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EFFECT OF FETAL CALF SERUM AND SERUM PROTEIN FRACTIONS ON THE UPTAKE OF LIPOSOMAL PHOSPHATIDYLCHOLINE BY RAT HEPATOCYTES IN PRIMARY MONOLAYER CULTURE

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Summary

We studied the effect of fetal calf serum and serum protein fractions on the interaction of phospholipid vesicles consisting of phosphatidylcholine, cholesterol and dicetylphosphate (molar ratio 7 : 2 : 1), with rat liver parenchymal cells in a primary monolayer culture. During incubation of such vesicles with fetal calf serum part of the labeled phosphatidylcholine is transferred to a lipoprotein particle similar to the one we identified previously as a derivative of high density lipoprotein (Scherphof, G., Roerdink, F.H., Waite, M. and Parks, J. (1978) Biochim. Biophys. Acta 542, 296—307). When the particle thus formed is incubated with the cells a transfer of the phospholipid label to the cells is observed. When vesicles are incubated with the cells in presence of serum such lipoprotein-mediated lipid transfer may conceivably contribute to the total lipid uptake observed. However, we found that the presence of fetal calf serum in the culture medium greatly diminished rather than increased the total transfer of liposomal lipid to the cells. Also bovine serum albumin and bovine β-globulins reduced this transfer, although to a lesser extent than whole serum. α-Globulins, on the other hand, were as effective as complete serum in reducing the uptake of liposomal phospholipid. A γ-globulin fraction failed to exhibit any effect on the uptake of [^{14}C]phosphatidylcholine by the cells.

All protein fractions which were able to inhibit cellular uptake of liposomal phospholipid were shown to bind to the phospholipid vesicles. Furthermore, lipid vesicles preincubated with fetal calf serum and then separated from it showed reduced transfer of labeled phosphatidylcholine to parenchymal cells.

These observations were taken to suggest that the diminished uptake of liposomal lipid may be caused by a modification of the liposomal surface membrane as a result of the binding of certain serum proteins. On the other

hand, we cannot rule out that plasma membrane modifications are involved in the mechanism of inhibition as well.

Introduction

Most mammalian cells require the addition of serum to the culture medium for optimal growth and maintenance of the cell lines in vitro. As a result serum components may adsorb to the cell surface [1—4] or may be ingested by the cells during culturing [5], thus affecting cellular properties. The presence of antibodies against serum proteins in antisera raised against cultured cells has been demonstrated [6,7].

It is quite conceivable therefore that interactions of structures such as liposomes with cultured cells are influenced by the presence of serum components on the cell surface or in the incubation medium. Recently, reports have been published concerning the interaction of serum components with liposomes and its consequences for cellular uptake and liposomal integrity [8—12]. When studying the interaction of phospholipid vesicles with rat hepatocytes in a primary monolayer culture, we observed a diminished uptake of liposomal lipid if fetal calf serum was present in the culture medium [13]. Similarly, Blumenthal et al. [14] demonstrated that the transfer of the fluorescent dye 6-carboxyfluorescein, trapped in the aqueous compartment of vesicles, to human lymphocytes was inhibited by fetal calf serum.

This study was undertaken to establish the nature and the site of action of the serum component(s) responsible for the reduction in transfer of liposomal lipid to rat liver parenchymal cells, with the aim to provide further insight into the mechanisms of liposome-cell interaction.

Materials and Methods

Materials. Egg-yolk phosphatidylcholine was purchased from Sigma. The purity of the preparations was routinely checked by thin-layer chromatography on silica gel. Cholesterol was obtained from Koch-Light; dicetylphosphate and stearylamine were products from Sigma and Merck, respectively. *Me*-^{14}C-labelled egg phosphatidylcholine (3.2 Ci/mol) was prepared as described by Stoffel [15]. Fetal calf serum was purchased from Flow Labs Ltd. Bovine serum albumin (Fraction V) and defatted bovine serum albumin were from Sigma. α-Globulins (bovine Cohn fraction IV) and β-globulins (bovine Cohn fraction III) were obtained from Koch-Light Labs Ltd. γ-Globulins (bovine Cohn fraction II) were a product from Serva.

Preparation of vesicles. Vesicles consisting of egg phosphatidylcholine, cholesterol and either dicetylphosphate or stearylamine (molar ratio 7 : 2 : 1, respectively) were prepared in 0.15 M NaCl/5 mM Tris-HCl, pH 7.4. Lipid suspensions were sonicated with a probe type sonifier (Branson Sonifier B-12) during 7 X 30 sec, power supply 70 W at 20° C. Multilamellar and unilamellar vesicles were separated by Sepharose 4B chromatography.

Isolation and culturing of rat-liver parenchymal cells. Rat hepatocytes were isolated by perfusion with collagenase via the portal vein as described before

[13]. Primary cell cultures of rat hepatocytes were initiated also as described in detail previously [13] and maintained in 4.0 ml HAM F-12 culture medium (Flow) buffered at pH 7.4 with $NaHCO_3$ (14.5 mM), 2-(*N*-morpholino)ethane sulfonic acid (12.5 mM, Sigma) and *N*-tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid (12.5 mM, Serva). The culture medium was supplemented with 15% fetal calf serum (Flow) when required, penicillin (100 units/ml) and streptomycin (100 μg/ml).

Incubation of vesicles with the cells. The procedure was carried out exactly as has been described previously [13]. Serum protein fractions were added to the culture medium in concentrations up to values to be found in 15% fetal calf serum [12] as indicated in the legends. After appropriate incubation times the medium was removed and the dishes were thoroughly washed with 0.9% NaCl. Cells were scraped from the plates with a rubber policeman in a small volume of 0.9% NaCl. Aliquots of the cell suspension were taken for determination of radioactivity and protein.

Other methods. Cell protein was determined according to the method of Lowry et al. [16]. Vesicle-bound protein was assayed with the fluorescent dye fluorescein as described by Böhlen et al. [17]. Immunoelectrophoresis and immunodiffusion were carried out on 1.5% agar gels in Tris/veronal buffer, pH 8.8 (18 g/l, Gelman Inst. Co., Ann Arbor, MI). Gels were stained with Coomassie Brilliant Blue after removal of non-precipitated proteins. As antiserum, rabbit anti-bovine serum (Flow) was used.

Results and Discussion

Effects of proteins on cellular uptake

Recently we demonstrated the release of phosphatidylcholine from liposomes in the presence of rat, human, or monkey plasma and the concomitant formation of a lipoprotein particle very similar to high density lipoprotein [11]. In preliminary experiments we found that the phospholipid associated with the particle formed in rat plasma was rapidly incorporated into isolated rat hepatocytes. This rose the question as to what extent the uptake of liposomal phospholipid by isolated rat hepatocytes as reported by us [13] in presence of fetal calf serum was related to the prior formation of the lipoprotein particle.

Fig. 1 demonstrates that a similar particle is formed when liposomes are incubated with fetal calf serum. Similar elution patterns are found at lower serum concentrations, provided that the lipid to protein ratio remains approximately the same. Particularly when small unilamellar vesicles are used, a considerable proportion of the liposomal phosphatidylcholine is recovered in a fraction eluting from the Ultrogel column behind the void volume which contains the remaining vesicles. This difference in reactivity between unilamellar and multilamellar vesicles can possibly be ascribed to the difference in available surface area and/or to the difference in curvature and packing of the lipid molecules in the two types of vesicle. A similar difference in reactivity between unilamellar and multilamellar vesicles has been observed by Morrisett et al. [18] in a study on the interaction of human very low density lipoproteins with phosphatidylcholine vesicles. Supposedly, physical parameters of the vesicles

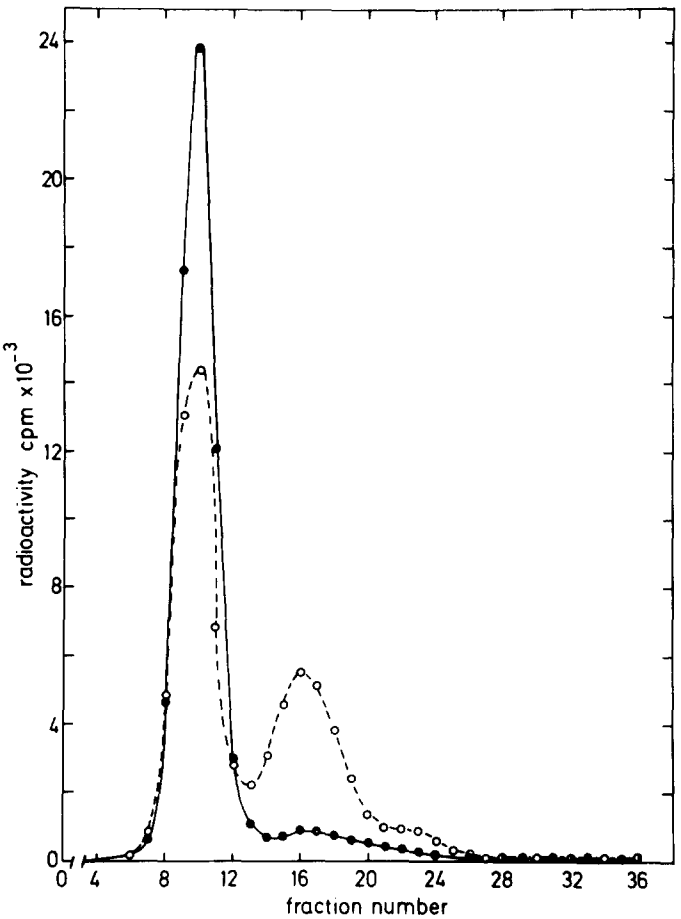

Fig. 1. Chromatography on Ultrogel AcA34 of unilamellar and multilamellar phospholipid vesicles after incubation with fetal calf serum. Phospholipid vesicles consisting of [^{14}C]phosphatidylcholine, cholesterol and dicetylphosphate in a molar ratio 7 : 2 : 1 were prepared by ultrasonic irradiation. Unilamellar and multilamellar vesicles were separately isolated by Sepharose 4B chromatography. 2.4 μmol lipid of each vesicle preparation were diluted to a final volume of 1 ml in 5 mM Tris/0.15 M NaCl, pH 7.4, 3 ml fetal calf serum was added and the mixture was incubated for 1 h at 37°C and subsequently chromatographed on Ultrogel. Fractions were collected and assayed for radioactivity. ○———○, unilamellar vesicles; ●———●, multilamellar.

determine the mode of interaction. We incubated pooled column fractions containing the non-liposomal radioactivity with rat hepatocytes in monolayer culture. The uptake of radioactivity by the cells was then measured and compared with uptake of radioactivity directly from radioactive phosphatidylcholine vesicles, i.e. in absence of fetal calf serum. Fig. 2 demonstrates that under those conditions (open symbols) the radioactive phospholipid is transferred to the cells slightly more rapidly from the vesicles than from the lipoprotein particle. The presence of fetal calf serum during the incubation (filled symbols) has a much more pronounced influence on the uptake from vesicles than on the uptake from the lipoprotein. The uptake from vesicles is decreased approximately 3-fold whereas the uptake from the lipoprotein is only slightly lowered.

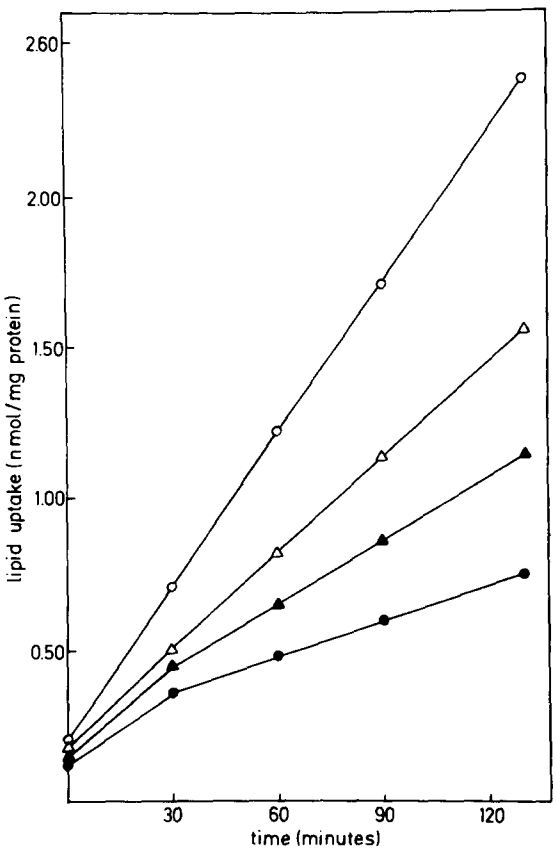

Fig. 2. Incorporation of [^{14}C]phosphatidylcholine mediated by the lipoprotein fraction and phospholipid vesicles into rat hepatocytes. A [^{14}C]phosphatidylcholine-labeled lipoprotein fraction was prepared as described in the legend to Fig. 1. Negatively charged unilamellar vesicles containing radioactive phosphatidylcholine were prepared as described in Materials and Methods. The two preparations containing equal amounts of [^{14}C]phosphatidylcholine (0.35 μmol lipid), were added to the cells (cell density $1.3 \cdot 10^6$—$1.4 \cdot 10^6$ cells/dish). After incubation times indicated the radioactivity associated with the cells was determined as described in Materials and Methods. ●———●, phospholipid vesicles in presence of 15% fetal calf serum; ○———○, phospholipid vesicles in absence of fetal calf serum; ▲———▲, lipoprotein fraction in presence of 15% fetal calf serum; △———△, lipoprotein fraction in absence of fetal calf serum.

It is obvious, however, that whatever uptake of phospholipid from vesicles is left in presence of serum could be accounted for by the lipoprotein-mediated mechanism (compare filled circles with open triangles), particularly when we presume an only partial transfer of liposomal phospholipid to the lipoprotein during the time of incubation.

The inhibitory effect of fetal calf serum is not confined to vesicles of negative surface charge. Also lipid uptake from positively charged vesicles is inhibited by serum as can be seen in Table I. These results also demonstrate that cellular phospholipid uptake from multilamellar vesicles, either positively or negatively charged, is inhibited by the serum to a somewhat greater extent than that from unilamellar vesicles.

The results of Fig. 2 allow us to conclude that although the formation of the lipoprotein particle may interfere with the incorporation of liposomal phos-

TABLE I

UPTAKE OF LIPOSOMAL LIPID BY RAT LIVER PARENCHYMAL CELLS IN ABSENCE AND PRESENCE OF FETAL CALF SERUM

Radioactive lipid vesicles (0.5 μmol) were incubated with rat liver parenchymal cells during 130 min and lipid uptake was assayed as described in Materials and Methods. Anionic vesicles consisted of phosphatidylcholine, cholesterol and dicetylphosphate, molar ratio 7 : 2 : 1. Cationic liposomes contained stearylamine instead of dicetylphosphate. Serum: 15% fetal calf serum (final concentration in culture medium).

Vesicles and charge	Uptake of lipid (nmol/mg protein)	
	+ serum	— serum
Unilamellar, negative	0.75 ± 0.04	2.57 ± 0.11
Unilamellar, positive	0.54 ± 0.03	1.97 ± 0.07
Multilamellar, negative	0.18 ± 0.02	1.13 ± 0.07
Multilamellar, positive	0.14 ± 0.02	0.85 ± 0.08

pholipid by the cells the overall effect of the fetal calf serum must involve other factors as well. We therefore investigated the separate effects of a number of other serum components on liposome-hepatocyte interactions.

Fig. 3 shows the effect of bovine serum albumin on phosphatidylcholine uptake from both uni- and multilamellar vesicles. At a concentration of 4 mg per ml culture medium this protein inhibits uptake from uni- and multilamellar vesicles to an extent of 40 and 55%, respectively, as compared to the uptake in absence of serum. Both numbers represent plateau values which do not increase at higher albumin concentrations. It should be recognized that in a medium containing 15% serum the albumin concentration is of the same order of magnitude. Yet, with 15% fetal calf serum the level of inhibition is significantly higher both for uni- and multilamellar vesicles as is also indicated in Fig. 3 (C_u and C_m, respectively). This suggests that not the albumin alone is responsi-

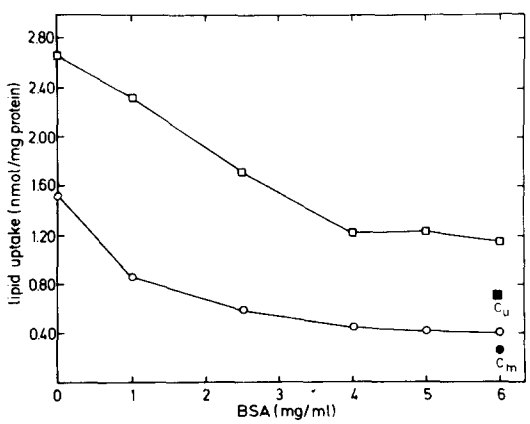

Fig. 3. Inhibition by bovine serum albumin (BSA) of the uptake of the liposomal marker, [^{14}C]phosphatidylcholine, into parenchymal cells. Uptake of negatively charged unilamellar (□———□) or multilamellar (○———○) vesicles was determined in presence of variable amounts of defatted albumin (concentration, 0—6 mg/ml; culture medium volume, 4 ml). No fetal calf serum was added. Incubation time was 130 min. C_u and C_m represent the uptake of unilamellar and multilamellar vesicles in presence of 15% fetal calf serum, i.e. in presence of approx. 6 mg albumin per ml culture medium.

ble for the diminished transfer of lipid in the presence of serum which is confirmed by the results presented in Fig. 4. These demonstrate that also α- and β-globulins are capable of inhibiting the uptake of liposomal phosphatidylcholine by cultured hepatocytes. The β-globulin fraction inhibits lipid uptake from both multi- and unilamellar vesicles up to about 40% at concentrations of 1.2 mg per ml culture medium, similar to the albumin fraction (Fig. 3). The α-globulins are even more potent inhibitors of lipid uptake. Less than 15% of the control values of cell-associated radioactivity is found in presence of 1.2 mg α-globulins per ml culture medium. Uni- and multilamellar vesicles are affected to similar extents. These results are partly at variance with those obtained by Tyrrell et al. [12]. They observed that α- and β-globulins enhanced the hepatic lipid uptake from anionic liposomes during rat liver perfusion while α-globulins also enhanced liposomal uptake by P815Y Mastocytoma cells in contrast to β-globulins which decreased the liposomal lipid uptake. These discrepancies with our results may be explained by their using cholesterol as a liposomal marker and/or by the different cell systems under investigation. For example, the contribution by non-parenchymal cells in the perfused liver system may be considerable. A difference in lipid uptake between different cell types was also found by Tyrrell et al. [12]. As also shown in Fig. 4 γ-globulins have no effect on the lipid uptake, confirming an observation by Blumenthal et al. [14].

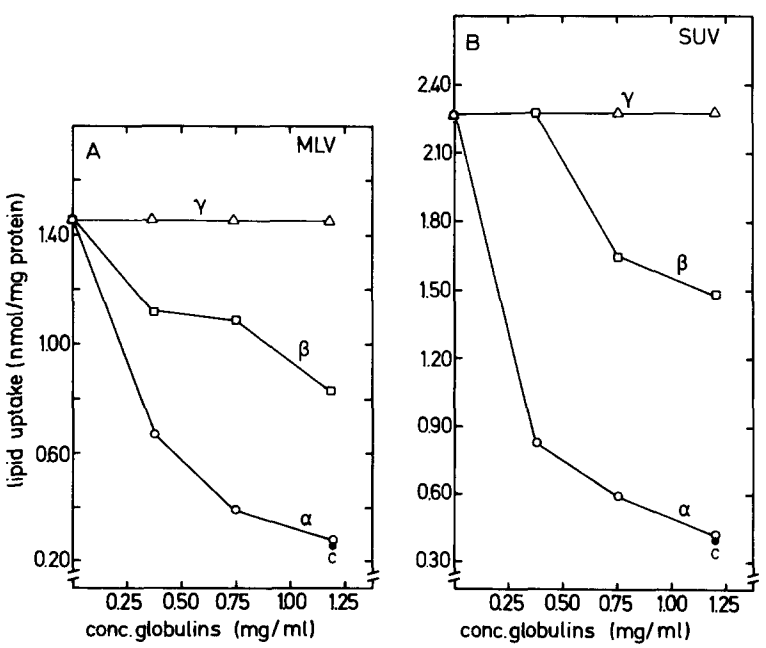

Fig. 4. Uptake of vesicles by parenchymal cells in presence of globulins. Vesicles, (negatively charged, composition as described in Materials and Methods) were incubated with parenchymal cells in presence of increasing concentrations of α- (○), β- (□) and γ-globulins (△). Lipid uptake was determined after an incubation period of 130 min. No fetal calf serum was added. (A) Shows the uptake of multilamellar vesicles (MLV) (0.5 μmol/dish). (B) Shows the uptake of unilamellar vesicles (SUV) (0.3 μmol/dish). C, is control (●) representing uptake in presence of 15% fetal calf serum.

Binding of proteins to liposomes

To get some insight into the mechanism of action of the inhibitory proteins we investigated their binding to phospholipid vesicles. Such binding might alter the surface properties of vesicles and thus their interaction with the cells [19]. After incubation of 1.5 μmole phospholipid vesicles, either unilamellar or multilamellar, for 1 h at 37°C with 60 mg of albumin in 2.5 ml NaCl/Tris vesicles were separated from the albumin on Ultrogel AcA34 and the presence of protein in the vesicle fraction was assayed. We found 9 μg protein per μmol phospholipid for unilamellar and 66 μg per μmol phospholipid for multilamellar vesicles. The presence of 0.4 mM Ca^{2+} (concentration in HAM-F12 medium) had no influence on the binding. In recent years several reports have been published concerning the interaction of albumin with phospholipid vesicles [9,20—26]. In none of them, binding of albumin to lipid vesicles was reported. This is most likely to be explained by either the relatively low albumin concentrations used [23,24] or by the use of a protein assay insufficiently sensitive for the detection of the small amounts of protein associated with the vesicles [9]. In some cases binding was not determined [22,25]. With phosphatidylserine vesicles, Kimelberg and Papahadjopoulos [22] demonstrated albumin-induced Na^+ leakage under various conditions of pH and protein concentrations. At pH 7.4, when both vesicles and protein are negatively charged, leakage of Na^+ was most pronounced at a high protein concentration, i.e. at a molar ratio of protein to phospholipid of 0.8. This molar ratio of protein to phospholipid is fairly close to the value used in our experiments, i.e. 0.66, while the vesicles, containing dicetylphosphate, were also negatively charged.

It would seem somewhat surprising that multilamellar vesicles bind more albumin than unilamellar vesicles because the available surface area per mole lipid is much higher for the latter. This difference in binding might be explained by differences in physical nature of the vesicles such as the area per lipid molecule or the radius of curvature. In the present study we confirmed our earlier observation [9] that liposomal phosphatidylcholine is transferred to albumin when liposomes are incubated with this protein. In addition, we observe now that this transfer is much lower from multilamellar than from unilamellar vesicles (not shown), essentially similar to the results in Fig. 1 on the formation of the lipoprotein particle. The difference in albumin binding we observed between multilamellar and unilamellar vesicles could therefore be explained in terms of a limited structural stability of the unilamellar type of vesicle as compared to the multilamellar one. Possibly, only a limited number of protein molecules can be bound to the vesicle surface without its falling apart, whereas the multilamellar vesicles can accomodate many more protein molecules resulting in increased permeability only [9]. As a consequence, the vesicles remaining in the void volume after incubation with albumin will have a much lower protein content in case they are unilamellar.

Uptake of protein-coated vesicles

When vesicles, after a preincubation with albumin or fetal calf serum and reisolation on Ultrogel AcA34, were incubated with hepatocytes the transfer of phospholipid from the vesicles to the cells appeared to be inhibited as is

shown in Fig. 5. The inhibitory effect of the preincubation with either albumin or whole serum is not as pronounced as when whole serum (filled circles in Fig. 5) or albumin (Fig. 3) is present during the incubation with the cells. These observations suggest that adsorption of protein(s) to the liposomal surface cannot completely explain the inhibitory effect observed in presence of fetal calf serum (compare filled symbols in Fig. 5). On the other hand, a 30-min incubation of albumin-pretreated vesicles in a medium without cells revealed, after reisolation of the vesicles on Ultrogel AcA34, that about 40% of the bound protein was lost in the medium. This would indicate that at least part of the protein is only loosely bound to the liposomes. Nevertheless the rates of cellular lipid uptake are considerably reduced as compared to that from non-pretreated vesicles in absence of serum. As demonstrated in Fig. 5, the effect of albumin alone is less pronounced than that of whole serum as might be expected from the results presented in Fig. 4 on the inhibiting effect of α- and β-globulins, thus confirming that other serum factors play a role as well. Immunoelectrophoretic analysis of phospholipid vesicles isolated after incubation in a medium containing the globulin fractions and albumin revealed (Fig. 6) that, in addition to albumin, also α- and β-globulins are present. Some γ-globulin may be bound as well despite its lack of effect on the uptake of phospholipid by the cells. These observations were confirmed by assaying the vesicles after preincubation with the protein fractions and subsequent reisolation on Sepharose 4B for total protein. The results are presented in Table II, showing that considerable amounts of the various proteins are bound to multilamellar

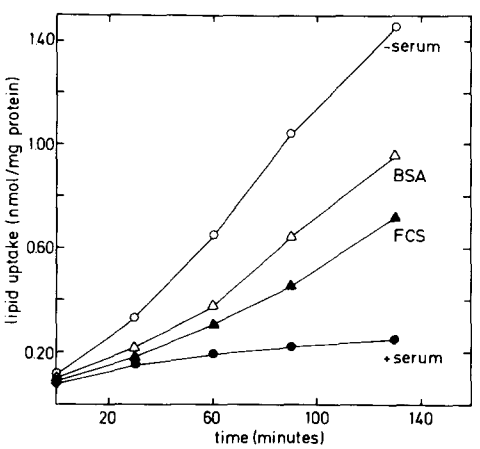

Fig. 5. Effect of preincubation of liposomes with fetal calf serum or albumin on uptake of liposomal phosphatidylcholine. Multilamellar vesicles (negatively charged, final concentration, 0.14 μmol/ml) were incubated in 5 mM Tris/0.15 M NaCl, pH 7.4, supplemented with fetal calf serum (final concentration, 15%) or albumin (final concentration, 6 mg/ml) for 1 h at 37°C. Vesicles treated with serum were isolated by Sepharose 4B chromatography and albumin-treated vesicles by Ultrogel AcA34 chromatography. Preincubated vesicles were incubated with the cells in medium without fetal calf serum (0.5 μmol lipid, $1.3 \cdot 10^6$—$1.4 \cdot 10^6$ cells/dish). Lipid uptake was determined at times indicated. ▲———▲, uptake of vesicles treated with fetal calf serum (FCS); △———△, uptake of bovine serum albumin-treated vesicles (BSA). For control values the uptake of untreated vesicles was determined in absence (○———○) or presence (●———●) of 15% fetal calf serum.

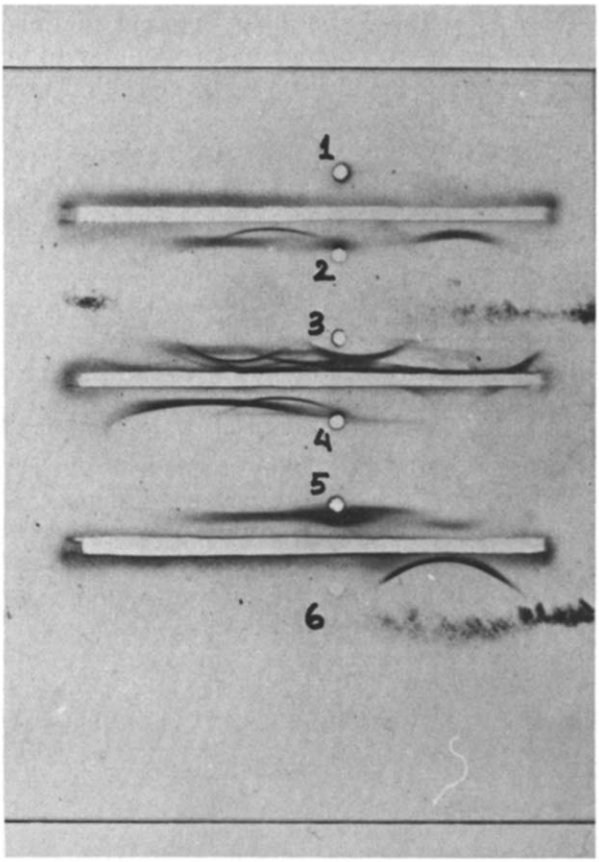

Fig. 6. Immunoelectrophoresis of vesicles after incubation with albumin, α- and β-globulins. Negatively charged multilamellar vesicles were incubated with the protein fractions for 1 h at 37° C (protein concentrations the highest as described in Table II). Vesicles were isolated by column chromatography on Sepharose 4B. The liposomal fraction was further concentrated by dialysis against solid Sephadex G-200. A few drops of 15% Triton X-100 were added and the fraction (containing approx. 30 nmol liposomal lipid) subjected to electrophoresis as described in Materials and Methods. The wells containing α- and β-globulins and albumin were treated similarly and contained 10 μg protein. After electrophoresis, the trough was filled with anti-bovine serum. 1, untreated vesicles; 2, α-globulins; 3, fetal calf serum; 4, β-globulins; 5, pretreated vesicles; 6, serum albumin.

vesicles at different protein concentrations. It should be noted that at the highest protein concentrations the protein/lipid ratio is the same as in the lipid uptake experiments of Fig. 4 at maximal protein concentrations while the intermediate protein concentrations for α- and β-globulins are those found in 15% fetal calf serum. Binding of α- and γ-globulins to lipid vesicles has also been observed by Black and Gregoriadis [8] and Weissmann et al. [27—29]. Binding of β-globulins with lipid vesicles has not been demonstrated so far although Tyrrell et al. [12] observed a high rate of leakage of methotrexate from vesicles during incubation with β-globulins. Our results suggest that binding of β-globulins to the vesicles may be the cause of such leakage. The inhibitory potency of the α-globulin fraction is approximately the same as that of whole serum (Fig. 4). Addition of any of the other inhibitory proteins,

TABLE II

BINDING OF SERUM PROTEIN FRACTIONS WITH NEGATIVELY CHARGED MULTILAMELLAR VESICLES

Lipid vesicles, 1.5 μmol lipid, consisting of phosphatidylcholine, cholesterol, dicetylphosphate (molar ratio 7 : 2 ; 1) were incubated with serum protein fractions during 1 h at 37°C in 2.5 ml 5 mM Tris/0.15 M NaCl, pH 7.4. Vesicles were separated from non-bound protein by Sepharose 4B chromatography. Protein was determined by the fluorescamine method [17], (n = 4).

Protein component incubated with vesicles	Protein concentration (mg/ml)	Protein bound (μg/μmol lipid ± S.E.)
Albumin	24	66 ± 7
	12	29 ± 6
	3	13 ± 2
α-Globulins	4.8	253 ± 12
	1.2	156 ± 8
	0.6	54 ± 4
β-Globulins	4.8	132 ± 5
	1.2	26 ± 4
	0.6	17 ± 3
γ-Globulins	4.2	82 ± 7

albumin or β-globulins, to the α-globulin fraction had almost no additional influence on the inhibition of uptake as can be seen in Table III. At concentrations at which the inhibitory effects on phospholipid uptake of separately added albumin or β-globulins are maximal (44 and 36% inhibition, respectively) these fractions, when present simultaneously, produce an even higher extent of inhibition (64%). This partly additive effect of the proteins suggests an additional site of action. As they both bind to the liposomes (Table II) and thus

TABLE III

EFFECT OF FETAL CALF SERUM, ALBUMIN, α-, β- AND γ-GLOBULINS ON UPTAKE OF LIPID VESICLES BY RAT LIVER PARENCHYMAL CELLS

Vesicles (unilamellar, negatively charged) were incubted with parenchymal cells. After 130 min lipid uptake was determined. Results are expressed as percentage of inhibition relative to that of fetal calf serum taking uptake in absence of serum as 0% inhibition and that in presence of serum as 100% inhibition. Protein fractions were added in the following concentrations: albumin, 6 mg/ml; α- and β-globulins, 1.2 mg/ml; γ-globulins, 1.5 mg/ml. Fetal calf serum: 15% (final concentration).

Fraction added	Percent of inhibition relative to that of fetal calf serum (=100%)
None *	0
Fetal calf serum	100
Albumin	44
α-Globulins	90
β-Globulins	36
γ-Globulins	0
Albumin + β-globulins	64
Albumin + α-globulins	100
α- + β-globulins	100
Albumin + α- + β-globulins	100

* Uptake in HAM F12 culture medium without addition of proteins or serum.

may act at the liposomal level, i.e. by virtue of their presence at the liposomal surface, one of them might act at the cellular surface as well. Previously we demonstrated that bovine albumin is not taken up or bound by rat hepatocytes [13] while others reported on the lack of binding of rabbit albumin to homologous hepatocytes [30] and fetal calf albumin to BHK-C13/21 fibroblasts [3]. On the other hand uptake of α- and β-globulins has been demonstrated, presumably after binding to the cell surface [5]. This would lend further support to the supposition that the β-globulins inhibit liposomal uptake by virtue of their binding to the liposomal surface as well as to the plasma membrane. Kerbel and Blakeslee [7] demonstrated that a component migrating in the α-globulin region could still be detected on the surface of cells even after extensive washing if cultured in presence of fetal calf serum. This further suggests involvement of the plasma membrane as a second site of action.

In conclusion, a number of serum proteins interfere with the interaction between liposomes and hepatocytes resulting in a decrease in the amount of liposomal lipid becoming associated with the cells. Such effects may be accomplished through different mechanisms of action either involving interactions with the liposomes or with the cells or both. The effect of lipoproteins on liposomes and its subsequent involvement in phosphatidylcholine uptake by the hepatocytes emphasizes that one should be cautious in interpreting association of liposomal lipid with cells as cellular uptake of liposomes.

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