acid deficiency, but to a lesser degree. The activities of ouabain-sensitive ($Na^+ + K^+$)-ATPase were higher in homogenates of salivary glands and kidneys of the deficient rats and those fed butter as compared with their controls. The results suggest a relationship between the double bond index of fatty acids as an indication of membrane lipid fluidity and allosteric modification of ($Na^+ + K^+$)-ATPase activity. However, other explanations for the observed changes in ($Na^+ + K^+$)-ATPase activity cannot be ruled out. There were no diet-related differences in the activities of γ-glutamyltranspeptidase or 5′-nucleotidase.

Introduction

A number of membrane-bound enzymes require for their function a hydrophobic environment supplied by lipid bilayer. The fluidity of this lipid environment appears to modulate the activity of such membrane-bound enzymes [1]. Changes in the fluidity of biological membranes have been achieved by several means such as lipid substitution or reconstitution techniques [2–5], by temperature acclimatization [6–9] and by manipulating the composition of the culture media or the diet]10–12]. In each case there have been modifications in the activity of membrane-associated enzymes.

($Na^+ + K^+$)-ATPase is known to require some phospholipids for its activity. The type of phospholipid [13,14] and nature of the fatty acid chain [15,16] seem to be important in activating this enzyme. In case of essential fatty acid deficiency, the activity of ($Na^+ + K^+$)-ATPase has been shown to be altered in several tissues such as liver and kidney [17] and brain synaptosomes [18]. Changes in enzyme activity were associated with alterations in the fatty acid composition of membrane lipids. We have previously observed similar changes in

$(Na^+ + K^+)$-ATPase activity in submandibular salivary gland homogenates and plasma membranes of essential fatty acid-deficient rats [19]. Since, it is known that in addition to essential fatty acid deficiency, other nutritional factors such as the type of dietary fat can also alter the tissue fatty acid composition, we have used this approach to study the relationship between $(Na^+ + K^+)$-ATPase activity and tissue fatty acid composition. In order to maximize the changes in tissue fatty acid composition, we have conducted feeding studies with rats in which the diets were fed for a long period of time. The feeding was initiated during latter part of pregnancy, continued throughout lactation and thereafter for 45 more weeks after weaning. In the present study, a comparison was also made between the essential fatty acid deficiency model and that of feeding saturated versus unsaturated fats. Since $(Na^+ + K^+)$-ATPase activity is very high in kidney, this tissue was included for comparative purposes, with submandibular salivary glands. In addition to $(Na^+ + K^+)$-ATPase, the activities of two other membrane-associated enzymes, 5′-nucleotidase and γ-glutamyltranspeptidase, were also measured.

Materials and Methods

Materials. All organic solvents were of analytical reagent grade and were glass-redistilled prior to use. The dietary ingredients were purchased from ICN, Cleveland, OH. Corn oil, butter and safflower oil were purchased from a local supermarket. Standards of phospholipids and methyl esters of fatty acids were purchased from Applied Science Inc., College Park, PA. All biochemical reagents, including Tris-HCl (Trizma-HCl), Tris-Base (Trizma Base), and enzyme substrates were obtained from Sigma Chemical Co, St. Louis, MO.

Nutritional studies

Experiment I. 15 day-pregnant, Sprague-Dawley rats (Holtzman Co, Madison, WI) were divided into 4 groups of 3–4 rats each and were fed ad libitum semipurified diets (basal diet, American Institute of Nutrition, 1976) containing 7% hydrogenated coconut oil (Group 1, essential fatty acid-deficient), 7% corn oil (group 2, control for group 1), 10% safflower oil (group 3, high in polyunsaturated fatty acids) or 10% butter (group 4, low in polyunsaturated fatty acids). Second day after delivery, the pups within each dietary group were randomized so that each foster litter contained 8 pups. The dams were maintained on the same dietary regime during lactation. The pups were weaned at 21 days of age, weighed, maintained individually in wire-bottom cages and were fed ad libitum the same diets as previously fed to their mothers. Rats were weighed once a week.

After feeding the diets for 45 weeks post-weaning, six male rats from each of the four groups were decapitated, submandibular salivary glands and kidneys were dissected, rinsed with cold physiological saline and weighed. The tissues were minced finely with scissors and homogenized in ice-cold 0.05 M Tris-HCl buffer (Trizma-HCl), pH 7.4 at 25°C (pH adjusted with Trizma base), in a Potter-Elvejhem Teflon-glass homogenizer (pestle clearance, 0.028 cm) using 20 strokes at 3000 rev/min for submandibular salivary glands and 10 strokes for kidney. Aliquots of the homogenate were used for enzyme assays and lipid extraction.

Experiment II. In another study, we prepared plasma membranes from the submandibular salivary glands of rats fed diets containing 10% safflower oil or butter for a period of 7–9 weeks (3 weeks of lactation and 4–6 weeks post-weaning). The salivary glands, pooled from each of the two dietary groups, were minced finely with scissors and homogenized with 20 strokes at 3000 rev/min in 9 volumes (w/v) of medium containing 0.32 M sucrose in 0.05 M Tris-HCl, pH 7.4 (at 25°C), 0.025 M KCl, 0.003 M $MgCl_2$ and 0.002 M $CaCl_2$, in a wide clearance (0.028 cm) Potter-Elvejhem Teflon-glass homogenizer. The homogenates were filtered through 4 layers of cheese cloth and plasma membranes were prepared by differential centrifugation method of Durham et al. [20]. Aliquot of plasma membranes were used for the assay of $(Na^+ + K^+)$-ATPase and 5′-nucleotidase activity as described under methods for enzyme assays.

Enzyme assays

All enzyme assays were done in duplicate or triplicate. The amount of protein used and the time of incubation were within the linear range of the enzyme reactions.

($Na^+ + K^+$)-ATPase (E.C. 3.6.1.3). The activity of ouabain-sensitive ($Na^+ + K^+$)-ATPase was determined using the method of Jorgensen [21]. The rate of inorganic phosphate release was measured in the presence of 3 mM $MgCl_2$, 3 mM Tris-ATP, 130 mM NaCl, 20 mM KCl, 30 mM histidine (pH 7.5) minus the rate of release in the same medium with 1 mM ouabain added.

Tissue homogenates representing 0.2–0.5 mg of protein or plasma membranes (30–50 μg protein) were preincubated for 5 min at 37°C in 0.9 ml of the above medium without Tris-ATP. At this time, 0.1 ml of Tris-ATP was added and the mixture further incubated for 10 min at 37°C (preliminary experiments had shown the reaction to be linear for at least 10 min). The reaction was stopped with the addition of 0.2 ml of 50% trichloroacetic acid to precipitate the proteins. After centrifugation at about 4000 × *g* for 10 min in a refrigerated centrifuge, the supernatant was transferred to another tube and inorganic phosphate was measured by a modification [22] of Fiske and Subba-Row's method [23].

5'-Nucleotidase (E.C. 3.1.3.5). The activity of this enzyme was determined by measuring the rate of release of inorganic phosphate from 5'-AMP according to the procedure described by Aranson and Touster [22].

γ – Glutamyltranspeptidase (E.C.2.3.2.2). The activity of this enzyme in tissue homogenates was measured according to the method of Orlowski and Meister [24].

Protein was determined by the method of Lowry et al. [25], using crystalline bovine serum albumin as a standard.

Fatty acid composition

Aliquots of tissue homogenates from experiment I and plasma membranes from experiment II were extracted for lipids using Bligh and Dyer's procedure [26]. Total lipid extracts were transesterified under nitrogen with boron trifluoride-methanol according to the procedure of Morrison and Smith [27]. The fatty acid composition of methyl esters was determined by gas chromatography on 180 × 0.2 cm glass columns packed with 10% SP-2330 on 100/120 chromosorb W, AW (Supelco Inc., Bellefonte, PA). The column temperature was 190°C, detector and injection port temperatures were 225°C. Log retention time versus carbon number and microhydrogenation techniques were used for the identification of fatty acid methyl esters.

Pooled samples of total lipid extracts from each group were subjected to column chromatography on Biosil-A, 100–200 mesh (Bio-Rad Lab., Richmond, CA) columns according to the procedure of Rouser et al. [28]. Phospholipid fractions were eluted with methanol, transesterified and the fatty acid composition was determined as described above for total lipids.

The double bond index was calculated from the sum of (percentage of each unsaturated fatty acid) × (number of double bonds).

Statistical analyses

All data were statistically analyzed and the significance of difference between the means (group 1 versus 2 and group 3 versus 4) was calculated using Student's *t*-test [29].

Results

Body weights and the tissue weights of rats fed various diets are shown in Table I. As compared with the controls (7% corn oil group), the rats fed the essential fatty acid-deficient diet (7% hydrogenated coconut oil) had lower final body weights and higher tissue weights when expressed on body weight basis. There was no significant difference in body or tissue weights between the groups of rats fed unsaturated oil (10% safflower oil) or a saturated fat (10% butter).

Activities of the three enzymes in salivary glands and kidney homogenates of rats fed various diets are shown in Table II. As compared to their respective controls, the specific enzyme activity of ouabain sensitive ($Na^+ + K^+$)-ATPase was significantly higher in salivary glands and kidney homogenates of rats fed an essential fatty acid-deficient diet (group 1 versus 2) or the one containing butter (group 3 versus 4). There was no significant difference among the various dietary groups in terms of γ-glutamyltranspeptidase or 5'-nucleotidase activity either in salivary gland or kidney homogenates. When comparing the two tissues against each other, the specific enzyme activity for 5'-nucleotidase was about the same, whereas it was

TABLE I

EFFECT OF LONG TERM FEEDING OF DIFFERENT DIETARY LIPIDS ON BODY, SUBMANDIBULAR SALIVARY GLAND(S) (SMSG) AND KIDNEY WEIGHTS OF RATS

Values are mean ± S.E. of six rats per group.

Dietary fat	Initial body wt. [a] (g)	Final Body wt. (g)	SMSG Wt.	Kidney Wt.
			(mg/100 g body wt.)	
7% Hydrogenated cocnut oil	50.0 ± 1.9	437.0 [b] ± 2.1	70.8 [c] ± 2.7	341.7 [c] ± 15.2
7% Corn oil	52.6 ± 0.8	614.2 ± 11.0	58.9 ± 1.7	280.5 ± 7.2
10% Safflower oil	57.5 ± 1.2	610.3 ± 14.2	56.1 ± 3.5	266.0 ± 11.5
10% Butter	53.3 ± 1.1	616.2 ± 18.3	53.9 ± 1.6	289.0 ± 13.5

[a] Body weight at weaning.
[b] $P < 0.001$.
[c] $P < 0.01$.

3–4-fold higher for $(Na^+ + K^+)$-ATPase and about 300–600-fold higher for γ-glutamyltranspeptidase in the kidney as compared with the submandibular salivary glands.

The fatty acid composition of total lipids of salivary gland homogenates is shown in Table III. As compared with controls (7% corn oil), in the essential fatty acid-deficient group (7% hydrogenated coconut oil), the proportions of 14 : 0, 16 : 1, 18 : 1 and 20 : 3ω9 were higher whereas those of 16 : 0, 18 : 0, 20 : 3ω6 and 20 : 4 were lower. Double bond index for total fatty acids was also lower in the salivary glands of the essential fatty acid-deficient rats.

The fatty acid patterns of total lipids in salivary gland homogenates of rats fed 10% butter were somewhat similar to those fed 7% hydrogenated coconut oil. The levels of 18 : 2 and 20 : 4, however, were not quite as low as in the deficient group. Also, the levels of 20 : 3ω9 were not quite as high as in the deficient group. Total lipids in salivary gland homogenates of rats fed diets containing butter had higher levels of 14 : 0, 16 : 1, 18 : 1, 20 : 3ω9 and lower levels of 18 : 2, 20 : 3ω6

TABLE II

THE ACTIVITIES OF $(Na^+ + K^+)$-ATPase, γ-GLUTAMYLTRANSPEPTIDASE AND 5′-NUCLEOTIDASE IN SMSG AND KIDNEY HOMOGENATES OF RATS FED DIFFERENT DIETARY LIPIDS

15 day-pregnant rats were placed on semipurified diets containing different lipids. Feeding was continued to the dams throughout lactation and thereafter to their pups for 45 weeks post-weaning. Rats were killed, kidneys and salivary glands were homogenized and the activities of $(Na^+ + K^+)$-ATPase, γ-glutamyltranspeptidase and 5′-nucleotidase were measured in tissue homogenates as described under methods. Enzyme activities are expressed as μmol product formed/mg protein per h. The values are mean ± S.E. of six rats in each group.

	$(Na^+ + K^+)$-ATPase		γ-Glutamyltranspeptidase		5′-Nucleotidase	
	SMSG	Kidney	SMSG	Kidney	SMSG	Kidney
7% Hydrogenated coconut oil	2.7 ± 0.1 [a]	9.2 ± 1.1 [b]	0.69 ± 0.08	232.8 ± 19.5	3.2 ± 0.2	2.6 ± 0.3
7% Corn oil	1.7 ± 0.2	4.0 ± 0.7	0.62 ± 0.03	193.4 ± 19.0	2.7 ± 0.3	2.3 ± 0.1
10% Safflower oil	1.2 ± 0.3	3.2 ± 0.2	0.59 ± 0.04	240.2 ± 17.5	3.1 ± 0.2	2.9 ± 0.4
10% Butter	2.7 ± 0.1 [a]	7.8 ± 0.7 [a]	0.43 ± 0.08	234.5 ± 10.7	2.7 ± 0.2	2.4 ± 0.3

[a] $P < 0.001$.
[b] $P < 0.01$.

TABLE III

FATTY ACID COMPOSITION OF SMSG HOMOGENATES OF RATS FED DIFFERENT DIETARY LIPIDS

Values are mean ± S.E. of five to six rats per group. –, not detected

Dietary lipid	Fatty acid									Double bond index
	14:0	16:0	16:1	18:0	18:1	18:2	20:3 (ω9)	20:3 (ω6)	20:4 (ω6)	
7% Hydrogenated coconut oil	1.5 [a] ± 0.14	25.6 [a] ± 0.48	8.4 [a] ± 0.52	10.2 [b] ± 0.45	32.8 [a] ± 1.16	1.6 [a] ± 0.17	13.2 [a] ± 0.92	– [a]	4.5 [a] ± 0.15	102.0 [a] ± 1.31
7% Corn oil	0.5 ± 0.04	28.4 ± 0.23	2.7 ± 0.23	12.9 ± 0.47	17.6 ± 0.87	14.8 ± 0.92	0.6 ± 0.06	3.1 ± 0.36	17.0 ± 1.18	129.0 ± 2.64
10% Safflower oil	0.4 ± 0.04	26.8 ± 0.47	2.0 ± 0.25	12.5 ± 0.51	13.7 ± 0.83	23.8 ± 1.37	0.9 ± 0.06	2.2 ± 0.19	16.3 ± 0.94	137.8 ± 1.07
10% Butter	0.8 [a] ± 0.07	28.3 ± 0.47	5.3 [a] ± 0.16	12.9 ± 0.34	28.3 [a] ± 0.58	4.5 [a] ± 0.07	3.2 [a] ± 0.15	1.6 [c] ± 0.07	13.5 [c] ± 0.25	111.0 [a] ± 0.30

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

TABLE IV

FATTY ACID COMPOSITION OF KIDNEY HOMOGENATES OF RATS FED DIFFERENT DIETARY LIPIDS

Values are mean ± S.E. of five to six rats per group. –, not detected.

Dietary lipid	Fatty acid										Double bond index
	14:0	16:0	16:1	18:0	18:1	18:2	20:3 (ω9)	20:3 (ω6)	20:4 (ω6)	> C20	
7% Hydrogenated coconut oil	0.7 [a] ± 0.03	17.6 ± 0.41	7.9 [a] ± 0.26	11.6 ± 0.13	26.9 [a] ± 0.28	3.5 [a] ± 0.15	11.9 [a] ± 0.39	– [a]	11.0 [a] ± 0.14	8.9	121.5 [a] ± 1.76
7% Corn oil	0.2 ± 0	19.1 ± 0.61	3.5 ± 0.22	11.8 ± 0.33	17.2 ± 0.94	15.3 ± 0.29	0.3 ± 0.04	0.7 ± 0.09	23.0 ± 1.17	8.9	146.3 ± 4.39
10% Safflower oil	–	20.9 ± 1.17	1.0 ± 0.60	11.0 ± 0.79	12.7 ± 0.47	23.7 ± 1.56	0.4 ± 0.02	0.6 ± 0.04	21.8 ± 1.45	6.7	151.3 ± 3.05
10% Butter	0.7 [a] ± 0.16	20.8 ± 0.51	5.2 [a] ± 0.39	11.2 ± 0.17	22.8 [a] ± 1.08	4.6 [a] ± 0.17	2.3 [b] ± 0.41	1.0 [c] ± 0.14	20.2 ± 0.76	10.0	127.9 [a] ± 2.80

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

TABLE V

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS OF SMSG AND KIDNEY HOMOGENATES OF RATS FED DIFFERENT DIETARY LIPIDS

Pooled from six rats within each group. –, not detected.

Dietary lipids	Fatty acid									Double bond index
	16:0	16:1	18:0	18:1	18:2	20:3 (ω9)	20:3 (ω6)	20:4 (ω6)	> C20	
SMSG										
7% Hydrogenated coconut oil	20.2	8.1	10.2	34.4	2.9	14.6	–	7.1	1.1	120.5
7% Corn oil	27.6	–	14.2	11.9	17.3	0.9	2.6	24.8	0.7	156.2
10% Safflower oil	27.4	–	13.2	14.9	13.4	0.6	3.6	23.2	2.5	147.1
10% Butter	22.9	5.3	11.6	25.7	5.9	3.8	2.2	17.5	4.1	130.8
Kidney										
7% Hydrogenated coconut oil	16.8	5.4	16.1	24.3	4.6	15.8	–	15.1	1.3	146.7
7% Corn oil	20.0	–	18.5	9.2	12.5	0.5	0.7	34.1	5.0	174.2
10% Safflower oil	22.6	–	17.8	10.9	9.8	0.4	0.6	32.6	5.4	163.9
10% Butter	23.0	–	15.6	16.8	5.9	2.5	1.3	28.7	5.3	154.8

and 20:4 when compared with those fed safflower oil. Also the double bond index was significantly lower in the total lipids of salivary gland homogenates in rats fed butter as compared with that of rats fed safflower oil.

TABLE VI

$(Na^+ + K^+)$-ATPase AND 5'-NUCLEOTIDASE ACTIVITIES OF PLASMA MEMBRANES ISOLATED FROM SMSG OF RATS FED SATURATED VERSUS UNSATURATED FATS

Plasma membranes were prepared by differential centrifugation from the submandibular salivary glands of rats fed 10% safflower oil or butter. The activities of ouabain-sensitive $(Na^+ + K^+)$-ATPase were measured by measuring the rate of release of inorganic phosphate in the presence of 3 mM $MgCl_2$, 3mM Tris-ATP, 130 mM NaCl, 20 mM KCL, 30 mM histidine, pH 7.5 minus the rate of release in the same medium with 1 mM ouabain added. 5'-Nucleotidase activity was determined by measuring the rate of release of inorganic phosphate from 5'-AMP according to the method of Aranson and Touster [22]. Enzyme activities are given as μmol P_i/mg protein/h (duplicate analyses of pooled samples). Values are for 6 weeks post-weaning. Data in parenthesis indicate enzyme activities measured 4 weeks post-weaning.

Dietary Lipid	$(Na^+ + K^+)$-ATPase	5-Nucleotidase
10% Safflower oil	10.4	10.6
	(10.5)	(21.4)
10% Butter	16.6	9.7
	(33.6)	(20.6)

Data on the fatty acid composition of total lipids in kidney homogenates are presented in Table IV. The fatty acid patterns in the kidney were generally similar to those in the salivary glands except it had relatively higher percentage of 20:4. Also, about 7–10% of the total fatty acids were higher than C_{20}. The types of differences between the dietary groups (7% hydrogenated coconut oil versus 7% corn oil and 10% safflower oil versus 10% butter) were essentially similar to those observed in the salivary glands i.e., the proportions of 14:0, 16:1, 18:1 and 20:3ω9 were higher whereas those of 18:2, 20:3ω6 and 20:4 were lower in kidney homogenates of rats fed hydrogenated coconut oil or butter compared with corn oil or safflower oil respectively. Also, the degree of unsaturation, as shown by the double bond index, was lower in total lipids of kidney homogenates of rats fed butter or hydrogenated coconut oil as compared with their respective controls.

The fatty acid composition of pooled samples of phospholipids of salivary glands and kidney homogenates is shown in Table V. The fatty acid patterns of phospholipids were similar to those observed in total lipids. Also, the differences in fatty acid composition between the essential fatty acid-deficient and the control groups and between groups fed safflower oil versus butter were essentially the same as in total lipids. The double bond

indices of phospholipids were somewhat higher than those of total lipids mainly because of higher percentage of unsaturated fatty acids in phospholipid fraction.

The activities of $(Na^+ + K^+)$-ATPase and 5'-nucleotidase in crude plasma membranes of salivary glands of rats in experiment II are shown in Table VI. $(Na^+ + K^+)$-ATPase activity was 1.5–3-times higher in the group fed 10% butter as compared with the group fed safflower oil. There was no difference in 5'-nucleotidase activity between the two groups. the fatty acid composition of total lipids and phospholipids in plasma membranes (data not shown) was essentially similar to that observed for these two groups in the whole homogenates in experiment I.

Discussion

Our data indicate that two different nutritional manipulations i.e. feeding of an essential fatty acid-deficient diet and a saturated fat such as butter, induced similar changes in the acyl group composition of tissue lipids and were associated with higher $(Na^+ + K^+)$-ATPase activity. Whereas, similar findings of higher $(Na^+ + K^+)$-ATPase activity have been reported in liver and kidney [17] and in brain synaptosomes [18] in essential fatty acid deficiency, the effects of long term feeding of a saturated fat such as butter have not yet been reported.

There can be at least three different explanations for the observed increase in the activity of $(Na^+ + K^+)$-ATPase in the tissues of rats fed an essential fatty acid-deficient diet or the one containing 10% butter (a) an increase in the number of enzyme molecules per mg protein (b) an increase in the activity of the individual ATPase molecules and (c) a change in the physical state of the membrane containing the ATPase molecules that affects the measurement of the activity.

$(Na^+ + K^+)$-ATPase activity of a crude tissue homogenate is known to depend upon the method of preparation because the membranes tend to form vesicles which limit the access of ATP, Mg^{2+}, Na^+ and K^+ to their appropriate site and may also limit the inhibitory action of ouabain [30,31]. However, we have also observed higher $(Na^+ + K^+)$-ATPase activity in plasma membranes prepared from submandibular salivary glands of rats fed an essential fatty acid-deficient diet [19] and from rats fed a diet containing 10% butter as the data shown in Table VI [32]. The fatty acid composition of the plasma membrane preparations in these studies was also quite similar to that of the tissue homogenates in the present study. Since vesicles can also be formed in plasma membranes, the possibility (c) above cannot be rigorously excluded. However, the present results on $(Na^+ + K^+)$-ATPase activity in tissue homogenates when considered in light of our data on plasma membranes tend to support the role of fluidity of membrane lipids in allosteric modification of $(Na^+ + K^+)$-ATPase activity [15,16,33].

The possibility that the tissue concentrations of $(Na^+ + K^+)$-ATPase molecules were altered as a result of feeding the various diets cannot be ruled out. It has previously been observed [17] that in liver and kidneys of rats fed an essential fatty acid-deficient diet for 7 weeks, there was an increase in the number of $(Na^+ + K^+)$-ATPase units per mg protein. Future studies using [^{3}H]ouabain binding assay with plasma membranes from rats fed various dietary lipids would be necessary in order to obtain a better understanding of the exact mechanism(s) for the observed increase in $(Na^+ + K^+)$-ATPase activity.

Our observation for higher $(Na^+ + K^+)$-ATPase activity in the salivary glands of essential fatty acid-deficient rats is consistant with our previous findings [34] of lower levels of Na^+ in saliva of essential fatty acid-deficient rats. Increase in the activity of this enzyme which is primarily located in salivary ducts would result in more efficient reabsorption of sodium ions and therefore lesser excretion in saliva.

Although the diet supplied marginal levels of linoleic acid in the group fed 10% butter, the rats fed this diet were not deficient in essential fatty acids, since their body weight gains were essentially similar to those fed 10% safflower oil and much higher than those fed 7% hydrogenated coconut oil. Also, the ratios of $20:3\omega9$ to $20:4$ in total lipids of kidney and salivary gland homogenates of rats fed 10% butter, although higher than those of rats fed safflower oil (0.113 versus 0.018 in kidney and 0.237 versus 0.055 in salivary glands) were still lower than 0.4. A ratio higher

than 0.4 in tissue lipids is considered to be indicative of essential fatty acid deficiency [35]. In the present study, 20 : 3ω9 to 20 : 4 ratio ranged from 1.08 to 2.93 in total lipids of kidney and salivary glands in the essential fatty acid-deficient rats. In view of the marginal essential fatty acid deficiency induced by feeding 10% butter, this nutritional model may have a greater relevance in terms of practical human nutrition since marginal essential fatty acid deficiency is more likely to exist in human population than frank essential fatty acid deficiency. Marginal essential fatty acid deficiency is also known to exist in patients with cystic fibrosis [36–38].

The activity of γ-glutamyltranspeptidase or 5′-nucleotidase in salivary glands or kidney homogenates was not affected by the type of dietary fat. We have observed [39] that in salivary glands of rats fed 13-*cis* retinoic acid the γ-glutamyltranspeptidase activity was not affected whereas, the $(Na^+ + K^+)$-ATPase activity was significantly decreased. Changes in $(Na^+ + K^+)$-ATPase activity were associated with modification in fatty acid composition of the gland. Thus, it appears that γ-glutamyltranspeptidase activity is not affected by a modification in its hydrophobic environment. Similarly, it has been observed that γ-glutamyltranspeptidase activity in fibroblasts was not affected by changes in phospholipid composition [40].

In contrast to $(Na^+ + K^+)$-ATPase activity, the 5′-nucleotidase activity of salivary glands and kidney homogenates was the same in essential fatty acid deficiency model as well as in saturated versus unsaturated fat model. The diet-induced differences in tissue fatty acid composition had no effect on the activity of this enzyme. We have observed [32] that in plasma membranes (data shown in Table VI) of submandibular salivary glands of rats fed either 10% butter or safflower oil during lactation and 4–6 weeks thereafter, the activity of 5′-nucleotidase was essentially the same whereas, the $(Na^+ + K^+)$-ATPase activity was much higher in the group fed butter. These changes in enzyme activities were associated with similar changes in fatty acid composition of total lipids and phospholipids as observed in the gland homogenates.

Arrhenius plots of 5′-nucleotidase activity are biphasic in nature. There is some controversy as to whether this is due to its lipid environment or it is a property of the enzyme itself. Dipple et al. [41] have shown that the break in Arrhenius plot of 5′-nucleotidase activity is due to the occurrence of a lipid phase separation localized in the outer half of the bilayer and modulating the activity of this membrane-bound enzyme. They observed an activation of 5′-nucleotidase by increasing the fluidity of the bilayer [42]. Merisko et al. [43] have shown that hepatic 5′-nucleotidase from isolated membranes and in partially purified fraction interacts with sphingomyelin and phosphatidylcholine; the first influences the stability of the enzyme and the second, the energy of activation. Their data indicate that the transition observed in Arrhenius plot of 5′-nucleotidase activity is the result of an interaction between the enzyme and phosphatidylcholine. Stanley and Luzio [44] have shown, however, that varying the fatty acid profiles, cholesterol and phospholipid content had no effect on the K_m and Arrhenius break temperature of 5′-nucleotidase of rat liver plasma membranes. It was suggested by these investigators that the biphasic nature of the Arrhenius plot of 5′-nucleotidase may be a property of the enzyme rather than its lipid environment. Our results on 5′-nucleotidase activity in tissues of rats fed different dietary lipids seem to be in agreement with the observations of Stanley and Luzio [44] and other investigators [45,46] who have shown that 5′-nucleotidase activity is independent of the membrane lipid composition. In our study, diet-induced modifications in lipid fluidity as measured by the double bond index of fatty acids, had no effect on 5′-nucleotidase activity.

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