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DEVELOPMENTAL CHANGES IN PLASMA MEMBRANE FLUIDITY IN CHICK EMBRYO HEART

HOWARD KUTCHAI, YECHEZKEL BARENHOLZ, THOMAS F. ROSS and DAVID E. WERMER

Departments of Physiology and Biochemistry, University of Virginia School of Medicine, Charlottesville, Va. 22901 (U.S.A.)

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SUMMARY

1. Decreases in the rate of transport of sugars (facilitated transport), amino acids (active transport), and urea (simple diffusion) occur in chick embryo heart during development. This work considers the possibility that changes in the plasma membrane fluidity during development contribute to the observed changes in transport activities.

2. Techniques were developed for subcellular fractionation of chick embryos and adult chickens.

3. The depolarization of the fluorescence of 1,6-diphenylhexatriene was used to estimate the fluidity of the lipid portion of plasma membrane enriched fractions of hearts from chick embryos at various stages of development and from adult hearts.

4. There is a pattern of decreasing membrane viscosity as development proceeds. Between 5–6 days and 10 days of embryonic life a 20 % decrease in viscosity of the plasma membrane-enriched fraction occurs. Between 10 and 20 days of embryonic life there is no significant change in viscosity. Between 20 days of development (1 day before hatching) and adulthood there is a further 55 % decrease in plasma membrane viscosity.

5. It is proposed that the changes in membrane fluidity observed may contribute to developmental changes in membrane transport activities, but other factors must also be involved.

INTRODUCTION

Previous work has shown that the rate of transport of sugars into chick embryo heart cells decreases over the course of embryonic development [1–4]. The ability of chick embryo heart cells to accumulate amino acids also decreases as development proceeds [5]. Urea, which presumably enters chick embryo heart cells by simple diffusion, also shows a pattern of decreasing permeability with development [3].

Since the rates of uptake of sugars (which enter by facilitated transport), amino acids (which are actively transported), and urea (which enters by simple

diffusion) all decrease during embryonic development, we were lead to consider the possibility that a change in some general property of the plasma membrane of the heart cells during development is responsible for all the transport changes observed. The fluidity of the lipid bilayer matrix of the plasma membrane (as defined by the rotational diffusion of fluorescent [6] or spin-labeled [7] probes) is one property that might be expected to play a role in the regulation of transport processes that occur via the mediation of a mobile carrier or by simple diffusion through the membrane. In the study reported here the fluidity of the lipid matrix of a plasma membrane-enriched fraction of chick embryo heart cells at various stages of embryonic life and in adult chickens was estimated by the depolarization of the fluorescence of 1,6-diphenylhexatriene. Significant decreases in the microviscosity of the lipid bilayer matrix of the plasma membrane-enriched fraction were found to occur during development, large decreases occurring between 5 and 10 days of embryonic life and between 20 days of embryonic life and adulthood.

METHODS AND MATERIALS

Subcellular fractionation of hearts from chick embryos and adult chickens

Embryonated hens eggs were obtained from the Grassy Knoll Hatchery (Standardsville, Va.) and kept in a commercial farm egg incubator in our laboratory. Adult hens were obtained through the University of Virginia Vivarium. Hearts were dissected from chick embryos at 5–6, 10, and 20 days of embryonic life (hatching occurs at 21 days). Hearts were dissected from adult hens after first injecting 2 ml of heparin in isotonic saline (5.2 units/ml) into a wing vein and then killing the animal by cervical dislocation. Hearts were immediately placed in 0.25 M sucrose (ice cold) and subsequently weighed and minced with fine scissors. Using 4–5 g of minced heart a 20 % (w/v) homogenate in 0.25 M sucrose was prepared by means of a "Willems" Polytron homogenizer (Brinkmann Instruments) with PT 20 ST head at half-maximal speed for 15 s. The homogenate was freed of clumps and connective tissue elements by filtration through nylon mesh held in a Swinney adaptor (Millipore Corp.) and then subjected to 75 min of centrifugation in the SS-34 rotor of a Sorvall RC-2B centrifuge at 48 246 $\times g$ (bottom of tube). The supernatant was discarded and the pellet resuspended in the original volume of 0.25 M sucrose. Subsequent fractionation was by a modification of the methods of Kidwai and associates [8]. Into the tubes for the Beckman SW 27 rotor 15 ml of 40 % (w/v) sucrose were pipetted. Then 15 ml of 25 % sucrose were layered on the 40 % sucrose and 9.5 ml of homogenate layered on top of the 25 % sucrose. The tubes were spun in the SW 27 rotor in a Beckman L3-50 ultracentrifuge at 128 574 $\times g$ (bottom of tube) for 90 min. Three very sharply defined bands were obtained. One at the top of the 25 % sucrose layer that we call the F_1 fraction which is enriched in plasma membrane, one at the interface between the 25 % and 40 % sucrose layers that is enriched in mitochondria and endoplasmic reticulum (we call this the F_2 fraction), and a band at the bottom of the tube that contains large amounts of contractile protein (insoluble in sucrose solutions) that we call the F_3 fraction. The fractions were removed by means of J-shaped Pasteur pipettes and diluted with 0.25 M sucrose and pelleted by centrifugation for 120 min at 48 246 $\times g$ (bottom of tube) in the Sorvall RC-2B centrifuge. The pellets were suspended in 0.025 M Tris/HCl (pH 7.5) by means of a Potter-Elvehjem homogenizer.

Assay of marker enzymes

The activities of 5′-nucleotidase and $(Na^+ + K^+)$-ATPase were used as markers for plasma membrane, the activity of succinic dehydrogenase as a mitochondrial marker, and the activities of NADH oxidase and non-specific esterase (*p*-nitrophenylacetate splitting) as markers for endoplasmic reticulum [9].

The activity of 5′-nucleotidase was assayed as follows [10]: variable amounts of subcellular fractions were mixed with 0.2 ml of buffer mixture (0.075 M Tris/HCl (pH 9), 0.01 M KCl, and 0.005 M $MgCl_2$) and 10 μl of 0.1 M 5′-adenosinemonophosphate (sodium salt). Mixtures were incubated for 30–60 min at 37 °C and then the reaction was stopped by addition of 100 μl of 5 % trichloroacetic acid and 100 μl of 9 M H_2SO_4. The tubes were centrifuged and 100 μl of supernatant transferred to acid-washed tubes for determination of the phosphate liberated by the method of Fiske and SubbaRow [11].

The activity of $(Na^+ + K^+)$-ATPase was determined in a mixture containing a variable amount of subcellular fraction and 3 mM ATP (sodium salt), 3 mM $MgCl_2$, 50 mM imidazole/HCl buffer (pH 7.4) and in the presence and absence of 10 mM KCl and 100 mM NaCl (total incubation volume 0.5 ml). Tubes were incubated for 20 min or longer at 37 °C and the reaction stopped by addition of 200 μl of 10 % trichloroacetic acid and the tubes centrifuged. Then 0.3 ml of supernatant was transferred to an acid-washed tube for determination of phosphate by the Fiske-SubbaRow method [11].

Succinic dehydrogenase was assayed by a modification of the method of Ells [12]. Into a 1 ml cuvette was pipetted 100 μl each of 0.5 M potassium phosphate buffer (pH 7.6), 0.1 M KCN in the phosphate buffer, 0.46 mM dichlorophenolindophenol and then 0.5 ml of water was added. A variable amount of subcellular fraction and 100 μl of 0.5 g/l phenazine methosulfate was added and the contents of the cuvette mixed thoroughly. The cuvette was placed in the Heath 701 spectrophotometer and the absorbance at 600 nm recorded until no further change took place. Then 100 μl of 0.2 M sodium succinate was added, the cuvette mixed by inversion, and the decrease of absorbance at 600 nm recorded versus time for several minutes. The number of μmol of succinate oxidized per min was calculated by dividing the slope of the absorbance record by 19.1 [12].

NADH oxidase was assayed as follows: to 1 ml of water was added 100 μl of 160 mM Tris/HCl (pH 7.4), 100 μl of 6.6 mM $K_3Fe(CN)_6$, 5 μl of 50 mM NADH, and variable amounts of subcellular fractions. The mixture was transferred to a cuvette and the decline of absorbance at 340 nm recorded on a strip chart recorder.

Nonspecific esterase activity was assayed with *p*-nitrophenylacetate (Sigma Chemical Co.) as substrate. To 1.5 ml of 0.025 M Tris/HCl (pH 7.5) a variable amount of subcellular fraction was added. While this mixture was being mixed on a vortex mixer, 5 μl of 0.5 M *p*-nitrophenylacetate (dissolved in methanol/acetone, 1/1) was added, the mixture transferred to a cuvette and the increase in absorbance at 401 nm was recorded on a strip chart recorder for about 10 min. There was significant hydrolysis in the absence of enzyme and this rate was subtracted from the rates obtained with the subcellular fractions present.

Protein was determined by a modified biuret method using the reagent described by Zamenhof [13]. To 1 ml of biuret reagent was added up to 50 μl of a subcellular fraction. The tubes were allowed to stand after mixing for 10–15 min and

then the absorbance at 350 nm was determined. Protein concentrations were determined relative to bovine serum albumin standards.

Electron microscopy of subcellular fractions

Subcellular fractions were pelleted by centrifugation for 75 min at 48 246 × *g* (bottom of tube) in the Sorvall RC-2B centrifuge and the supernatant discarded. The pellets were fixed at 4 °C for 2 h in 7.3 mM glutaraldehyde in 60 mM cacodylate buffer (pH 7.4), rinsed for 1 h in cacodylate buffer, post-fixed in phosphate-buffered osmium tetroxide, and rinsed twice with 0.9 % NaCl. The specimens were then dehydrated in graded solutions of ethanol, then treated with propylene oxide, and finally embedded in epon. Sections were cut with a Porter-Blum microtome (Sorvall, Inc.) and viewed with a Zeiss EM-9A electron microscope.

Fluorescence labeling of the membranes

To 3.5 ml of water was added an aliquot of the plasma membrane-enriched fraction containing 50–600 μg of protein (most experiments used greater than 200 μg of protein). Then 2 μl of 1 mM 1,6-diphenylhexatriene (Aldrich, Puriss grade) in tetrahydrofuran was added and the suspension mixed thoroughly by means of a vortex mixer. Assuming the membrane fraction to be composed of equal weights of lipid and protein and assuming the average lipid molecule to have a molecular weight of 750, the ratio of lipid molecules to 1,6-diphenylhexatriene molecules ranged from 33.5 to 400. Control experiments described below showed that the fluorescence anisotropy was nearly constant in this range of membrane protein concentration. The suspension was transferred to a cuvette which was placed in the thermoregulated (at about 40 °C) cuvette holder of an Hitachi-Perkin Elmer spectrophotofluorimeter (Model MPF-3). The sample was excited with 360 nm light and emitted light was collected at 430 nm. The total fluorescence intensity was monitored and found to increase gradually and to stabilize after 15–20 min of incubation at 40 °C. After the fluorescence intensity became stable polarization measurements were performed as described below.

Fluorescence polarization measurements

The fluorescence polarization of the diphenylhexatriene in the suspension of plasma membrane-enriched vesicles was determined as described by Shinitzky and Barenholz [15]. The MPF-3 spectrophotofluorimeter was modified for polarization measurements with the assistance of Mr. Gene Stubbs. Fluorescence intensity and polarization were obtained by measuring $I_{\parallel}$ and $I_{\perp}$ (where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities detected through polarizers oriented parallel and perpendicular to the direction of polarization of the excitation beam). The fluorescence anisotropy (r) is defined by $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$.

Readings of $I_{\parallel}$ and $I_{\perp}$ were recorded on a strip chart recorder and showed no decrease during the time of the measurement. Measurements with membrane suspensions in the absence of 1,6-diphenylhexatriene showed that the contribution of light scattering to the intensity at 430 nm was less than 1 % of the fluorescence intensities observed at the largest concentration of membrane protein used.

After obtaining stable readings at about 40 °C the thermostat on the Lauda MK-2 circulator that controlled the temperature of the sample compartment was

turned to 0 °C. The temperature of the sample fell at approx. 0.3 °C/min and was recorded continuously by means of a Yellow Springs Instrument Co. thermistor and readout device. Every 1–1.5 °C, readings were taken of $I_{\parallel}$, $I_{\perp}$, and the temperature (±0.1 °C). The excited-state lifetime was estimated from the total fluorescence intensity as described by Shinitzky et al. [6].

Analysis of fluorescence data

From the experimentally determined values of $I_{\parallel}$ and $I_{\perp}$, values of the anisotropy (r) were calculated. The microviscosity in the neighborhood of the probe molecules was then calculated from the Perrin equation [6, 15].

$$r_0/r = 1 + C(r)T\tau/\eta \quad (1)$$

where r_0 is the limiting value of r at high viscosity (low temperature), T is the absolute temperature, τ is the excited state lifetime, η is the microviscosity, and $C(r)$ is a parameter that depends on the shape of the fluorophore and on r. The function $C(r)$ was determined using solutions of 1,6-diphenylhexatriene in White American Oil as described by Shinitzky et al. [6].

Arrhenius plots of the natural log of the calculated microviscosity vs. $1/T$ were constructed. The data were fitted by a single straight line and showed no indication of phase-transition behavior. The slope of the least-squares fit to the Arrhenius plot was taken to be E_a/R where E_a is the activation energy of the microviscosity and R is the gas constant.

RESULTS

Electron microscopic appearance of subcellular fractions

The electron microscopic appearance of the subcellular fractions we obtained is shown in Fig. 1. The most prominent component of the F_3 fraction (bottom of the

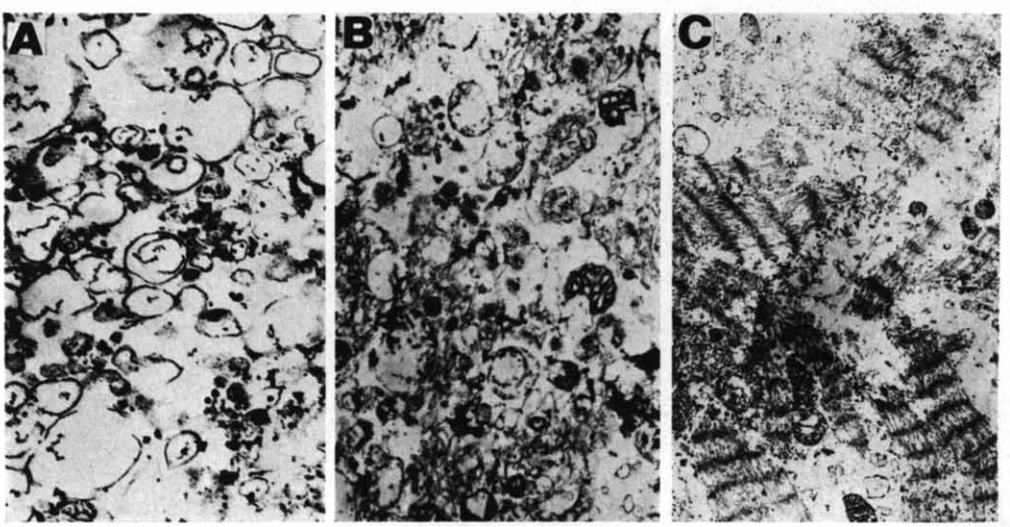

Fig. 1. Electron micrographs of subcellular fractions obtained as described in the text. Panel A: F_1 fraction, 6671 ×; Panel B: F_2 fraction, 2287 ×; Panel C: F_3 fraction, 1255 ×.

centrifuge tube) is contractile protein as seen by the presence of apparently intact sarcomeres. A significant number of mitochondria also occur in F_3. The F_2 fraction (interface between the 25 and 40 % sucrose layers) consists largely of mitochondria and various membrane-bounded vesicles. The F_1 fraction (top of the 25 % sucrose layer) consists almost entirely of membrane-bounded vesicles. The thickened membrane of some is suggestive of the presence of basement membrane in association with plasma membrane vesicles.

Assay of marker enzymes

Table I gives typical values for the specific activities of the marker enzymes in our subcellular fractions from 20-day heart. The activities of 5'-nucleotidase and $(Na^+ + K^+)$-ATPase were consistently enriched in the F_1 fraction. The results with 5'-nucleotidase were more consistent and its relative specific activities in the F_1 fraction were larger than those for $(Na^+ + K^+)$-ATPase.

The activity of succinate dehydrogenase was completely absent from the F_1 fraction and this correlates with the absence of mitochondria in the electron microscope sections of this fraction. The largest amount of succinate dehydrogenase always was present in the F_2 fraction, with a smaller amount (about 20 % of the total) in the F_3 fraction.

The endoplasmic reticulum markers we used, NADH oxidase and nonspecific esterase (*p*-nitrophenylacetate splitting), were consistently enriched about 2–3 fold in the F_2 fraction and the F_1 fraction contained less of these activities than the homogenate.

TABLE I

SPECIFIC ACTIVITIES OF THE VARIOUS MARKER ENZYMES

The figures in parentheses give the specific activity relative to that of the homogenate. Values given (μmol/min/mg protein) are typical for subcellular fractions of heart of 20-day chick embryos.

Fractions	5'-Nucleotidase	$(Na^+ + K^+)$-ATPase	Succinate dehydrogenase	Nonspecific esterase	NADH oxidase
F_1	0.0282 (5.41)	0.201 (3.48)	0 (0)	0.00054 (0.50)	0.0196 (0.69)
F_2	0.0023 (0.44)	0.0705 (1.22)	0.000672 (2.11)	0.00243 (2.75)	0.0729 (2.57)
F_3	0.0021 (0.40)	0.0410 (0.71)	0.000064 (0.20)	0.00104 (0.96)	0.0065 (0.23)
Hgn	0.0052	0.0577	0.000318	0.00108	0.0284

Depolarization of the fluorescence of 1,6-diphenylhexatriene

1,6-diphenylhexatriene is widely used as a fluorescent probe of the lipid bilayer portion of cell membranes and model membrane systems. It is well suited for this function because of its hydrophobicity and because its fluorescence is undetectable in aqueous solution [6]. Control experiments of the type shown in Fig. 2 in which progressively greater amounts of protein were added to a constant concentration of 1,6-diphenylhexatriene showed that the fluorescence anisotropy was essentially

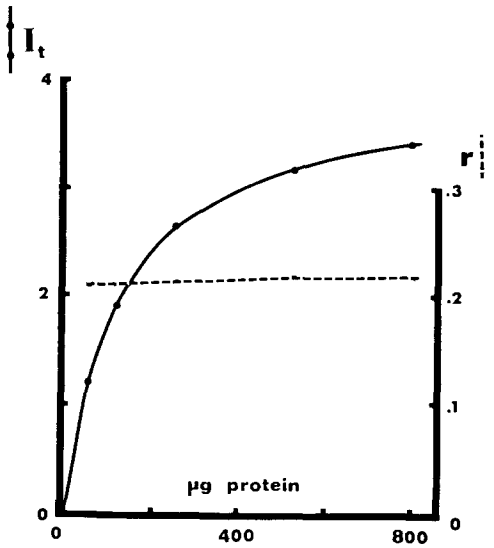

Fig. 2. Results of an experiment in which a progressively greater amount of plasma membrane-enriched fraction was added to 2 nmol 1,6-diphenylhexatriene. Total fluorescence intensity (I_t) and the anisotropy of fluorescence (r) are plotted against the amount of membrane protein added.

constant over the range of protein concentrations used in our experiments. This suggests that even at high ratios of 1,6-diphenylhexatriene to membrane protein, probe-probe interaction is not a significant factor and the probe is not partitioning into hydrophobic regions with different fluidities from that of the hydrophobic domains in which 1,6-diphenylhexatriene exists when the ratio of probe to membrane protein is lower.

Note also that Fig. 2 shows that the total fluorescence intensity continues to rise as membrane protein is added. This suggests that at all levels of membrane protein used, some 1,6-diphenylhexatriene exists in aqueous solution that partitions into the membrane lipid when more membrane is added. This means that we cannot use the technique of assuming that the fluorescence lifetime is proportional to the total fluorescence intensity [6] unless we can rule out the possibility that as the temperature changes the partition of the probe between aqueous solution and membrane lipid also changes. To determine whether the partition of 1,6-diphenylhexatriene between the membrane and free solution changed in our experiments as the temperature was lowered, the following experiment was performed. 100 μl of plasma membrane-enriched fraction (300 μg protein) was mixed with 11 ml water and 5 μl of 1 mM 1,6-diphenylhexatriene in tetrahydrofuran and thoroughly vortexed. The mixture was kept at 37 °C for 30 min and then split into two equal parts. One was kept at 37 °C; the other cooled to 0 °C in an ice bath for 30 min, sedimented at 48 000 × g (bottom of tube) at 0 °C and resuspended in an equal volume of water after the supernatant had been discarded. The total fluorescence intensity of both samples was then monitored at 30 °C. The sample that had been kept at 37 °C had a total fluorescence intensity that was 2.7 % larger than the sample that had been cooled, sedimented, and resuspended. A number of factors (incomplete sedimentation of the cooled mem-

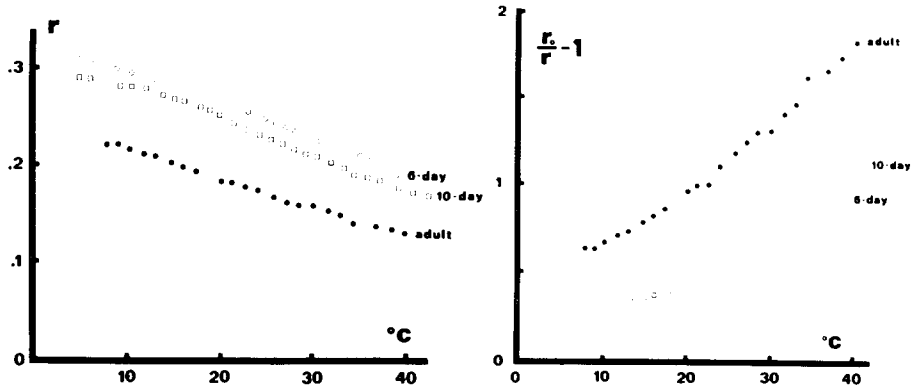

Fig. 3. The results of typical fluorescence depolarization experiments with chicken heart plasma membrane-enriched fraction at various stages of development. The anisotropy (r) is plotted vs. temperature in panel A. In panel B $r/r_0 - 1$ is plotted vs. temperature where r_0 is the limiting value of r at low temperatures.

branes, irreversible aggregation on pelleting, etc.) might account for the minor discrepancy between the two samples. That the calculated microviscosities are not grossly in error due to changes in 1,6-diphenylhexatriene partitioning with temperature is also suggested by the relative constancy of the microviscosities obtained in experiments in which the ratio of 1,6-diphenylhexatriene to membrane protein varied widely. For example, in 7 experiments with 10-day heart membrane fractions the amount of protein varied 12 fold (from 50 to 600 μg of membrane protein). The Arrhenius plots obtained in all these experiments were quite similar (standard errors of the slope and y-intercept values were less than 4 % of their mean values) and the calculated viscosities at 37 °C had a standard error that was less than 6 % of the mean value.

The results of typical experiments with 6-day, 10-day, and adult heart membrane fractions are shown in Fig. 3. Fig. 3a shows that as development proceeds from 6 to 10 days there is a decrease (about 7 %) in the fluorescence anisotropy. Plasma membrane fractions from adult heart show still smaller values of the anisotropy, about 25–30 % less than those of 10-day hearts. Plasma membrane-enriched fractions of hearts from 20-day embryos were also studied and showed anisotropy values slightly larger than, but not statistically different from 10-day heart. As can be seen from Eqn. 1, the microviscosity of the membrane is proportional to the anisotropy. Fig. 3b presents the same data in terms of $r_0/r - 1$, where r_0 is the limiting value of the anisotropy for low temperatures, which is inversely related to the microviscosity.

Fig. 4 shows an Arrhenius plot of the natural logarithm of the calculated microviscosity versus the reciprocal of the absolute temperature calculated from the data of a typical experiment. A single straight line fits the data and there are no regions that appear to represent transitions of the membrane lipids from gel to liquid crystalline states. In all our experiments a single straight line fit the Arrhenius plot data with a correlation coefficient at least as large as 0.98. Using the regression line slope and y-intercept values the apparent microviscosity at any temperature can be estimated.

Fig. 5 is a summary of our data for F_1 subcellular fractions from chick embryo hearts at 5–6, 10, and 20 days of embryonic life and from adult chicken heart. The

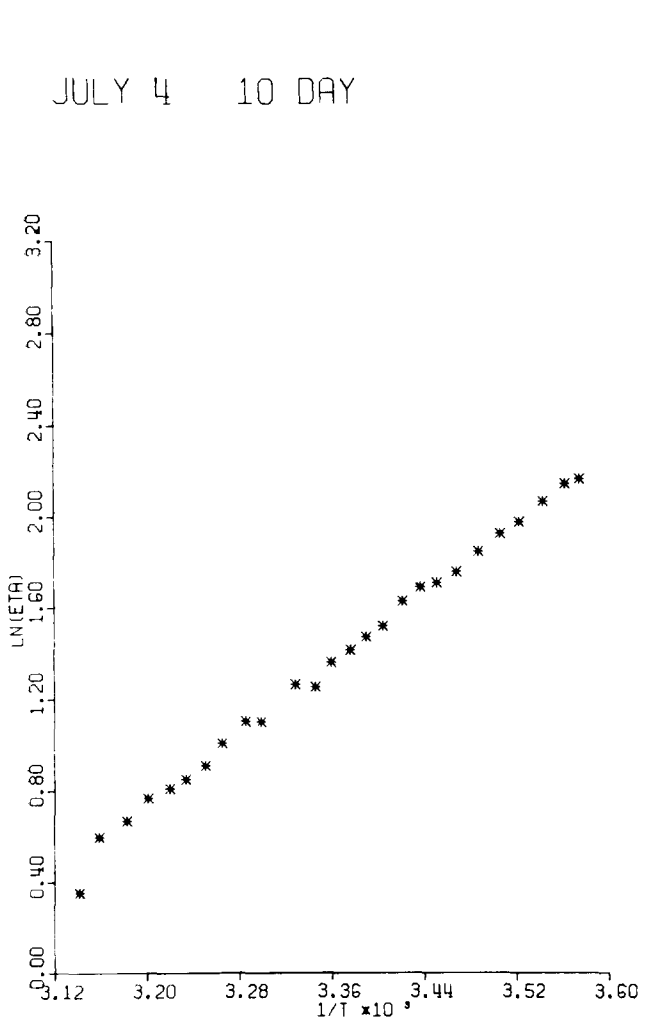

Fig. 4. A typical Arrhenius plot from one of our experiments (in this case with the F_1 fraction of 10-day embryo heart). The natural log of the calculated viscosity is plotted vs. 1000 times the reciprocal of the absolute temperature.

lines were obtained by averaging the values for the slope and y-intercept values obtained from the Arrhenius plots of the data from the individual experiments. Note that as the chicken develops from 5–6 to 10 days there is a marked decrease in the apparent viscosity of the plasma membrane-enriched fraction. Between 10 and 20 days of embryonic life there is a small increase in the apparent viscosity of the F_1 membranes, but this difference is not statistically significant. Between the 20th day of embryonic life (hatching occurs at 21 days) and adulthood there is another large decrease in the apparent microviscosity of the plasma membrane-enriched fraction.

Table II shows some of the data in tabular form. Note that as development proceeds there is a steady decrease in the apparent activation energy of the microviscosity. Values of the apparent viscosity at 25 and 37 °C, calculated from the average regression lines in Fig. 5 are shown for comparison. Note that between 5 and 6 days

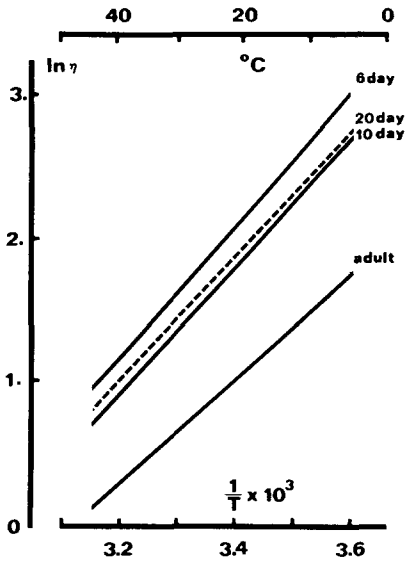

Fig. 5. Average Arrhenius plots obtained by averaging the slopes and intercepts of the plots obtained in the individual experiments for F_1 fractions of heart at various stages of development.

TABLE II

FLUORESCENCE DEPOLARIZATION DATA FOR PLASMA MEMBRANE-ENRICHED FRACTIONS

The values of fluorescence anisotropy (r) at 25 and 37 °C were calculated directly from experimentally determined values of $I_{\parallel}$ and $I_{\perp}$. The values of the activation energy (E_a) of the viscosity and the viscosity (η) were determined from the average Arrhenius plot of $\ln(\eta)$ vs. $1/T$ for all the experiments in a given age group. The statistical significance of differences between mean values was determined by the Student's t-test.

Age	No. of experiments	$r(25) \pm$S.E.	$r(37) \pm$S.E.	$\eta(25)$ (poise)	$\eta(37)$ (poise)	E_a (kcal/mol)
5–6-day embryo	4	0.2431 ± 0.0032	0.1980 ± 0.0047	6.43	3.52	9.16
10-day embryo	7	0.2297 ± 0.0061*	0.1870 ± 0.0053*	5.06	2.82	8.90
20-day embryo	5	0.2345 ± 0.0048**	0.1930 ± 0.0043**	5.52	3.18	8.40
Adult	5	0.1709 ± 0.0051***	0.1369 ± 0.0044***	2.34	1.44	7.36

* Different from the value immediately above it with $P = 0.05$.
** Not significantly different from the value immediately above it.
*** Different from the value immediately above it with $P = 0.001$.

of embryonic life and the adult state there is greater than 50 % decrease in the apparent microviscosity of the plasma membrane-enriched fraction of the heart.

DISCUSSION

The values we obtained for the viscosity of the membranes of the plasma membrane-enriched fraction of adult chicken heart are not markedly different from

the viscosities of rat and mouse lymphocytes [14] or human erythrocytes [15]. The viscosities of the plasma membrane-enriched fractions of chick embryo hearts, however, are almost 3-times greater than for adult heart. The significance of the elevated plasma membrane viscosity of the embryo is obscure at present, but raises questions of whether the viscosity of the membranes of other cellular organelles of chick embryo heart are also elevated and whether the plasma membranes of other organs in the embryo also have highly viscous membranes.

As was mentioned in the Introduction the rate at which chicken embryo heart cells take up sugars, amino acids, and urea decreases as development proceeds. It is possible that the changes in the fluidity of the plasma membrane lipid bilayer matrix play a role in regulating the metabolism by the embryo of substances whose utilization is limited by the rate of membrane transport or by regulating the activity of certain key membrane-bound enzymes as has been suggested for the $(Na^+ + K^+)$-ATPase [16]. The fact that changes in urea and sugar transport do not correlate perfectly with changes in membrane fluidity in chick embryo heart (transport decreases between 10 and 20 days in the absence of a change in fluidity) suggests that, if changes in fluidity contribute to transport changes during embryonic development, other factors must also be involved in the regulation of transport rates.

We have demonstrated that marked changes in fluidity of the lipid bilayer matrix of the plasma membrane of chicken heart cells occur during the course of development from 5–6 day embryo to adult chicken. Previous work in our laboratory has shown that as development proceeds from 5–6 to 20 days of embryonic life there is a progressive increase in the unsaturation of the fatty acids of the plasma membrane enriched fraction [17]. We believe that the increasing unsaturation of plasma membrane fatty acids contributes to the increased fluidity during development. Other factors that may play a role include the membrane concentrations of cholesterol and sphingomyelin and the protein composition of the plasma membrane.

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