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EFFECT OF LIPOSOMES CONTAINING CHOLESTEROL ON ADENYLATE CYCLASE ACTIVITY OF CULTURED MAMMALIAN FIBROBLASTS

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Summary

Liposomes prepared with cholesterol and dipalmitoyl phosphatidylcholine were incubated with a clone of normal rat kidney fibroblast of cells in culture. The cells took up [^{14}C]cholesterol in proportion to the concentration of liposomes in the incubation medium, and the uptake increased with time over the four hours of study. Two cell membrane enzymes, adenylate cyclase and ($Na^+ + K^+$)-ATPase, exhibited decreased activity after treatment with cholesterol-containing liposomes. The decrease in adenylate cyclase activity was directly proportional to the uptake of [^{14}C]cholesterol. When a variety of subclones of NRK 5W were examined some were found to respond to cholesterol treatment and some did not. These data are consistent with the view that membrane cholesterol content plays a role in controlling the activity of some plasma membrane enzymes.

Introduction

Much recent evidence demonstrates the importance of lipids in the control of the activity of membrane enzymes. Specific phospholipids and cholesterol have been shown to play a role in modulating the activity of a number of different membrane enzymes from various tissues (see reviews, refs. 1—3).

Although the role of lipids in maintaining enzyme activity is just beginning to be defined, it is clear that within membranes lipids provide a fluid matric into which proteins insert [4]. It has recently been shown that alterations of the cholesterol content of such membranes can change the fluid nature of these membranes [5] and perhaps exert a constraint upon enzyme activity [3].

Liposomes prepared by sonication of purified phospholipids and cholesterol can fuse with cells in culture [6—9] and alter the lipid composition of these cells by as much as 10—15% [9]. With this technique specific lipids can be introduced into cultured cells in a relatively short period of time. We have

undertaken to determine if interaction of various fibroblasts lines in cell culture with liposomes of known composition is capable of altering the activity of adenylate cyclase.

We have focused on the enzyme adenylate cyclase and the effect of cholesterol-containing lipid vesicles upon its activity since (a) the product of adenylate cyclase, cyclic AMP has been demonstrated to exert an important role in the control of the behavior of cells in culture [10] and (b) changes in cholesterol content have been postulated as an explanation for some of the plasma membane alterations seen in transformed cell lines [11,12].

Materials and Methods

Cholesterol, ergosterol, egg-yolk lecithin, and dihydrocortisone were obtained from Sigma; dipalmitoyl phosphatidylcholine from Grand Island Biological and Silica Gel Q1 plates from Quantum Industries. Polystyrene latex beads (1.1 μm, Dow), were a gift from Dr. E. Korn. Zymosan particles from Nutritional Biochemicals. [α-^{32}P]ATP and cyclic-[^{3}H]AMP from New England Nuclear.

Growth of cells

Normal rat kidney cells were obtained from Dr. E. Scolnick (NIH) and subcloned in our laboratory. They were usually grown on 60 mm plastic Falcon dishes in Dulbecco-Vogt modified Eagle's medium with 10% calf serum at 37°C in a 5% CO_2 humidified atmosphere. Cells were planted at $5 \cdot 10^3$ cells/cm^2; the medium was changed every 48 h, and 24 h before utilization. Density at harvest ranged from $5 \cdot 10^4$ to $1 \cdot 10^5$ cells/cm^2. Six subclones of kidney cells referred to as 5W, S, A, C, G, and K, as well as a line of kidney cells transformed with Kirsten sarcoma virus were employed for the current studies. Cells were counted with a Coulter cell counter after removal by trypsinization.

Liposomal preparation

Liposomes were prepared by the method of Cooper et al. [5] with the following modification: the appropriate phospholipid, 40 mg of either egg yolk lecithin or dipalmitoyl phosphatidylcholine was dissolved in $CHCl_3$ to which 20 mg cholesterol was added (1 : 1, cholesterol : phospholipid, w/w); or 80 mg cholesterol (2 : 1, cholesterol : phospholipid, w/w) with 2 μCi [^{14}]cholesterol. The mixture was dried under a stream of N_2. To the dried mixture, 10 ml of 100 mM NaCl/15 mM KCl/0.1 mM EDTA/10 mM dextrose (pH 7.4) was added and then sonicated with a Model 185 sonicator (Heat Systems, Inc. Plainview, N.Y.) under N_2 for 1 h at 60 W. The mixture was centrifuged at 32 000 $\times g$ for 20 min to remove unsuspended lipid. Temperature throughout was maintained at 3°C for egg yolk lecithin mixtures and between 35 and 45°C for dipalmitoyl phosphatidylcholine. Aliquots of the supernatant were then removed for assay of phospholipid phosphorus by the method of Bartlett [13], cholesterol by the method of Rudell and Morris [14] and for determination of radioactivity. Where indicated, liposomes without cholesterol (phospholipid alone) or with 80 mg of other steroids (ergosterol or hydrocortisone) were similarly prepared. Liposomal preparations were extracted with chloroform/methanol (2 : 1, v/v)

and the extract chromatographed on Silica gel G in a solvent system of chloroform/methanol/acetic acid/water (20 : 12 : 3 : 1, v/v) with appropriate standards. The lipid regions were identified with iodine vapor, removed and the content of lipid phosphorus [13] and radioactivity determined. More than 95% of the radioactivity ran with the cholesterol standards, and more than 96% of the phospholipid phosphorus chromatographed with the standard phospholipid.

The liposomes were analyzed by chromatography on a Sepharose 4B column (Fig. 1). Phospholipid vesicles composed of egg yolk lecithin eluted in a homogenous profile at about 50% of the column volume, whereas 2 : 1, cholesterol : phospholipid liposomes exhibited a more heterogenous elution profile, with most of the lipid appearing in the void volume. Membrane filtration of the cholesterol-containing liposomes was performed on 0.45 μm filters. Electron micrographs of cholesterol-containing liposomes stained negatively with uranyl acetate showed a heterogenous population of vesicles of sizes varying from 300 Å to 4500 Å (Fig. 2).

$CaCl_2$ was added to the liposomal preparations to a final concentration of 2 mM just prior to use and the concentration of lipid adjusted to the concentration noted in the text with Ca^{2+}-containing sonication buffer. Lipid vesicle concentration is expressed either as the concentration of phospholipid for liposomes composed only of phospholipid, or as the concentration of cholesterol in a suspension of cholesterol-containing liposomes.

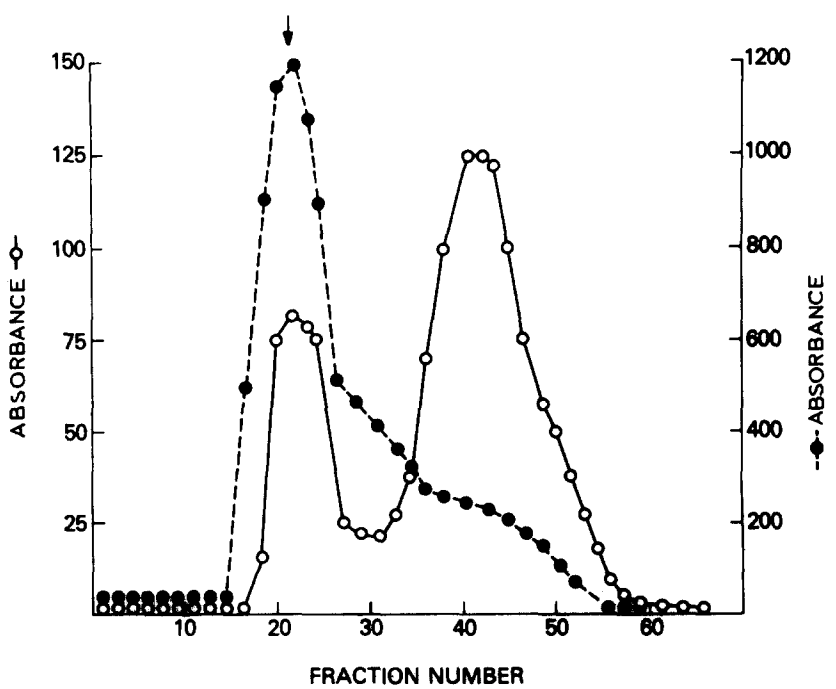

Fig. 1. Sepharose 4B chromatography of liposomes containing egg yolk lecithin alone (○) or cholesterol and egg yolk lecithin at a molar ratio of 2 : 1 (●). Arrow indicates column void volume as determined with dextran blue.

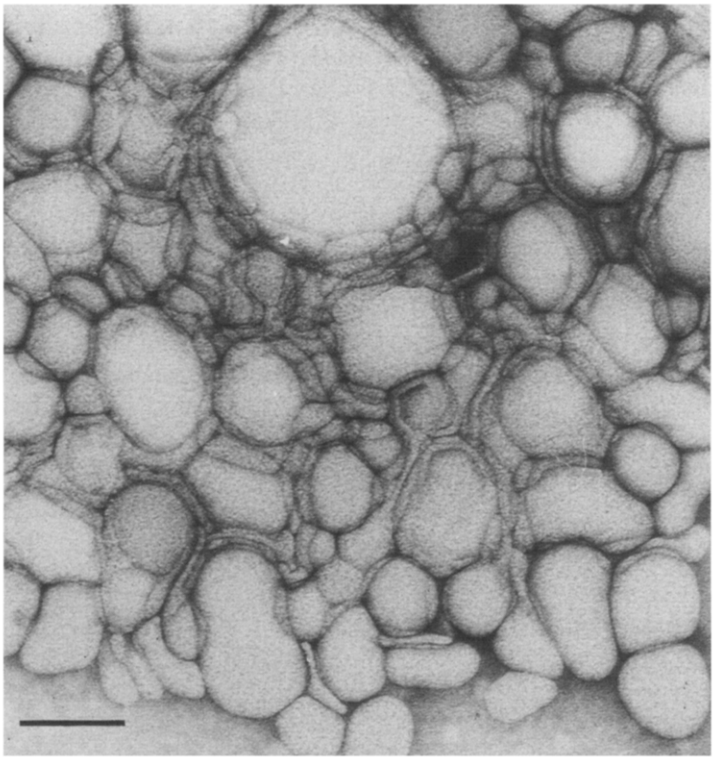

Fig. 2. Liposomes of 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine negatively stained with uranyl acetate. Magnification X 128000; bar is 1000 Å.

Treatment of cells

Prior to treatment, cells were washed 3 times with 5 ml of the sonication buffer. Then 3 ml of either the liposome suspension in sonication buffer or sonication buffer alone was added to the cells and incubated with them for 4 h at 37°C unless otherwise noted. The cells were harvested by scraping with a rubber policeman and washed twice with 3 ml of the sonication buffer by centrifugation at 600 × *g* for 10 min at 23°C. Cells used for adenylate cyclase assays or other enzyme assays were then suspended in 0.25 M sucrose/10 mM Tris · Cl (pH 7.7) and homogenized in a tight-fitting Dounce homogenizer. An aliquot of this homogenate was assayed for protein by the method of Lowry et al. [15]. Cells used for measurement of [^{14}C]cholesterol uptake were washed twice more, dissolved in 250 μl of 10% sodium dodecyl sulfate (SDS) and counted in Aquasol. Total cell number was obtained by counting a nontreated companion dish. Cholesterol uptake is expressed as molecules of cholesterol/cell. Control cells were incubated in control buffer, but a liposomal suspension was added just prior to harvesting. The addition of liposomes did not affect the amount of protein recovered from the culture dishes.

Enzyme assay

Adenylate cyclase activity was assayed by measuring the conversion of [α-^{32}P]ATP to cyclic-[^{32}P]AMP as previously described [16]. The incubation

mixture contained 0.2 mM ATP ($3—4 \cdot 10^6$ cpm)/5 mM $MgCl_2$/8 mM theophylline/0.1% bovine serum albumin/10 mM Tris · Cl (pH 7.7) and 25—50 μg of cell protein. Enzyme incubation was for 5 min at 37°C and the reaction stopped with 0.5 ml 2% SDS/42 mM ATP/12.5 mM cyclic AMP/approx. $1 \cdot 10^4$ cpm cyclic-[^{3}H]AMP and then by addition of trichloroacetic acid to 10% (v/v). Cyclic AMP was separated from other nucleotides as described [17]. Enzyme activity was calculated from ^{32}P radioactivity corrected for ^3H recovery and expressed as a percentage of a simultaneously-run control. When noted, 5 μg/ml prostaglandin E_1 and 10^{-5} M GTP or 10 mM NaF were present. Na^+- K^+-stimulated ATPase (($Na^+ + K^+$)-ATPase) was assayed in a medium containing 67 mM NaCl/3.3 mM KCl/5 mM MgATP (pH adjusted with imidazole)/30 mM Tris · Cl (pH 7.5). The assay was initiated with the cell homogenate. The reactions were stopped with cold trichloroacetic acid and the phosphate liberated was determined by the method of Fiske and Subba Row [18]. To determine the basal Mg^{2+}-ATPase activity 1 mM ouabain was substituted for the 3.3 mM KCl; ($Na^+ + K^+$)-ATPase activity was calculated as the difference between total ATPase and basal Mg^{2+}-ATPase activity.

Cholesterol content

Rat kidney 5W cells treated for 4 h with cholesterol-rich liposomes, as well as untreated control cells, were removed by scraping, washed, and centrifuged at $600 \times g$ (three times) with sonication buffer. The cell pellet was then extracted as described by Lo and Levey [19]. Cholesterol in the extract was measured by the method of Rudel and Morris [14] and phospholipid as phospholipid phosphorus by the method of Bartlett [13] assuming an average molecular weight of 725 for phospholipids. Cholesterol is expressed as total μg cholesterol extracted from two 60 mm cell culture dishes.

Stimulation of phagocytosis

Washed, boiled Zymosan particles and washed latex beads (1.1 μm) were incubated with cells as previously described [20,21]. Interaction of latex beads with rat kidney 5W cells was monitored using phase contrast microscopy. These cells so treated were then assayed for adenylate cyclase activity. To inhibit phagocytosis, 2,2-dinitrophenol at 10^{-5} M and 2-deoxyglucose at 10^{-2} M were preincubated with rat kidney 5W cells for 30 min prior to addition of cholesterol-containing liposomes and during the subsequent 4 h incubation. Adenylate cyclase activity of the cells so treated was determined.

Results

Cellular uptake of [^{14}C]cholesterol from cholesterol-containing liposomes

The uptake of [^{14}C]cholesterol from 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes was proportional to the concentration of cholesterol incubated with the rat kidney 5W cells over a cholesterol concentration range of 0.26—3.0 mg/ml. The uptake of cholesterol increased with time and was 80% greater at 4 h than at 2 h. [^{14}C]Cholesterol incorporation was twice as great with 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes as with 1 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes, when sus-

pensions containing equal phospholipid cholesterol : dipalmitoyl phosphatidylcholine liposomes concentrations were compared.

Alteration of adenylate cyclase activity by cholesterol-containing liposomes

When rat kidney 5W fibroblasts were exposed to 2 : 1, cholesterol : dipalmitoyl liposomes at increasing cholesterol concentrations, there was a progressive fall in adenylate cyclase activity when the latter was measured in one of 3 ways: without activators, with fluoride, or with prostaglandin E_1 plus GTP. The fall in enzyme activity was proportional to the uptake of [^{14}C]cholesterol with a correlation coefficient of 0.92 (Fig. 4, closed circles). The maximum fall in basal activity observed was to less than 15% of the control value. The ability of liposomes with equimolar amounts of cholesterol and phospholipid to decrease enzyme activity was also proportional to the amount of [^{14}C]cholesterol taken up. The decrease in enzyme activity was independent of the degree of saturation of the fatty acid group of the phospholipid, since 2 : 1, cholesterol : egg yolk lecithin liposomes elicited a fall in basal adenylate cyclase similiar to that of 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine vesicles (Fig. 4, open circles).

The adenylate cyclase activity progressively fell with time over 4 h and followed the same time course as the uptake of [^{14}C]cholesterol.

Adenylate cyclase activity fell by 37% at 2 h and by 54% at 4 h. The uptake of cholesterol and the fall in adenylate cyclase were both temperature depen-

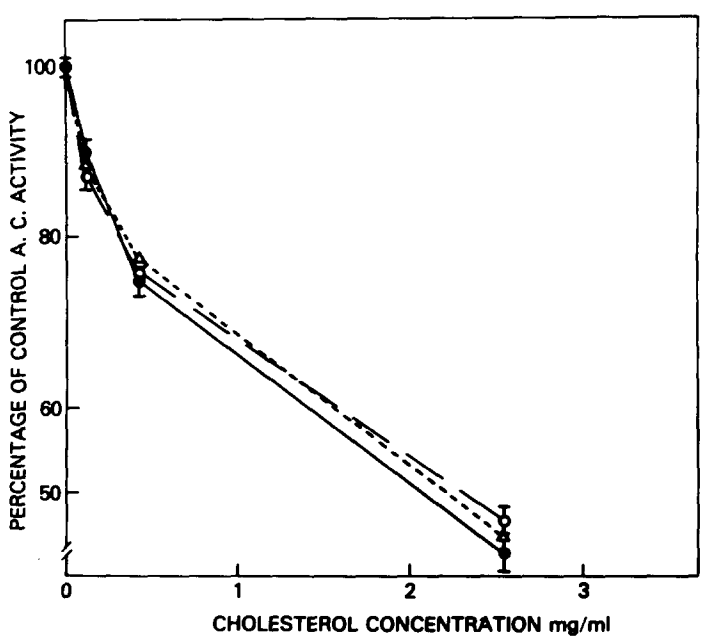

Fig. 3. Effect of cholesterol-rich liposomes on adenylate cyclase (A.C.) activity of rat kidney 5W cells as a function of cholesterol concentration. Data is expressed as a percentage of simultaneously-run control enzyme activities measured without activators (○) or with fluoride (△) or with PGE_1 plus GTP (●). Values are the mean ± S.E.M.

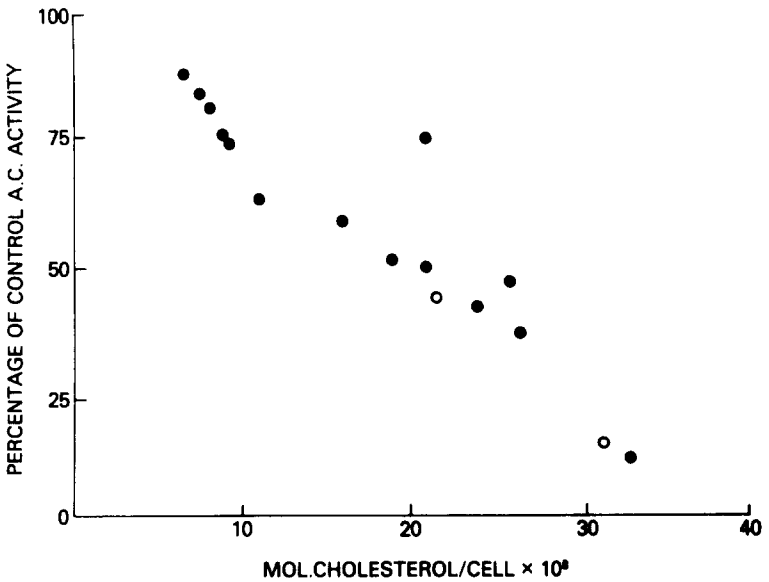

Fig. 4. Correlation of the decrease in basal adenylate cyclase (A.C.) activity with cholesterol uptake per cell. Each point represents the average of duplicates from separate experiments. ●, 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes; ○, 2 : 1, cholesterol : egg yolk lecithin liposomes.

dent. Incubation of cells at 3°C was accompanied by a fall both in [^{14}C]cholesterol uptake and in the degree of enzyme inhibition (Table I).

Since it seemed possible that the cells were phagocytosing the liposomal preparations, we attempted to assess the role of phagocytosis by incubating the cells with Zymosan particles or latex beads. These were equally without effect upon adenylate cyclase activity after 4 h of incubation with cells. However, phase contrast microscopy failed to reveal extensive particle uptake as is seen with macrophages in culture [21]. We undertook to inhibit phagocytosis by the addition of 2,4-dinitrophenol or 2-deoxyglucose. Addition of either 2,4-dinitrophenol ($1 \cdot 10^{-5}$ M) or 2-deoxyglucose ($1 \cdot 10^{-2}$ M) for 30 min before liposome

TABLE I

EFFECT OF 2,4-DINITROPHENOL, 2-DEOXYGLUCOSE AND TEMPERATURE ON CHOLESTEROL UPTAKE AND ADENYLATE CYCLASE ACTIVITY OF RAT KIDNEY 5W CELLS, TREATED WITH 2 : 1, CHOLESTEROL : DIPALMITOYL PHOSPHATIDYLCHOLINE LIPOSOMES

Each value is the average of duplicate determinations.

Liposome preparation	Incubation temperature	Cell treatment	Cholesterol uptake (mol/cell)	Adenylate cyclase activity (% of control)
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	37	None	$19 \cdot 10^8$	45
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	37	Dinitrophenol ($1 \cdot 10^{-5}$ M)	$21 \cdot 10^8$	40
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	37	2-deoxyglucose ($1 \cdot 10^{-2}$ M)	$18 \cdot 10^8$	53
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	3	None	$11 \cdot 10^8$	73

addition and during a 4 h incubation was without effect on the decrease in adenylate cyclase activity or on the uptake of [^{14}C]cholesterol (Table I).

Cholesterol-rich liposomes are known to be more heterogenous in size and contain larger liposomes than phospholipid liposomes. The effects observed upon adenylate cyclase activity could have been due to these larger vesicles. Therefore, we filtered the cholesterol-rich dipalmitoyl liposomes through a 0.45 μm milipore filter. This filter allowed more than 80% of the cholesterol to pass through. The filtrate produced the expected decrease in enzyme activity.

Effect of liposomes without cholesterol or with other steroids

Liposomes of phospahtidylcholine alone were incubated with rat kidney 5W cells for 4 h. Tabel II shows that egg yolk lecithin liposomes had a small stimulating effect on enzyme activity. Dipalmitoyl phosphatidylcholine liposomes alone produced a slight inhibition of activity. Only when cholesterol was present with the phospholipids was striking inhibition observed. Liposomes cannot be made from cholesterol alone and therefore could not be tested.

Dipalmitoyl phosphatidylcholine liposomes containing ergosterol (a compound with a close structural similarity to cholesterol) had an inhibitiory effect on adenylate cyclase activity similar to that observed with cholesterol. Liposomes prepared with hydrocortisone were without effect.

Effect of 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes on other cellular enzyme activities

The effect of liposomes on two other cellular enzymes was examined. As seen in Table III, liposome-treated cells exhibited a significant fall in (Na^+ + K^+)-ATPase activity that was in the same range as the fall in adenylate cyclase. On the other hand there was a small increase in Mg^{2+}-stimulated ATPase activity. The level of 5'-nucleotidase activity was quite low but was not significantly altered (data not shown).

Cholesterol content of liposome-treated cells

In order to determine if the presence of [^{14}C]cholesterol in the liposome-treated cells represented uptake rather than lipid exchange, total cellular cholesterol was measured. The cholesterol content of cells treated with 2.6 mg/ml

TABLE II

ADENYLATE CYCLASE ACTIVITY IN RAT KIDNEY 5W CELLS TREATED WITH LIPOSOMES OF PHOSPHATIDYLCHOLINE ALONE AND CHOLESTEROL-CONTAINING LIPOSOMES

Each value represents the average of duplicates from 1 experiment.

Liposome preparation	Cholesterol (mg/ml)	Dipalmitoyl phosphatidyl-choline (mg/ml)	Egg yolk lecithin (mg/ml)	Adenylate cyclase activity (% of control)
Egg yolk lecithin	—	—	4	112
Dipalmitoyl phosphatidylcholine	—	—	—	83
Cholesterol : egg yolk lecithin	2.5	—	2.0	14
Cholesterol : dipalmitoyl phosphatidylcholine	2.3	2.0	—	42

TABLE III

EFFECT OF CHOLESTEROL-RICH LIPOSOMES UPON 3 DIFFERENT FIBROBLAST ENZYMES

Each value represents the mean of duplicates from 2 different experiments. Adenylate cyclase was measured without activators.

Enzyme	Control	2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	% of control
$(Na^+ + K^+)$ATPase nmol PO_4/30 min per mg protein	362	224	62
Mg^{2+}-ATPase nmol PO_4/30 min per mg protein	668	707	106
Adenylate cyclase pmol/5 min per mg protein	190	100	53

of 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes was 128 μg compared to 118 μg in control cells. An intermediate value was obtained for cells treated with 0.5 mg/ml of cholesterol-rich liposomes (Table IV). Although the increase in cholesterol content of cells incubated with cholesterol-rich liposomes is not statistically significant, it agrees well with the predicted increase in cholesterol content calculated from the [^{14}C]cholesterol uptake. Similarly, phospholipid phosphorus increased in the treated cell populations when compared to control: this increase was less than 10%. This data is consistent with cellular uptake of liposomes containing cholesterol.

The subcellular localization of [^{14}C]cholesterol was examined in partially purified plasma membranes prepared by two different methods [22,23] and compared to the total cellular radioactivity. Plasma membranes contained from 10 to 35% of the total cellular radioactivity depending upon the isolation procedure employed.

Effect of liposomes on other cell clones

4 other subclones of kidney cells designated Cl A, Cl C, Cl G, and Cl K were treated with 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes at

TABLE IV

CHOLESTEROL CONTENT OF CONTROL AND 2 : 1, CHOLESTEROL : DIPALMITOYL PHOSPHATIDYLCHOLINE-TREATED RAT KIDNEY 5W CELLS

Each value is the mean of 4 determinations + S.E.M.

Liposomal preparation	Cholesterol concentration in liposomal suspension (mg/ml)	Cholesterol uptake (mol/cell)	Cholesterol in total cell extract (μg)
Control	0	0	118 ± 6
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	0.5	$10.5 \cdot 10^8$	123 ± 4
2 : 1 Cholesterol : dipalmitoyl phosphatidylcholine	2.5	$21 \cdot 10^8$	128 ± 5

a cholesterol concentration of 2.4 mg/ml. Clones A and C showed a 50% fall in basal, fluoride, and PGE_1 + GTP-stimulated activity, whereas simultaneously-run clones G and K were unaffected. Rat kidney clone S was also unaffected by cholesterol-containing liposomes even though [^{14}C]cholesterol uptake (measured simultaneously, $25 \cdot 10^8$ mol/cell) was similar to that seen with the 5W clone. ($Na^+ + K^+$)-ATPase activity in this cell type was also unchanged (data not shown).

There was no correlation between basal adenylate cyclase activity of the different subclones and their response to cholesterol-containing liposomes. In addition, treatment of the transformed cell line which has been reported to have low basal, and hormonally unresponsive adenylate cyclase, with 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes was without effect upon enzyme activity. To determine if the liposome preparation was interacting with the transformed cell line, [^{14}C]cholesterol uptake was simultaneously measured and found to be $31 \cdot 10^8$ molecules/cell.

Discussion

Adenylate cyclase is a membrane-bound enzyme whose catalytic activity is dependent upon the presence of specific lipids [2]. It has been postulated that cholesterol has a role in regulating adenylate cyclase activity [24,25].

The present data suggest that in certain cell types, exposure to liposomes containing cholesterol can decrease enzyme activity proportionate to the amount of cholesterol taken up by the cells. This effect appears to be independent of the nature of the acyl group of the phosphatidylcholine liposome in which the cholesterol is contained. Further, the decrease in activity is reflected in basal, fluoride-stimulated, and PGE_1 + GTP-stimulated activity suggesting that the effect is due to alteration of the catalytic subunit of the enzyme.

Recently Sinha, Shattil and Coleman reported a decrease in hormonally responsive adenylate cyclase in human platelets exposed to cholesterol-rich, but not equimolar cholesterol-phosphatidylcholine liposomes [25]. The magnitude of change of cholesterol content in platelets was much greater than that seen in the present study.

The inability of cholesterol-containing liposomes to alter adenylate cyclase activity in certain of our fibroblasts clones and in the Kirsten virus-transformed fibroblasts line is not explained. The measured uptake of [^{14}C]cholesterol in one of the nonresponsive cell types is the same as that of the responsive line. Further, in rat kidney S cells, when adenylate cyclase activity is found to be unaffected by cholesterol-containing liposomes, ($Na^+ + K^+$)-ATPase activity is similarly unaffected. This suggests that the ability of cholesterol-containing liposomes to alter membrane enzyme activity may be a function of the initial membrane lipid content or composition. Possibly in certain cell lines the membrane is relatively saturated with cholesterol and the added cholesterol is forced into an abnormal interaction with membrane enzymes [27]. Thus, only a small increase in cellular cholesterol content could produce large changes in enzyme activity in the responsive cell lines. Some alternative explanations for the inability in unresponsive cells include, failure of liposomal cholesterol to localize to the plasma membrane or increased esterification of cholesterol in unresponsive clones [28].

Mammalian cells in culture [8,9] and *Acanthamoeba castellanii* [7] exposed to liposomes containing phosphatidylcholines with acyl chains of different lengths and degress of saturation have been demonstrated to interact with liposomes in a number of different ways. Direct cell fusion, pinocytosis, and a combination of fusion with discharge of the contents of a multilamellar liposomes have been observed [6—9]. Another possible interaction of liposomes with cells is via the exchange of specific liposome components such as cholesterol, with the plasma membranes of platelets and red cells [5,25,29].

The nature of the interaction of mammalian fibroblasts with the cholesterol-containing liposomes employed in the present study has not been completely defined. However, electron micrographs showing liposomal-cell fusion (data not shown) and the presence in the plasma membrane fraction of a significant portion of the [^{14}C]cholesterol taken up suggests that these liposomes interact at least in part with the plasma membranes. The fact that another membrane enzyme ($Na^+ + K^+$)-ATPase shows a decrease in enzyme activity in parallel with that seen with adenylate cyclase, while Mg^{2+}-stimulated ATPase was not affected, further supports a relatively specific effect on the plasma membrane. Cholesterol-containing phosphatidylcholine liposomes have previously been found to inhibit the activity of rabbit kidney ($Na^+ + K^+$)-ATPase [26].

The cholesterol-rich liposomal preparation used in these studies is a morphologically heterogenous population as seen by uranyl acetate negatively-stained electron micrographs as well as by Sepharose 4B chromatography. This is in contrast to liposomes prepared with egg yolk lecithin alone, which were smaller and more uniform in size. This increase in size, which has been previously reported for cholesterol-containing liposomes [30], does not account for the effects we observed, since cholesterol-rich liposomes filtered through a 0.45 Millipore filter were fully effective in decreasing cellular enzyme activity. Cells exposed to liposomes constituted of 1 : 1, cholesterol : dipalmitoyl phosphatidylcholine incorporated approximately half the amount of [^{14}C]cholesterol as that of cells exposed to 2 : 1, cholesterol : dipalmitoyl phosphatidylcholine liposomes, which suggests that a similar number of liposome-cell interactions occured in each case. Further, the decrease in adenylate cyclase activity seen in experiments utilizing 1 : 1, cholesterol : phospholipid liposomes remained proportional to the [^{14}C]cholesterol uptake.

There are reports of altered cholesterol content in transformed cell lines [30, 31] which are of particular interest since free cholesterol content is closely regulated for a given cell type [33]. In addition we have observed decreased adenylate cyclase activity in some clones of transformed cells [17]. It is possible that changes in membrane cholesterol may, in part, be responsible for the alterations observed. In support of this notion is the recent observation that by removing adenylate cyclase from a membrane-bound form with detergent treatment it is possible to reverse the observed difference in enzyme activity between certain normal and transformed cells [34].

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