RESULTS AND DISCUSSION

Echinocytosis is an anomaly in size of erythrocytes, physically caused by inclusion of additional lipid in the outer leaflet of the cell membrane (Fig. 1a). The outer leaflet has a greater surface area than the inside thus inducing outgrowths. The action can be induced artificially by incubation of live red blood cells in an energy- lacking environment or with nonionic detergent solution.

Echinocytosis might be a symptom of some severe diseases. Most of them are connected with liver disabilities or misdiagnosed blood disorders and other problems. Echinocytes are created (naturally occurring RBC) to echinocytes created in the laboratory by using detergents. Selective isolation of RBC from whole blood was achieved by using polylysine coated slides. This treatment makes the slides positively charged thereby attracting negatively charged erythrocytes.

METHODS

Two sets of experiments were performed. In both of them the sample of blood (200 µl) was collected from a 24 y.o. female and mixed with anticoagulant (15% Na2HPO4 solution). Echinocytosis was induced by 2.6 mM KC1, 15.4 mM Na2HPO4, and 1.77 mM KH2PO4 pH 7.4 – solutions in human blood. Fixation was performed as in Fig. 2.

Glass slides were covered with polylysine to enable selective isolation of RBC (Tyler et al., 2000). One of the slides was designated for ESEM (stored at 4ºC). Two others were dehydrated with the use of critical point drying or chemical drying with hexamethyldisilazane. Spurr coating was also performed on dehydrated specimens. The specimens were observed in a Quanta 200-SEM in ESEM mode as well as in normal high-vacuum mode. In ESEM 2 degrees C and 1 Torr chamber pressure were selected for a relative humidity of 95%. This ensured the specimen remained hydrated during SEM examination. In regular mode 20 KV and spot size 4.0 were used. Image J was used to take measurements. Photoshop CS4 enabled us to adjust pictures for optimal contrast and color.

LITERATURE CITED