Changes in $(Na^+ + K^+)$-ATPase Activity of Ehrlich Ascites Tumor Cells Produced by Alteration of Membrane Fatty Acid Composition†

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ABSTRACT: The fatty acid composition of plasma membrane derived from Ehrlich ascites tumor cells was altered in vivo by changing the dietary lipid of the tumor-bearing mice. The activity of (sodium + potassium)-adenosinetriphosphatase ($(Na^+ + K^+)$-ATPase), in partially purified plasma membranes, was measured as a function of temperature. Arrhenius plots of the data were biphasic. Striking differences, dependent on the membrane fatty acid composition, were observed in the transition temperatures and in the energies of activation below the transition temperature. The transition temperatures for the $(Na^+ + K^+)$-ATPase of plasma membrane derived from tumor cells grown in mice fed a regular chow diet containing a mixture of fatty acids (PM_C), a 16% sunflower oil diet (PM_{SU}), or a 4% tristearin diet (PM_{TS}) were 20, 21, and 13.5 °C, respectively. Energies of activation above the transition temperature were about the same (~20 kcal/mol) in all three cases, but the energy of activation below the transition temperature was about twice as high for the $(Na^+ + K^+)$-ATPase of PM_{TS} (~60 kcal/mol) as for the $(Na^+ + K^+)$-ATPase of either PM_{SU} or PM_C (~30 kcal/mol). There were no significant differences in the cholesterol or phospholipid content in the three types of plasma membranes. However, there were marked differences in the fatty acyl composition of PM_{TS} as compared with either the control (PM_C) or PM_{SU} membranes. The content of oleate (18:1ω9) and eicosatrienoate (20:3ω9) was higher and that of linoleate (18:2ω6) and arachidonate (20:4ω6) lower in PM_{TS} than in PM_C or PM_{SU}. It is concluded that dietary lipid induced changes in membrane fatty acyl composition can change the activity of the plasma membrane $(Na^+ + K^+)$-ATPase.

Large alterations in the fatty acid composition of Ehrlich ascites tumor cells can be produced by varying the type of fat fed to the tumor-bearing host (Liepkalns and Spector, 1975). These changes were also found in a membrane fraction derived from the tumor cells. This system provides a convenient means for producing homogeneous populations of mammalian cells containing appreciable differences in membrane fatty acid composition.

An important component of the plasma membrane of animal cells is the enzyme system, $(Na^+ + K^+)$-ATPase (EC 3.6.1.3), which functions in the active transport of Na^+ and K^+ in vivo (Skou, 1964). $(Na^+ + K^+)$-ATPase requires lipids for activity, but the specificity and exact function of the lipids have not been established (cf. Jorgensen, 1974b; Schwartz et al., 1975). Arrhenius plots for several membrane-bound enzymes, including $(Na^+ + K^+)$-ATPase, are biphasic (Fourcans and Jain, 1974) or multiphasic (Gruener and Avi-Dor, 1966; Wisnieski et al., 1974) and the slopes either intersect or are discontinuous at unique transition temperatures. From electron spin resonance experiments on spin-labeled membrane lipids, Grisham and Barnett (1973) concluded that this transition temperature for $(Na^+ + K^+)$-ATPase was due to a lipid-phase transition. Kimelberg and Papahadjopoulos (1972, 1974) reached similar conclusions from experiments with reconstituted systems. The latter authors also suggested that cholesterol may modulate the fluidity of membrane lipids, which in turn could be a means of controlling the activity of membrane bound enzymes.

In the present study, we demonstrate a correlation between the plasma membrane fatty acyl composition and the transition temperature and activation energy of $(Na^+ + K^+)$-ATPase. We also describe a purification procedure which yields plasma membranes with a significantly higher specific activity for $(Na^+ + K^+)$-ATPase than previously reported for the $(Na^+ + K^+)$-ATPase of Ehrlich ascites tumor cells.

Experimental Procedure

Growth and Treatment of Cells. Ehrlich ascites tumors were grown in male CBA mice fed either a control diet (4.5% fat, made up of 35% saturated, 31% monoenoic-ω9 and 30% polyenoic-ω6 fatty acids), a diet containing 16% sunflower oil (58% polyenoic-ω6 fatty acids), or a diet containing 4% tristearin as the only lipid component (Liepkalns and Spector, 1975; Brenneman et al., 1975). Mice were placed on these diets at least 4 weeks prior to tumor inoculation and were maintained on the diets during tumor growth. The tumors were harvested 14 days after transplantation. The tumor cells were separated from the ascites plasma and washed (McGee and Spector, 1974).

Preparation of Cell Homogenates and the Microsomal Fraction. Washed ascites tumor cells in 0.25 M sucrose containing 5 mM HEPES·NaOH (pH 7.5)[1] were disrupted by nitrogen cavitation and the homogenate made 1 mM with respect to EDTA as described by Wallach and Kamat (1966). In this method of cell disruption, the cell suspension

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[1] Abbreviations used are: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

is incubated in a Parr pressure bomb for 30 min at a N_2 pressure of 800 psi. The cell suspension is then expelled slowly and the cells disrupt due to the rapid decompression of intracellular N_2 (Wallach and Kamat, 1966). The homogenate was centrifuged at 6000*g* for 15 min (4 °C) to remove nuclei, mitochondria, and larger particles. The pellet was resuspended in 5 mM HEPES·NaOH (pH 7.5), 0.25 M sucrose, and 1 mM EDTA and centrifuged again as above. The combined supernatants were centrifuged at 100 000*g* (Beckman Ti-60 rotor, 40 000 rpm) for 45 min (4 °C). The sediment was washed successively in 5 mM Tricine·NaOH (pH 8.1)[1] and 1 mM Tricine·NaOH (pH 8.1) to remove trapped soluble proteins (Wallach and Kamat, 1966). The final microsomal pellets were suspended and gently homogenized in 10 mM HEPES·NaOH (pH 7.5), 0.25 M sucrose, and 1 mM EDTA at a protein concentration of about 10 mg ml^{-1} to give a uniform suspension. This microsomal fraction was either further purified immediately or stored frozen at −20 °C. There was no significant loss in $(Na^+ + K^+)$-ATPase activity when the microsomal fraction was stored under these conditions.

Sodium Dodecyl Sulfate Activation of $(Na^+ + K^+)$-ATPase. The microsomal fraction was incubated with sodium dodecyl sulfate in the presence of ATP (Jorgensen, 1974a). The optimal activation of latent $(Na^+ + K^+)$-ATPase activity by detergents is dependent on several factors including detergent and protein concentrations, pH, and temperature (Jorgensen and Skou, 1971; Jorgensen, 1974a). The optimum concentration of sodium dodecyl sulfate for the activation of $(Na^+ + K^+)$-ATPase was determined for each microsomal preparation by incubating various concentrations of sodium dodecyl sulfate with the microsomal fraction in 30 mM HEPES·NaOH (pH 7.5), 3 mM ATP, 0.5 mM EDTA, and 0.12 M sucrose for 20 min at room temperature. The protein concentration was 2–5 mg ml^{-1}. ATP protects $(Na^+ + K^+)$-ATPase against irreversible inactivation by sodium dodecyl sulfate (Jorgensen, 1974a). After the incubation period, aliquots were withdrawn for assay of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities.

Density Gradient Centrifugation. The microsomal fraction was incubated with an optimal concentration of sodium dodecyl sulfate as described above and was then layered on a 25-ml linear gradient of 12–44% (w/v) sucrose in 10 mM Tricine·NaOH (pH 8.1) and 1 mM EDTA in 3–6 ml portions. The sample was overlayed with 10 mM Tricine·NaOH (pH 8.1) and 1 mM EDTA. The tubes were centrifuged for 12 h at 40 000 rpm in a Beckman Ti-60 fixed angle rotor maintained at 4 °C. After centrifugation, the bottom of each tube was punctured with a 22-gauge needle, and 30-drop fractions were collected. The fractions containing the peak of $(Na^+ + K^+)$-ATPase were diluted four- to fivefold with 10 mM Tricine·NaOH (pH 8.1) and 1 mM EDTA and were centrifuged in a Ti-60 rotor for 12 h at 40 000 rpm (4 °C). The pellet was resuspended by homogenization in 10 mM HEPES·NaOH (pH 7.5), 0.25 M sucrose, and 1 mM EDTA to a protein concentration of 1–2 mg ml^{-1} and was stored frozen at −20 °C.

Enzyme Assays. The optimum pH for $(Na^+ + K^+)$-ATPase activity, using 30 mM Tricine·NaOH buffer, was 8.0–8.2. ATPase assay mixtures contained 30 mM Tricine·NaOH (pH 8.1), 3 mM ATP (Na salt), 3 mM $MgCl_2$, 120 mM NaCl, and 20 mM KCl. The difference between the activities in the presence and absence of 2 mM ouabain was taken as the activity of $(Na^+ + K^+)$-ATPase. The activity in the presence of 2 mM ouabain or in the absence of K^+ was taken as the activity of Mg^{2+}-ATPase. The amount of ATP hydrolyzed was determined by measuring the amount of P_i liberated (Fiske and Subbarow, 1925). NADH-cytochrome *c* reductase activity was measured as described by Solomonson and Vennesland (1972). Succinate dehydrogenase activity was measured using ferricyanide as the electron acceptor (Veeger et al., 1969).

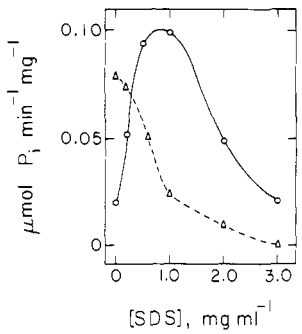

FIGURE 1: Effect of sodium dodecyl sulfate on $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities of Ehrlich ascites tumor cells. The microsomal fraction was incubated with different concentrations of sodium dodecyl sulfate, and enzyme activities were measured as described in Experimental Procedure. The protein concentration in the incubation mixtures was 2.8 mg ml^{-1}. (O—O) $(Na^+ + K^+)$-ATPase. (Δ- - -Δ) Mg^{2+}-ATPase.

Analytical Measurements. Protein was estimated by a modification of the method of Lowry (Bailey, 1967) after precipitation and washing once with 5% TCA at 0 °C. Lipids were extracted from the membrane fractions with a chloroform–methanol mixture (Folch et al., 1957). Cholesterol was measured by the method of Searcy and Bergquist (1960), and phospholipids were estimated by the method of Raheja et al. (1973). After saponification and methylation (Morrison and Smith, 1964), fatty acid methyl esters were separated by gas–liquid chromatography (Liepkalns and Spector, 1975).

Electron Microscopy. Samples prepared for electron microscopy were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 (Sabatini et al., 1963), for 1 h, washed for 15 min in 0.1 M cacodylate buffer, pH 7.2, and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2, for 1 h. They were then dehydrated in ethanol and embedded in Spurr's embedding medium (Spurr, 1969). Gray to light gold sections were cut on a Sorval MT-2B ultramicrotome and mounted on uncoated 300- or 400-mesh copper grids. Staining was done with uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963). The sections were examined in a Hitachi HU-125F electron microscope.

Results

Activation of $(Na^+ + K^+)$-ATPase by Sodium Dodecyl Sulfate. Treatment of the microsomal fraction of Ehrlich ascites tumor cells with sodium dodecyl sulfate results in an activation of $(Na^+ + K^+)$-ATPase and an inactivation of Mg^{2+}-ATPase, thus resulting in a pronounced increase in the ratio of $(Na^+ + K^+)$-ATPase to Mg^{2+}-ATPase activity. The effect of sodium dodecyl sulfate on $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities of the microsomal fraction is illustrated in Figure 1. Incubation of the microsomal fraction with increasing concentrations of sodium dodecyl sulfate caused an increase and then a decrease in the activity of $(Na^+ + K^+)$-ATPase. As shown in Figure 2, treat-

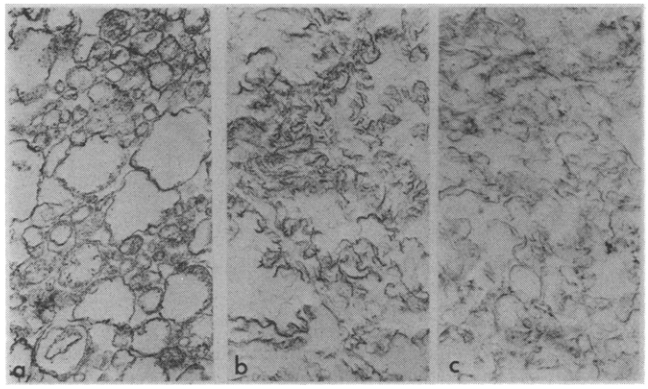

FIGURE 2: Electron microscopy of: microsomal fraction before sodium dodecyl sulfate treatment; (a) microsomal fraction before sodium dodecyl sulfate treatment; (b) microsomal fraction after sodium dodecyl sulfate treatment; and (c) purified plasma membrane fraction of Ehrlich ascites tumor cells. Details of the sodium dodecyl sulfate treatment, preparation of the membrane fractions, and preparation of the samples for electron microscopy are given in experimental procedure.

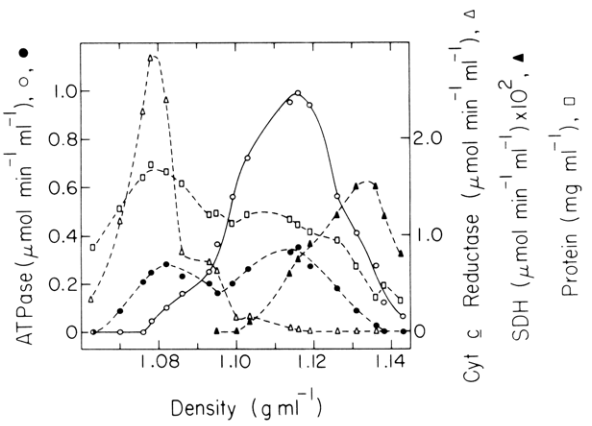

FIGURE 3: Purification of $(Na^+ + K^+)$-ATPase by sucrose density gradient centrifugation. Enzyme activities and protein concentration are plotted vs. the density of the sucrose solution which was determined for each fraction with an Abbe refractometer: (—⊙—) $(Na^+ + K^+)$-ATPase; (- - -●- - -) Mg^{2+}-ATPase; (- - -△- - -) NADH-cytochrome *c* reductase; (- - -▲- - -) succinate dehydrogenase; (- - -□- - -) protein.

ment with sodium dodecyl sulfate also results in the opening of closed microsomal vesicles. Sodium dodecyl sulfate had similar effects on the membrane fraction of Ehrlich ascites tumor cells obtained from mice fed either the control or the two test diets.

Purification of $(Na^+ + K^+)$*-ATPase.* In addition to activating the $(Na^+ + K^+)$-ATPase, low concentrations of sodium dodecyl sulfate also solubilize or dissociate extraneous membrane proteins associated with the membrane fraction. These effects of sodium dodecyl sulfate formed the basis for the further purification of $(Na^+ + K^+)$-ATPase. The microsomal fraction was treated with sodium dodecyl sulfate at a concentration that resulted in a maximal activation of the $(Na^+ + K^+)$-ATPase and an inactivation of the Mg^{2+}-ATPase. This mixture was then centrifuged on a linear sucrose gradient to separate $(Na^+ + K^+)$-ATPase from soluble proteins and other membrane fractions. $(Na^+ + K^+)$-ATPase activity remains associated with the plasma membrane fraction (Figure 2c). The results of a typical fractionation are illustrated in Figure 3. The activities of NADH-cytochrome *c* reductase, succinate dehydrogenase, and Mg^{2+}-ATPase were low and variable. In some preparations,

Table I: Purification of $(Na^+ + K^+)$-ATPase from Ehrlich Ascites Tumor Cells[a]

Fraction	Total Activity[b] (μmol/min)	Specific Activity (μmol/min per mg protein)	Yield (%)	Purification (-fold)
1. Regular Diet (2)				
Microsomal	9.6	0.08	100	1.0
Plasma membrane	6.5	0.67	68	8.4
2. 16% Sunflower Diet (3)				
Microsomal	5.0	0.05	100	1.0
Plasma membrane	3.3	0.35	66	7.0
3. 4% Tristearin Diet (3)				
Microsomal	7.5	0.06	100	1.0
Plasma membrane	5.8	0.43	77	7.2

[a] Details of the purification procedure are given in Experimental Procedure. The diet is that of the host animal from which the tumor cells were obtained. The number in parenthesis after each diet refers to the number of different preparations, and the values are the combined data from these preparations. [b] Activity after activation with an optimal concentration of sodium dodecyl sulfate.

these activities could not be detected after treatment with sodium dodecyl sulfate. A part of the Mg^{2+}-ATPase activity appears to be associated with the plasma membrane, while the NADH-cytochrome *c* reductase and succinate dehydrogenase are associated with other membrane fractions, presumably arising from endoplasmic reticulum or mitochondrial contaminants.

The results of several purifications of the microsomal fraction of tumor cells derived from mice maintained on normal, 16% sunflower, or 4% tristearin diets are summarized in Table I. We did not routinely measure the ATPase activity and protein content of unfractionated cell homogenates but, because of the extensive washing of the microsomal fraction to remove entrapped protein, the extent of purification with respect to the cell homogenate would probably be considerably higher than shown in this table. A good recovery of activity was achieved, indicating that the $(Na^+ + K^+)$-ATPase remains active after removal of excess sodium dodecyl sulfate. The specific activity of the purified $(Na^+ + K^+)$-ATPase of tumor cells grown in mice fed a normal diet appears to be somewhat higher than that of cells grown on either of the test diets.

Lipid Composition of Purified $(Na^+ + K^+)$*-ATPase.* The lipid compositions of the purified plasma membrane fractions of tumor cells derived from mice fed the regular, 16% sunflower, or 4% tristearin diets (PM_C, PM_{SU}, and PM_{TS}, respectively) are summarized in Table II. The cholesterol–phospholipid and lipid–protein ratios were similar in all three cases. Marked differences were observed, however, in the fatty acyl compositions of PM_C, PM_{SU}, and PM_{TS}. PM_{TS} had a greatly increased content of 18:1ω9 relative to PM_C as well as components that were tentatively identified as 20:3ω9 and 22:3ω9. Moreover, in PM_{TS}, the content of 18:2ω6 was greatly reduced and the content of 20:4ω6 was significantly reduced as compared with control (PM_C) values. Differences in the fatty acyl composition of PM_{SU} relative to control values also were observed, but they were not as striking as in the case of PM_{TS}.

Temperature–Activity Relationships of Purified $(Na^+ + K^+)$*-ATPase.* The $(Na^+ + K^+)$-ATPase activity associated with PM_C, PM_{SU}, and PM_{TS} was measured at different temperatures, and the results are given in the form of Ar-

Table II: Lipid Composition of Purified Plasma Membranes from Ehrlich Ascites Tumor Cells[a]

	PM_C	PM_{SU}	PM_{TS}
Cholesterol–phospholipid (w/w)	0.35	0.33	0.36
Lipid–protein (w/w)	0.81	0.71	0.76
Fatty acid (% of total)			
16:0	13.0	11.4	11.3
16:1ω7	2.1	2.9	5.6
18:0	19.8	15.7	11.1
18:1ω9	19.7	12.9	36.5
18:2ω6	19.0	22.3	4.0
18:3ω3–20:1ω9	1.2	<1.0	
20:2ω9	1.0	5.0	2.2
20:3ω9			8.1
20:4ω6	11.0	9.5	6.1
22:3ω9			4.0
22:4ω6	3.1	8.3	2.4
22:5ω6	1.6	3.3	1.8
22:5ω3	1.1	1.3	<1.0
22:6ω3	5.5	4.7	3.2

[a] Purification and analytical procedures are described in Experimental Procedure. PM_C, PM_{SU}, and PM_{TS} refer to purified plasma membrane fractions of tumor cells obtained from host animals which had been maintained on diets of regular chow, 16% sunflower, or 4% tristearin, respectively. Lipid refers to the sum of cholesterol and phospholipid.

Table III: Transition Temperatures and Energies of Activation for $(Na^+ + K^+)$-ATPase of Plasma Membranes with Different Fatty Acyl Compositions

	PM_C	PM_{SU}	PM_{TS}
T_{tr} (°C)	20.3	21.6	13.6
Activation energy below T_{tr} (kcal/mol)	33.4	27.7	60.0
Activation energy above T_{tr} (kcal/mol)	18.0	19.7	21.8

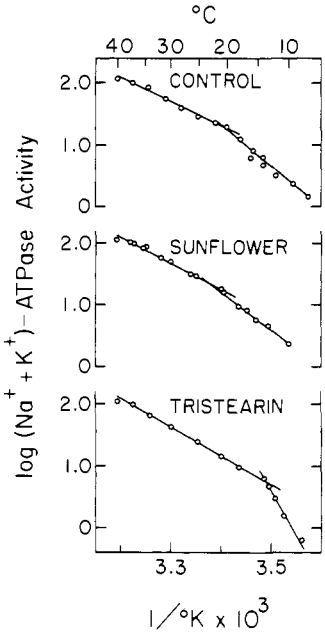

FIGURE 4: Arrhenius plots of $(Na^+ + K^+)$-ATPase from purified plasma membrane of Ehrlich ascites tumor cells obtained from host animal maintained on diets of regular chow, 16% sunflower oil, or 4% tristearin. The $(Na^+ + K^+)$-ATPase activity measured at 37 °C was arbitrarily set at 100 to facilitate comparison between different preparations. All of the points for the tristearin plot and many of the points for the sunflower and control plots are the average of two determinations which, in most cases, varied by less than 5% from each other. The data of all points between 7 and 37 °C were analyzed by the method of least-squares (Bogartz, 1968) and were fitted best by two straight lines intersecting at a unique transition temperature. The regression coefficients for the slopes of the lines above the transition temperature were −0.996, −0.998, and −0.999, respectively, for the control, sunflower, and tristearin plots, and the regression coefficients for the slopes of the lines below the transition temperature were −0.984, −0.991, and −0.988, respectively.

rhenius plots as shown in Figure 4. The data of Figure 4, analyzed by the method of least-squares (Bogartz, 1968), were fitted best in all three cases by a biphasic plot of two intersecting straight lines. The correlation coefficients for the slopes of the points above the transition temperatures were $>|-0.99|$ in all three cases, while the correlation coefficients for the slopes of the points below the transition temperatures were $>|-0.98|$ in all three cases. The transition temperatures and the energies of activation calculated from these slopes are given in Table III. As was the case for the fatty acyl composition, the greatest differences in both transition temperatures and energy of activation were observed in PM_{TS} relative to the control, PM_C, and to PM_{SU}. The values for PM_C and PM_{SU} were similar, in agreement with the fact that their fatty acyl compositions were quite similar. The energy of activation below the transition temperature was greatly increased and the transition temperature was considerably lower in PM_{TS} as compared with either PM_C or PM_{SU}. By contrast, the energies of activation above the transition temperature were similar in all three cases.

Discussion

The activation of $(Na^+ + K^+)$-ATPase by low concentrations of sodium dodecyl sulfate probably is due to the exposure of additional enzyme active sites. This may result from either the opening of artifactually closed vesicles as shown by electron micrographs (Figure 2) or by the removal of an inhibitor of $(Na^+ + K^+)$-ATPase associated with the plasma membrane. Our results on the membrane morphological changes accompanying sodium dodecyl sulfate activation of $(Na^+ + K^+)$-ATPase are in agreement with earlier observations of Rostgaard and Moller (1971) using deoxycholate activation of the $(Na^+ + K^+)$-ATPase from ox kidney. Further support for the view that the activating effect of detergents is due to exposure of additional enzyme active sites comes from the demonstration that the molar activity of $(Na^+ + K^+)$-ATPase is not changed in the process of enzyme activation (Jorgensen and Skou, 1971). It also has been shown that the activation of $(Na^+ + K^+)$-ATPase by deoxycholate or by sodium dodecyl sulfate is not associated with the binding of detergent to the microsomal particles (Jorgensen and Skou, 1971; Jorgensen, 1974a).

The partially purified $(Na^+ + K^+)$-ATPase preparation has the appearance of plasma membrane fragments in electron micrographs (Figure 2c), and the cholesterol–phospholipid ratio is characteristic of plasma membranes (Coleman and Finean, 1966; Jorgensen, 1973). Both the electron micrographs and the enzyme assays indicate that the purified plasma membrane fraction is relatively free of contamination by other cellular components. Most of the Mg^{2+}-ATPase is removed or inactivated by sodium dodecyl sulfate treatment. A part of the Mg^{2+}-ATPase activity, however, sedimented with the plasma membrane fraction on sucrose gradients. This activity probably is due to an ATPase associated with the outer surface of Ehrlich ascites tumor cells (Ronquist and Ågren, 1975). There is considerable evidence that the hydrolysis of ATP catalyzed by $(Na^+ + K^+)$-AT-

Pase occurs on the inner (cytoplasmic) surface of the cell membrane (Schwartz et al., 1975). The specific activity for the $(Na^+ + K^+)$-ATPase of Ehrlich cells purified by the procedure described herein is considerably higher than that reported using other methods (Forte et al., 1973; Colombini and Johnstone, 1973; Molnar et al., 1969; Kamat and Wallach, 1965). It is still much lower, however, than that of purified membrane-bound $(Na^+ + K^+)$-ATPases from tissues rich in sodium pump activity (cf. Jorgensen, 1974b; Schwartz et al., 1975).

We found that the Ehrlich cell Na^+ plus K^+ stimulated ATPase activity was completely inhibited by ouabain. By contrast, Wallach and Ullrey (1964) reported only a 70% inhibition by 1 mM ouabain and Forte et al. (1973) observed only a 50–60% inhibition by 20 μM ouabain for the $(Na^+ + K^+)$-ATPase of Ehrlich cells. Under the conditions of our assay, we observed no significant difference in the temperature–activity relationships when the $(Na^+ + K^+)$-ATPase activity was determined by omitting K^+ rather than inhibiting with ouabain. Charnock et al. (1975) have reported that the energies of activation for the $(Na^+ + K^+)$-ATPase of rabbit kidney are different when determined by cation activation rather than by ouabain inhibition.

The biphasic Arrhenius plots over the temperature range 7 to 37 °C of the $(Na^+ + K^+)$-ATPase activity in Ehrlich cells are in agreement with the results of other studies on the $(Na^+ + K^+)$-ATPase from several different sources (Charnock et al., 1971, 1973, 1975; Grisham and Barnett, 1973; Kimelberg and Mayhew, 1975; Priestland and Whittam, 1972; Smith, 1967; Tanaka and Teruya, 1973; Taniguchi and Iida, 1972). These $(Na^+ + K^+)$-ATPases also undergo a large change in activation energy near 20 °C as was observed in the present study for the Arrhenius plot of the $(Na^+ + K^+)$-ATPase activity in PM_c (control diet). Gruener and Avi-Dor (1966) observed a second discontinuity at about 6 °C for the $(Na^+ + K^+)$-ATPase of rat brain. It was not possible in the present study to obtain accurate measurements of the $(Na^+ + K^+)$-ATPase activity at these lower temperatures due to a decrease in the ratio of $(Na^+ + K^+)$-ATPase to Mg^{2+}-ATPase activity. Wisnieski et al. (1974) observed seven breaks in the Arrhenius plot, over the temperature range 4–40 °C, for $(Na^+ + K^+)$-ATPase in the plasma membrane of a cultured mouse fibroblast line when the activity was measured at smaller temperature intervals. Therefore, we cannot exclude the possibility that additional minor discontinuities might be observed if the temperature–activity relationships of the $(Na^+ + K^+)$-ATPase of Ehrlich cells were examined in more detail. Our results, however, clearly demonstrate that a marked change (~7 °C) in the major transition temperature can be caused by changes in the dietary lipid of the tumor-bearing mice. Likewise, they demonstrate that a twofold difference in the activation energy for $(Na^+ + K^+)$-ATPase below the transition temperature also occurs. These changes in the temperature–activity relationships were shown to be accompanied by changes in the fatty acyl composition of the plasma membrane. By contrast, no significant changes were observed in the phospholipid or cholesterol content of the plasma membrane.

Previous studies with artificial membrane systems and bacteria have demonstrated that the transition temperature of lipid-associated enzymes decreases as the degree of lipid unsaturation increases (Fourcans and Jain, 1974). Based upon these data, we expected a lower transition temperature for the $(Na^+ + K^+)$-ATPase activity in the membranes prepared from cells grown in mice fed the sunflower oil diet as compared with the tristearin diet. As shown in Figure 4 and Table III, however, the transition temperature actually was higher in the PM_{SU}. Although the mechanism of this finding is unknown at present, the fatty acid composition data in Table III suggest a possible explanation. In addition to containing more polyunsaturated fatty acids, PM_{SU} also are richer in saturated fatty acids, particularly stearate. The increase in the unsaturated fatty acids 18:1ω9, 20:3ω9 and 22:3ω9 relative to the ω6 and ω3 series of fatty acids in PM_{TS} presumably reflects a compensatory metabolic shift in the cells grown on tristearin in an attempt to maintain optimum fluidity. Therefore, it is possible that the phospholipid fatty acids associated with the PM_{TS} $(Na^+ + K^+)$-ATPase actually may contain more total unsaturation, accounting for the higher transition temperature that was observed. On the other hand, the fatty acyl composition of the total plasma membrane lipid shown in Table II may not adequately reflect the fatty acyl groups intimately associated with the ATPase complex.

The present study has demonstrated that alteration of the plasma membrane fatty acyl composition in vivo results in altered properties of the $(Na^+ + K^+)$-ATPase associated with these membranes. Our results are consistent with a number of reactivation studies with lipid-depleted $(Na^+ + K^+)$-ATPase preparations which suggest that the fatty acyl groups as well as the polar head groups of phospholipids are important determinants of enzyme activity. Walker and Wheeler (1975), in agreement with earlier studies (Fenster and Copenhaver, 1967; Hokin and Hexum, 1972; Kimelberg and Papahadjopoulus, 1972; Roelofsen and van Deenen, 1973; Tanaka, 1969; Wheeler and Whittam, 1970), found that only the acidic phospholipids reactivated a lipid-depleted $(Na^+ + K^+)$-ATPase. The amount of reactivation, however, depended on the nature of the fatty acyl groups as well as the polar head groups of these acidic phospholipids. Tanaka and Teruya (1973) showed with reconstituted systems that the temperature–activity relationship of $(Na^+ + K^+)$-ATPase was determined not by the enzyme source but by the lipid used to activate the enzyme. Other workers (Kimelberg and Papahadjopoulos, 1974; Warren et al., 1974) have also concluded that the fatty acyl groups and membrane fluidity are important parameters for $(Na^+ + K^+)$-ATPase activity. The transition temperature is far below the normal physiological temperature, and the energy of activation for $(Na^+ + K^+)$-ATPase above the transition temperature is similar for membrane preparations with differing fatty acyl compositions. Therefore, the $(Na^+ + K^+)$-ATPase probably is functioning in vivo in a largely fluid lipid environment at physiological temperatures. Kimelberg and Mayhew (1975) showed that the activity of $(Na^+ + K^+)$-ATPase was increased in virally transformed cells. When this occurred, the transition temperature and energy of activation for $(Na^+ + K^+)$-ATPase were altered. In a related study, Barnett et al. (1974) found differences in membrane fluidity and structure of normal and transformed cells based on experiments using spin labels. These observations, together with the present results, support the view that changes in the fatty acyl composition of the cell membrane can have an important influence on the activity of the $(Na^+ + K^+)$-ATPase.

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References

Bailey, J. L. (1967), Techniques in Protein Chemistry, 2nd ed., Amsterdam, Elsevier, p 340.
Barnett, R. E., Furcht, L. T., and Scott, R. E. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1992.
Bogartz, R. S. (1968), *Psych. Bull. 70,* 749.
Brenneman, D. E., McGee, R., Liepkalns, V. A., and Spector, A. A. (1975), *Biochim. Biophys. Acta 388,* 301.
Charnock, J. S., Almeida, A. F., and To, R. (1975), *Arch. Biochem. Biophys. 167,* 480.
Charnock, J. S., Cook, D. A., Almeida, A. F., and To, R. (1973), *Arch. Biochem. Biophys. 159,* 393.
Charnock, J. S., Cook, D. A., and Casey, R. (1971), *Arch. Biochem. Biophys. 147,* 323.
Charnock, J. S., Cook, D. A., and Opit, L. J. (1971), *Nature (London), New Biology 233,* 171.
Coleman, R., and Finean, J. B. (1966), *Biochim. Biophys. Acta 125,* 197.
Colombini, M., and Johnstone, R. M. (1973), *Biochim. Biophys. Acta 323,* 69.
Fenster, L. J., and Copenhaver, J. H. (1967), *Biochim. Biophys. Acta 137,* 406.
Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66,* 375.
Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem. 226,* 497.
Fourcans, B., and Jain, M. K. (1974), *Adv. Lipid Res. 12,* 147.
Forte, J. G., Forte, T. M., and Heinz, E. (1973), *Biochim. Biophys. Acta 298,* 827.
Grisham, C. M., and Barnett, R. E. (1972), *Biochim. Biophys. Acta 266,* 613.
Grisham, C. M., and Barnett, R. E. (1973), *Biochemistry 12,* 2635.
Gruener, N., and Avi-Dor, Y. (1966), *Biochem. J. 100,* 762.
Hokin, L. E., and Hexum, T. D. (1972), *Arch. Biochem. Biophys. 151,* 453.
Jorgensen, P. L. (1973), *Methods Enzymol. 32,* 277.
Jorgensen, P. L. (1974a), *Biochim. Biophys. Acta 356,* 36.
Jorgensen, P. L. (1974b), *Q. Rev. Biophys. 7,* 239.
Jorgensen, P. L., and Skou, J. C. (1971), *Biochim. Biophys. Acta 233,* 366.
Jorgensen, P. L., Skou, J. C., and Solomonson, L. P. (1971), *Biochim. Biophys. Acta 233,* 381.
Kamat, V., and Wallach, D. F. H. (1965), *Science 148,* 1343.
Kimelberg, H. K., and Mayhew, E. (1975), *J. Biol. Chem. 250,* 100.
Kimelberg, H. K., and Papahadjopoulos, D. (1972), *Biochim. Biophys. Acta 282,* 277.
Kimelberg, H. K., and Papahadjopoulos, D. (1974), *J. Biol. Chem. 249,* 1071.
Liepkalns, V. A., and Spector, A. A. (1975), *Biochem. Biophys. Res. Commun. 63,* 1043.
McGee, R., and Spector, A. A. (1974), *Cancer Res. 34,* 3355.
Molnar, J., Markovic, G., Chao, H., and Molnar, Z. (1969), *Arch. Biochem. Biophys. 134,* 524.
Morrison, W. R., and Smith, L. N. (1964), *J. Lipid Res. 5,* 600.
Priestland, R. N., and Whittam, R. (1972), *J. Physiol. 220,* 353.
Raheja, R. K., Kaur, C., Singh, A., and Bhatia, I. S. (1973), *J. Lipid Res. 14,* 695.
Reynolds, E. S. (1963), *J. Cell Biol. 17,* 208.
Roelofsen, B., and van Deenen, L. L. M. (1973), *Eur. J. Biochem. 40,* 245.
Ronquist, G., and Agrên, G. K. (1975), *Cancer Res. 35,* 1402.
Rostgaard, J., and Moller, O. J. (1971), *Exp. Cell Res. 68,* 356.
Sabatini, D. D., Bensch, K., and Barnett, R. J. (1963), *J.. Cell Biol. 17,* 19.
Schwartz, A., Lindenmeyer, G. E., and Allen, J. C. (1975), *Pharmacol. Rev. 27,* 3.
Searcy, R. L., and Bergquist, L. M. (1960), *Clin. Chim. Acta 5,* 192.
Skou, J. C. (1964), *Prog. Biophys. 14,* 133.
Smith, M. W. (1967), *Biochem. J. 105,* 65.
Solomonson, L. P., and Vennesland, B. (1972), *Biochim. Biophys. Acta 267,* 544.
Spurr, A. R. (1969), *J. Ultrastruct. Res. 26,* 31.
Tanaka, R. (1969), *J. Neurochem. 16,* 1301.
Tanaka, R., and Teruya, A. (1973), *Biochim. Biophys. Acta 323,* 584.
Taniguchi, K., and Iida, S. (1972), *Biochim. Biophys. Acta 274,* 536.
Veeger, C., Der Vartanian, D. V., and Zeylemaker, W. P (1969), *Methods Enzymol. 13,* 81.
Walker, J. A., and Wheeler, K. P. (1975), *Biochim. Biophys. Acta 394,* 135.
Wallach, D. F. H., and Kamat, V. B. (1966), *Methods Enzymol. 8,* 164.
Wallach, D. F. H., and Ullrey, D. (1964), *Biochim. Biophys. Acta 88,* 620.
Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974), *Biochemistry 13,* 5501.
Watson, M. L. (1958), *J. Biophys. Biochem. Cytol. 4,* 475.
Wheeler, K. P., and Whittam, R. (1970), *J. Physiol. 207,* 303.
Wisnieski, B. J., Parkes, J. G., Huang, Y. O., and Fox, C. F. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4381.