The degree of development of hypertrophy of the rat adrenal gland varies considerably depending both on the animals' age and the time elapsing after the operation. It is interesting to note that instability of development of compensatory hypertrophy also was observed in relation to ovaries of infantile golden hamsters [1] and the kidney in young rats [2]. A more penetrating analysis of the character of compensatory hypertrophy of the adrenal in the early postnatal period will be the subject of future research.

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SURFACE ACTIVITY OF PLASMA MEMBRANES

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Plasma membranes (PM) isolated from smooth-muscle tissues are vesicular formations [4, 6, 9]. They are characterized by activity of various enzymes connected with ATP hydrolysis and regulation of the ionic composition of the cytoplasm. However, direct experimental investigation of the functions of ion-transporting enzymes incorporated into the structure of PM is difficult. Attempts to insert such enzymes into flat lipid membranes have not yielded consistently reproducible results [5].

In the investigation described below surface activity of membrane preparations and their interaction with phospholipid monolayers, simulating the surface of the bilayer, were studied.

EXPERIMENTAL METHOD

PM were isolated from smooth-muscle tissue of the rabbit small intestine by differential centrifugation in a sucrose density gradient and were characterized electron-microscopically and biochemically as described previously [4]. The level of ATPase activity of PM was verified by a potentiometric method [1, 2]. The PM preparation was kept in medium containing 25% glycerol (by volume), 0.1 mM EDTA, 0.2 mM $CaCl_2$, and 5 mM imidazole, pH 7.2, at 0-4°C. ATPase activity persisted for about 1 month under these conditions.

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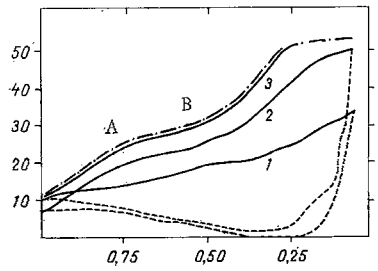

Fig. 1

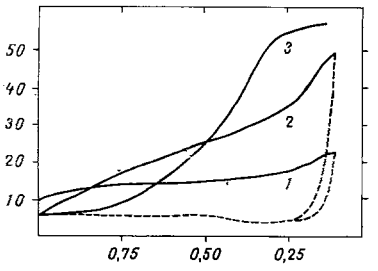

Fig. 2

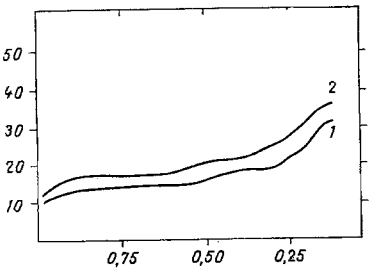

Fig. 3

Fig. 1. Cyclic compression isotherms of PM monolayer obtained as a result of injection of vesicles into subphase (line of dots and dashes) and monolayer formed by application of vesicles directly to surface of electrolyte-air phase boundary (continuous line). 1, 2, 3) Sequence of cycles. Velocity of movement of barrier 5.2 cm/min. Abscissa, ratio S/S_0; ordinate, $\Delta\sigma$ (in dynes/cm).

Fig. 2. Cyclic compression isotherms of monolayers from total fraction of PM phospholipids. 1) Velocity of compression 5.2 cm/min, 2) 13 cm/min, 3) 39 cm/min. Remainder of legend as to Fig. 1.

Fig. 3. Cyclic compression isotherms of original lecithin monolayer (1) and of lipoprotein monolayer (2) obtained by adsorption of PM injected into subphase beneath lecithin monolayer. Velocity of compression 5.2 cm/min. Remainder of legend as to Fig. 1.

The surface activity of PM and their interaction with phospholipid monolayers were investigated at 18°C on the phase partition boundary between an aqueous solution of electrolyte and air [8]. The monolayer was formed in a Teflon cuvette measuring 385 × 140 × 12 mm. An electrolyte of the following composition was used as subphase: 5 mM Tris-HCl, 10 mM KCl, pH 7.2. The lipids chosen for testing were ovolecithin, synthetic dipalmitoyllecithin and dioleyllecithin (from Koch Light, England), and also the total phospholipid fraction obtained by ourselves from the PM preparation by the method of Kates [3].

Monolayers were formed by two methods: by application of a suspension of vesicles (1 mg protein) directly to the surface of the subphase or by introduction of the same quantity of the preparation into a volume of electrolyte so that its direct contact with the phase partition boundary between lipid and air was excluded. Phospholipid monolayers were formed by application of portions of a solution of lecithin in hexane (0.1 mg/ml) to the surface of the subphase until the assigned packing density of the molecules in the monolayer was achieved. Insertion of the membrane preparations into the phospholipid monolayers was judged by the change in surface tension and the boundary potential jump in response to injection of PM into the subphase. Surface tension was measured by means of a half-immersed platinum disk (perimeter 9 cm) connected to a KhM-6 mechanotron [7]. The sensitivity of the measuring system was 0.1 dyn/cm. The boundary jump of potential was recorded with a dynamic capacitor [8]. The resolution of the instrument was 2 mV.

EXPERIMENTAL RESULTS

Lipid vesicles are known to possess surface activity [10] and to be adsorbed on air–water phase boundaries. Under these circumstances their spherical structure is disturbed and the bilayer membranes spread out along the interphase with the formation of monolayers. A similar approach was used to study vesicular preparations of PM of smooth-muscle cells. After injection of membranous vesicles, which also possess surface activity and are adsorbed on electrolyte-air phase boundaries, into the subphases a steady-state of adsorption was established in the course of 4-6 h. The surface tension decreased under these circumstances by 5-6 dynes/cm, and the boundary potential drop changed by about 180 mV relative to its initial level (positive in air). A similar result was achieved when the preparation was applied directly to the surface of the subphase. In this case the PM quickly flowed over the partition surface. The potential jump as a result changed by 180-200 mV and the surface tension fell by 3-25 dynes/cm depending on the number of PM falling on the interphase. In all cases adsorption ended with filling of the surface of the subphase with material of the membranes and the formation of densely packed monolayers with closely similar values of charge density in them and with identical orientation of the molecules on the phase boundary.

Significant differences were observed in the kinetics of formation of the steady state during adsorption of vesicles injected into the subphase and applied to its surface. In monolayers formed by the first method, consistently reproducible changes of two-dimensional pressure were recorded during their cyclic compression and

TABLE 1. Numerical Values of Changes in Surface Tension $\Delta\sigma$ and Boundary Potential $\Delta\psi$ of Lecithin Monolayers after Injection of PM into Subphase

Type of monolayer	Initial $\Delta\sigma$, dynes/cm	$\Delta\sigma$ of reaction, dynes/cm	$\Delta\psi$ of reaction, mV
Ovolecithin	3,5	16,8	59
	13,7	10,8	153
	32,4	4,1	12
Dioleyllecithin	2,7	6,5	24
	11,0	4,2	21
Dipalmitoyllecithin	3,9	9,4	14
	10,0	6,1	22

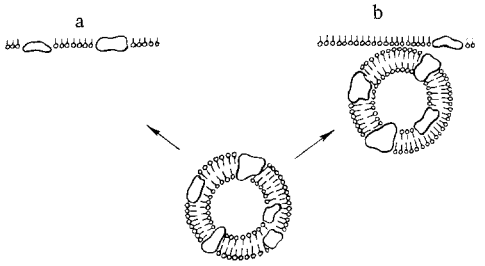

Fig. 4. Scheme of adsorption of PM on phase boundary.

expansion (Fig. 1). In monolayers obtained by the second method the course of the isotherms changed from its initial type (curve 1) to the steady-state type (curve 3) in the course of the cycles, and at the 3rd-5th cycle the course of these isotherms began to coincide with those of compression of the monolayers formed by the first method. The transition from the first compression cycle to the next, with equal ratios between areas (S/S_0) was accompanied not by a decrease in two-dimensional pressure or by its constancy, as is recorded in "classical" monolayers, but by an increase. In other words, membranes of the vesicles, when falling on the surface, are destroyed during compression by the barrier and the lipoprotein complexes are organized into a true monolayer, which requires a greater area than that of the original vesicles.

On injection of PM directly into the subphase adsorption of vesicles was accompanied by their destruction, and no transformation in the course of the compression isotherms of the monolayers was thus observed. The lipoprotein monolayer differed not only from the vesicular layer (Fig. 1), but also from the monolayer of phospholipids extracted from PM (Fig. 2). Under equal conditions of compression the phospholipid monolayer was more elastic and more soluble in the subphase than the lipoprotein monolayer. The modulus of elasticity ($\Delta\sigma/\Delta S$) and the two-dimensional pressure in it ($\Delta\sigma$) in different stages of contractions of the area were always less than for the lipoprotein monolayer. Collapse in a phospholipid monolayer was recorded only if the velocity of compression exceeded 13 cm/min. The pressures at the beginning of collapse coincided in both cases, but no intermediate plateau AB (Fig. 1) characteristic of the lipoprotein monolayer was observed on the compression isotherm of the phospholipid monolayer, indicating structural changes in the latter. Probably under these circumstances the protein underwent denaturation.

If the surface of the electrolyte was filled with the lipid monolayer, adsorption of vesicles took place much faster than on a free surface, and was accompanied by a change in the two-dimensional pressure and the surface potential. Steady-state adsorption was established in the course of 60-120 min. The lower time level corresponds to a high packing density of the monolayers (about 30 dynes/cm), the upper to a loosely packed state (3-5 dynes/cm). It will be clear from Table 1 that, irrespective of the nature of the phospholipids in the monolayer, injection of the PM preparations into the subphase caused an increase in the two-dimensional pressure and a decrease in the boundary potential. The maximal change in these parameters was recorded on monolayers of ovolecithin, whereas the minimal effect was observed on monolayers from synthetic lipids. With an increase in the initial packing density of the molecules, the rise of pressure and the fall of boundary potential were exhibited to a lesser degree. Hence it follows that adsorption of PM vesicles on phospholipid monolayers

leads to penetration of their substance and its accumulation in the plane of the polar groups bordering the subphase. Clearly, with higher values of two-dimensional pressure, less of the substance of PM will penetrate into the monolayer.

Compression isotherms of a lecithin monolayer with initial two-dimensional pressure of 10 dynes/cm and of this same monolayer after injection of the membrane preparation into the subphase are given in Fig. 3. It will be clear from Fig. 3 that, despite displacement of the "zero" level of two-dimensional pressure in the lipoprotein monolayer from this same level into the phospholipid monolayer, the course of the compression isotherm of the first monolayer remains similar to the compression isotherm of the latter. On the whole, the curve is only displaced upward relative to the previous one by a constant value. This indicates the appearance of an adsorption layer, consisting of vesicles, in the region of the polar groups of the phospholipids. The vesicular layer increases the kinetic barrier for solution of lipid and protein molecules from the outer monolayer, in turn the phospholipids in the outer monolayer prevent denaturation of the protein penetrating into it. The following scheme of adsorption of PM on a pure surface of an electrolyte and adsorption in the presence of lipid monolayers can be put forward on the basis of these results (Fig. 4).

The greater part of the lipids in the membranes is evidently bound with proteins. During adsorption of vesicles on the clean surface a monolayer is formed and this is accompanied by a gain of energy and by denaturation of the protein molecules (Fig. 4a). If a phospholipid monolayer already exists on the phase boundary, molecules of lipids and proteins will flow from the vesicles into the monolayer. On completion of the adsorption process the excess of vesicles is concentrated in the zone of polar groups of the lipoprotein monolayer in the native state (Fig. 4b). Under these circumstances the presence of lipid on the surface impedes protein denaturation.

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