dition, in some cases instead of perfusion, nutrient substances were injected once only into different parts of the gastrointestinal tract.

This abdominal preparation, consisting of an alimentary complex including stomach, duodenum, small and large intestines, and pancreas, can thus be used to study many problems in normal and pathological physiology, pharmacology, and in biology as a whole.

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PREPARATION OF LIPOSOMES BY REVERSE-PHASE EVAPORATION AND BY FREEZING AND THAWING

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KEY WORDS: liposomes, diethylenetriaminopenta-acetic acid (DPTA).

Preparation of liposomes containing large quantities of drugs is an essential condition for their use as a method of transporting substances into the cells and of achieving a marked

TABLE 1. Dependence of Incorporation of [^{14}C]DTPA into Liposomes on Method of Forming Suspension

Type of liposomes according to method of obtention	Incorporation of [^{14}C]-DTPA, %	Volume of aqueous phase in liposomes, μl/μmole phosphatidylcholine
[^{14}C]-DTPA added to suspension of liposomes	<0,01	—
"Bangham" liposomes	19,3±1,5 (100%)	3,78±0,29
"Bangham" liposomes frozen and thawed 10 times	22,9±1.4 (118%)	4,48±0,27
[^{14}C]-DTPA coprecipitated with lipids before addition of aqueous phase	23,5±4,0 (122%)	4,62±0,78
Liposomes obtained with reverse-phase evaporation and freezing and thawing	54,6±4,4 (281%)	10,6±1,3

Legend. Liposomes formed with phosphatidylcholine and cholesterol each in a concentration of 54 mM.

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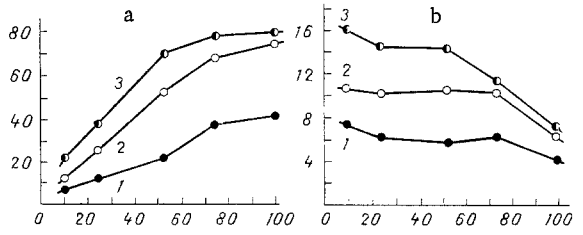

Fig. 1. Content of [^{14}C]DTPA (a) and relative volume of aqueous phase (b) in liposomes. Abscissa, phosphatidylcholine concentration (in mM); ordinate: a) content of [^{14}C]DTPA in solution (in %); b) μl/μmole phosphatidylcholine; 1) "Bangham" liposomes (ratio phosphatidylcholine/cholesterol 1 mole:1 mole), formed with reverse-phase evaporation and freezing and thawing; 3) liposomes (phosphatidylcholine/cholesterol/stearic acid 1 mole: 1 mole: 0.15 mole), formed with reverse-phase evaporation and freezing and thawing.

therapeutic effect. The use of laminated "Bangham" liposomes [3] for this purpose cannot be justified because they incorporate a comparatively small volume of the aqueous phase (0.2-4 μl/μmole phospholipids) and, for that reason, they incorporated a very small quantity of hydrophilic compounds [3, 5, 8, 9]. Moreover the structure of "Bangham" liposomes is very heterogeneous, and they vary in size from 0.5 to 10 μ [2, 3]. Ultrasonic treatment of "Bangham" liposomes enables single lamella liposomes under 1 μ in diameter to be obtained, but the content of substances in single lamella liposomes is reduced by more than 10 times [5, 7]. Among known methods of obtaining liposomes [4, 7] the most successful is their formation by reverse-phase evaporation [9], followed by their extrusion through polycarbonate filters [6]. As a result, liposomes with a volume of aqueous phase of up to 10-12 μl/μmole phospholipids are formed [6, 9]. However, in the original method, during phase reversal, lipids and encapsulated material of organic solvents (ether, chloroform, etc.) are exposed at the same time to ultrasound [6]. Treatment with ultrasound is known to be accompanied by chemical degradation of lipids as a result of the development of peroxidation of the lipids and hydrolysis of covalent bonds [2], and combining treatment with both factors may lead to inactivation or destruction of the therapeutic preparations. Experience gained during my research has shown that in some cases, in particular if the concentration of phospholipids exceeds 20-30 mg/ml, after phase reversal the system remains for a long time in the amorphous gel state, and hardly any is transformed spontaneously into a suspension of liposomes. Such transformation takes place after several cycles of freezing and thawing. Freezing and thawing are usually used to reduce the size of Bangham liposomes, but by contrast with ultrasonic treatment, freezing and thawing is not accompanied by destruction of lipids [2].

In the present investigation the characteristics of liposomes obtained by a modified method with reverse-phase evaporation and freezing and thawing were determined, and in addition, incorporation of a water-soluble complexone, namely the trisodium-calcium salt of diethylenetriaminopenta-[2-^{14}C]-acetic acid ([^{14}C]-DPTA), which is used to eliminate certain highly toxic radionuclides from the body, into liposomes of different types were compared.

EXPERIMENTAL METHOD

Liposomes were formed from an equimolar mixture of chromatographically pure egg phosphatidylcholine and cholesterol in a 75-mM solution of Na_3Ca[^{14}C]-DPTA. Bangham liposomes were obtained by mechanical dispersion of a dry film of lipids in a solution of DTPA [3]. The reverse phase evaporation was carried out by addition of an equal volume of diethyl ether to the suspension of Bangham liposomes, and evaporating the ether *in vacuo* after brief (5-10 min) vigorous shaking. The system thus obtained was quickly frozen in liquid nitrogen and thawed in hot water [2]. The freezing and thawing cycle was repeated 10 times. To determine incorporation of [^{14}C]-DTPA into the liposomes, the suspension was filtered through "Synpor" membrane filters (from Chemapol, Czechoslovakia) with a pore size of 0.25 μ (rate of filtration not more than 1 ml/min), the filter was washed with 5 ml of a 300-mM solution of glucose, and activity of the filter was measured in 5 ml of Bray's scintillator. For light microscopy the liposomes were stained by the addition of 1-anilinonaphthalene-8-sulfonate (from Serva, West Germany) to the suspension up to a concentration of 10^{-6} M. The investigation was carried out in a special quartz cuvette 30 μ deep. For electron microscopy the liposomes were stained with phosphotungstic acid (from Serva) [1].

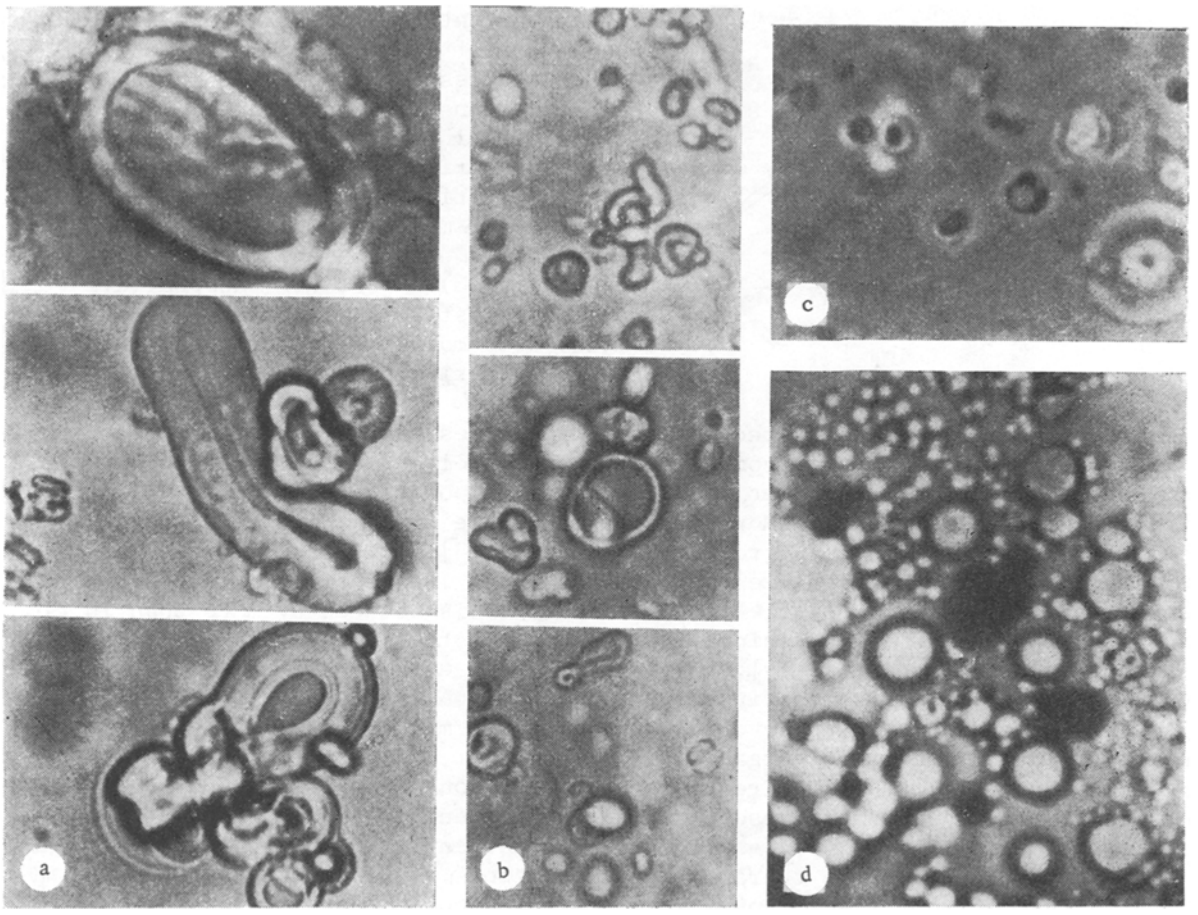

Fig. 2. Ultrastructure of different types of liposomes: a) "Bangham" liposomes; b) "Bangham" liposomes frozen and thawed 10 times; c, d) liposomes formed by reverse-phase evaporation and freezing and thawing. a, b, d) Light microscopy, 1500 ×; d) electron microscopy, negative staining, 10,000 ×.

EXPERIMENTAL RESULTS

Comparison of incorporation of [^{14}C]-DTPA into the different types of liposomes showed that liposomes formed by the modified method with reverse-phase evaporation and freezing and thawing contained twice to three times as much of the complexone as other types of lipids, and that liposomes with a negative surface charge (with stearic acid) contained 20-25% more than neutral liposomes (Fig. 1a). The relative volume of the aqueous phase in the liposomes obtained was 10-14 µl/1 µmole phosphatidylcholine. By increasing the concentration of lipids during formation of the suspension to 50-70 millimoles, it was possible to incorporate up to 70-80% of the [^{14}C]-DPTA present in the solution into the liposomes (Fig. 1a), and under these circumstances the relative volume of the aqueous phase in the liposomes was virtually unchanged (Fig. 1b). It is evident that the vesicles in the suspensions differed very little in size and number of bimolecular layers of lipid in the membrane. During the formation of "denser" suspension, however, incorporation of [^{14}C]-DTPA increased only insignificantly (Fig. 1a), whereas the relative volume of aqueous phase decreased (Fig. 1b). Most likely of all, as a result of interaction of liposomes with each other, their structure changed, probably on account of an increase in the number of layers of lipids in the liposome membrane.

During light microscopy the "Bangham" liposomes appeared as structures of different shapes, consisting of a thick lipid membrane and a relatively small intraliposomal space (Fig. 2a). The structure of the liposomes remained the same after freezing and thawing, although their size was greatly reduced (Fig. 2b). Liposomes formed with reverse-phase evaporation and freezing and thawing were practically indistinguishable in the light microscope (Fig. 2c), and only a few of the largest vesicles were visible. The results of electron microscopy show (Fig. 2d) that these liposomes varied in size for 0.2 to 1.5 µ, and they were surrounded by a thin lipid membrane, probably of one or two layers.

The cholesterol molecule is known to occupy an area of 38 A^2, the phosphatidylcholine molecule in the presence of cholesterol an area of 58 A^2, and according to the equation D = 3V/S, where V is the total encepsulated volume and S the total surface area of lipid [9], it can be calculated that the mean diameter (D) of single lamella liposomes is 450-540 A, in good agreement with the results of the microscopic investigation.

Ultrasonic treatment is thus not essential for reverse-phase evaporation. The freezing and thawing procedure facilitates transformation of the amorphous gel-like system formed with high concentrations of lipids after phase reversal into a suspension of liposomes 0.2-1.5 μ in diameter, containing 10-14 μl of aqueous phase/μmole phospholipids. By increasing the lipid concentration, up to 70-80% of the total volume of the aqueous phase can be incorporated into liposomes. Since incorporation of hydrophilic compounds into liposomes is largely determined by the encapsulated volume, this method can be recommended for the production of liposomal forms of other therapeutic preparations.

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INEQUALITY OF LUNG VENTILATION DETERMINED BY TRANSCUTANEOUS MEASUREMENT OF pO_2 IN ARTERIAL BLOOD DURING OXYGEN INHALATION

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KEY WORDS: transcutaneous partial pressure of oxygen, inequality of ventilation, lungs.

The simplest and most accessible method of discovering inequality of lung ventilation is oxyhemometric determination of the oxygen desaturation time of arterial blood after inhalation of oxygen [1, 3, 8, 9]. However, by this method adequately clear results can be obtained only in patients in whom the oxygen saturation of the arterial blood is depressed during inhalation of air [9]. The practical value of the parameter studied in that case (the blood desaturation time) is considered to be doubtful because of the great variability and poor reproducibility of this parameter [7, 8]. A more informative parameter during inhalation of pure oxygen is the partial pressure of oxygen in arterial blood (p_aO_2). However, dynamic and continuous measurement of p_aO_2 is fraught with technical difficulties (catheterization of arteries, the need to use special catheter electrodes, and so on). For continuous determination of p_aO_2 the writers have used the method of transcutaneous determination of the partial pressure of oxygen in arterial blood (Tcp_aO_2), first suggested by Huch et al. [11]. The degree of inequality of lung ventilation was estimated from the time of lowering of p_aO_2 (blood desaturation time) after inhalation of oxygen.

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