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The Role of Serum Albumin in the Uptake of Fatty Acids by Cultured Cardiac Cells from Chick Embryo

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Fatty acids enter the cultured cardiac cells from chick embryo through two mechanisms, one involving a saturable process, the other resembling passive diffusion. Studies of the saturable component, at fixed concentrations of palmitate or of oleate and at increasing concentrations of albumin, show that this protein increases the rate of palmitate uptake with a maximum at palmitate/albumin molar ratios between 7 and 10 while it decreases the rate of oleate uptake under all conditions. Albumin cannot be replaced by other serum proteins; its effect is specific to saturated fatty acids, can be mimicked by the detergent Tween 40 and involves the binding of the fatty acid to the protein, prior to its delivery to the cell. Both with labelled saturated and unsaturated fatty acids the presence of albumin lowers the proportion of unesterified fatty acids and enhances the proportion of esterified fatty acids recovered in the cardiac cell after uptake. A similar effect of albumin was also found with hepatocytes and permanent cell lines. A specific role for serum albumin is presented, which assumes a 'dispersing' effect of this protein towards dimers (or higher aggregates) of saturated fatty acids and the entry of fatty acids into the cell as monomers.

As shown recently, fatty acids enter cultured cardiac cells through two mechanisms, one involving a saturable process and another resembling passive diffusion [1]. Some observations upon the role of serum albumin indicated that the rate of uptake was controlled by the total fatty acid concentration, that is the fatty acid unbound and bound to the protein; serum albumin was also observed to have a potent stimulating effect on the uptake of stearate as compared to that of oleate. This observation was unexpected and attributed to the physical properties attached to the unsaturation degree of fatty acids [1]; therefore we decided to investigate further the role of serum albumin reported below. Our results show on the one hand that this protein prevents an accumulation of unesterified saturated and unsaturated fatty acids in the cells. They show on the other hand that the stimulatory effect of serum albumin upon the uptake rate of saturated fatty acids can be mimicked by a detergent, Tween 40, and could be due to the disruption of dimers and/or microaggregates of fatty acids.

MATERIALS AND METHODS

Methods

Chick embryo heart cells were prepared and cultured on medium B as previously described [1]. Rat hepatocytes were prepared according to Lecam *et al.* [2]; they were suspended in medium B containing 10% fetal calf serum and antibiotics [1]. The medium was changed after 4 h in order to eliminate unattached cells and cell debris. [^{3}H]Leucine (0.5 μCi per 35-mm culture dish containing an average of 2.5×10^6 cells) was added and the cells were used for uptake of ^{14}C-labelled fatty acids after 24 h; all uptake rates were subsequently normalized by double labelled counting. Fibroblasts from the original strain 3T3-M [3] and its transformed 3T6 counterpart [4] were cultured in Dulbecco-Vogt's modified Eagle's medium and used after 32 passages and 14 passages respectively; as for hepatocytes, the rates of ^{14}C-labelled fatty acid uptake were normalized versus the amount of [^{3}H]-leucine incorporated. The preparation of the different fatty acid–protein complexes, the uptake of labelled fatty acids and the analysis of radioactive lipids were performed as previously reported [1]. The procedure of Stanley and Williams [5] was used for the determination of the intracellular concentration of ATP. When needed, cultured heart cells were treated for 60 min at 37 °C in minimal medium [1] containing 0.3 mM dinitrophenol and 0.1 mM iodoacetate. Under these conditions the cells remain fully viable, as shown by Trypan blue exclusion, although their ATP concentration decreases by two orders of magnitude (from an average of 9 mM to below 80 μM). The uptake of fatty acid was subsequently performed in

the presence or in the absence of the inhibitiors with identical results.

Materials

NCTC 135 medium and fetal calf serum were from Gibco, trypsin and Eagle's minimum essential medium from Institut Pasteur Production and crude collagenase from Worthington Biochemicals. ^{125}I-labelled bovine serum albumin (1.32 Ci/g), [1-^{14}C]-linoleate and [U-^{14}C]uridine were purchased from Amersham Centre. [1-^{14}C]Acetate, [1-^{14}C]laurate, [1-^{14}C]palmitate, [1-^{14}C]oleate, D-[U-^{14}C]glucose and L-[2-^{14}C]tryptophan were from the Commissariat à l'Energie Atomique.

Bovine serum albumin (fraction V, fatty-acid-poor) was a product of Sigma and further delipidated according to Chen [6]. Chicken serum albumin, bovine β-globulin (fraction IV) and bovine immunoglobulin (fraction II) were purchased from Miles Laboratories Ltd.

RESULTS

Uptake of Saturated and Unsaturated Fatty Acids at Increasing Albumin Concentrations

As shown in Fig. 1 the presence of serum albumin, at two fixed concentrations of palmitate (1 and 10 μM), stimulates the rate of fatty acid uptake by the cardiac cell with a maximum at palmitate/albumin molar ratios between 7 and 10 (Fig. 1 A and B). The curves of Fig. 1 C and 2 C give, as a function of fatty acid/albumin molar ratio, the concentration of total unbound fatty acid and the distribution of the bound form between the different macroscopic species, that is the different fatty acid–albumin complexes of stoichiometry 1:1, 2:1 . . . up to 8:1 for palmitate and for oleate. No parallelism was observed between the concentration of unbound palmitate or oleate (Fig. 1 C and 2 C) and the uptake rate (Fig. 1 B and 2 B), which suggests that not only unbound fatty acid but also fatty acid bound to albumin are recognized in order to enter the cell.

The fact that the increase in the uptake rate of palmitate occurs when the concentration of the macroscopic complexes increase and while at the same time the initial concentration of unbound fatty acid decreases in the uptake medium, also suggests that bound palmitate is better recognized than unbound palmitate. It is in agreement with experiments reported in Fig. 7 and previously [1], which indicate that the presence of albumin decreases the K_m value by one order of magnitude while the V value remains unchanged. Below a palmitate/albumin molar ratio of approximately 7, that is by increasing albumin concentration, a decrease in the uptake rate appears.

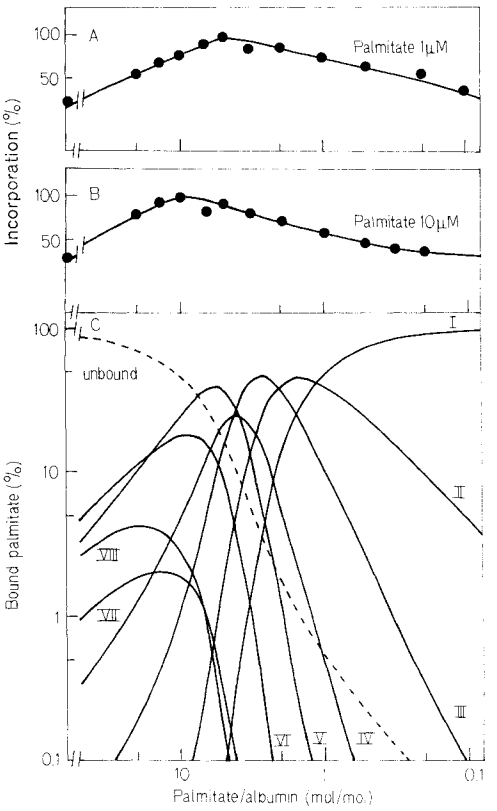

Fig. 1. *Effect of serum albumin on the uptake of palmitate.* The initial rates of uptake were determined at 37 °C as previously described [1]. The different solutions of palmitate-albumin complexes were pre-equilibrated for at least 60 min at 25 °C before use. The 100% values refer to 0.022 nmol incorporated · min^{-1} · (10^6 cells)$^{-1}$ in (A) and 0.13 nmol incorporated · min^{-1} · (10^6 cells)$^{-1}$ in (B). Note the same logarithmic scale of the ordinates for the uptake rate (A and B) and for the concentrations of unbound palmitate and of the different macroscopic palmitate-albumin complexes (numbered I-VIII in C). The concentrations of albumin varied from 0–10 μM in A (1 μM palmitate) and from 0–100 μM in B and C (10 μM palmitate). The concentrations of the complexes were calculated by mean of the classical Adair equation [7], using the stepwise equilibrium constant values determined by Spector et al. [8], and correspond to the experiments of B. The unbound palmitate concentrations used for computation were chosen by an iterative procedure similar to that described by Wosilait and Nagy [9]

This low decrease is correlated by a decrease in the concentration of the different macroscopic fatty acid–albumin complexes of high stoichiometry (approximately from 8:1 to 4:1) and by an increase in the concentration of the complexes of low stoichiometry (approximately from 3:1 to 1:1). It is very likely that, at the level of individual sites, this decrease is correlated by a displacement of palmitate from a majority of low and medium affinity sites to an increasing majority of high affinity sites of albumin for fatty acids. Therefore, at palmitate/albumin molar ratio between 3 and 0.1, the latter sites 'compete' with the cardiac cell for the fatty acid and the rates of uptake become similar or even lower to that observed in the absence of albumin.

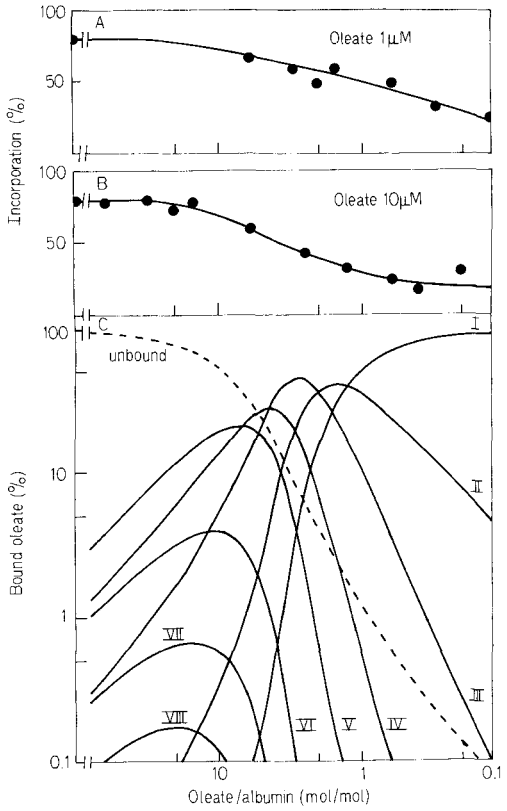

Fig. 2. *Effect of serum albumin on the uptake of oleate.* The conditions are identical to those described in Fig. 1. The 100% values refer to 0.025 nmol incorporated · min^{-1} · $(10^6$ cells$)^{-1}$ in (A) and to 0.15 nmol incorporated · min^{-1} · $(10^6$ cells$)^{-1}$ in (B). The legends are identical to those of Fig. 1. The first eight macroscopic complexes are numbered I–VIII; their concentrations correspond to the experiments of B

As indicated by the curves of Fig. 2, no stimulation of oleate uptake (present at two fixed concentrations) occurs but on the contrary the inhibitory effect of albumin is visible under all conditions, mainly when the oleate-albumin complexes of high affinity become the major species. As for palmitate, there is no parallelism between the decrease in the uptake rate (Fig. 2B) and the decrease in the concentration of unbound oleate (Fig. 2C). These results indicate that both bound and unbound oleate can be recognized by the cardiac cell.

The recognition of both bound and unbound fatty acid is also supported by the experiments reported in Fig. 3A: the addition to the uptake medium first of albumin, then of palmitate, shifts the control curve (palmitate alone) to a curve similar to that obtained with the preequilibrated palmitate-albumin complex. Moreover this conclusion is strengthened by the curve of Fig. 3B showing that the β-globulin (and immunoglobulin, not shown), which does not significantly bind fatty acids, has a negligible effect upon the uptake rate; on the contrary the stimulatory effect of chicken serum albumin upon palmitate uptake is identical to that of bovine serum albumin (not shown). Therefore both the stimulatory and inhibitory effects of albumin are relevant to its affinity for fatty acid. The stimulatory effect of serum albumin is specific to saturated fatty acids. No stimulation occurs when glucose, leucine, acetate or uridine are the substrates. Moreover no stimulation is observed for tryptophan, although this compound binds to serum albumin with a good affinity ($K_a = 1.6 \times 10^4$ M^{-1} [10]) (not shown). Since on the one hand bound fatty acid can be recognized in order to enter the cell, and since on the other hand serum albumin does not enter the cell [1] and is not metabolized (not shown), it is very likely that fatty acid does enter the cell after dissociation from the protein.

Nature of the Products Formed in the Absence and in the Presence of Serum Albumin

The curves of Fig. 4 indicate that, at all concentrations of palmitate with a constant palmitate/albumin molar ratio, the presence of albumin stimulates the total uptake of the fatty acid. The rate of labelled palmitate incorporated into polar and neutral lipids (diglycerides plus triglycerides), as well as its percentage by taking the total recovery of esterified and unesterified palmitate as 100% basis, are increased in the presence of albumin. The stimulation factor, defined as the percentage of labelled fatty acid recovered into esters in the presence of albumin versus the percentage of labelled fatty acid recovered into esters in the absence of albumin, was calculated to be 1.6 at 5 μM palmitate and 2.6 at 100 μM palmitate. A similar stimulation of the incorporation of palmitate into esters was observed at equal rates of uptake (Fig. 1) obtained at 10 μM palmitate in the absence of albumin or in the presence of 50 μM albumin (not shown). On the other hand, when albumin is present, the rate of formation of radioactive aqueous products does not vary significantly. However it must be pointed out that the stimulatory effect of albumin is not an effect upon the esterification processes *per se*, but is an effect linked to an event occurring prior to the fatty acid entry: an identical stimulation was observed when the cardiac cells were poisoned by iodoacetate plus dinitrophenol (see Methods); under these conditions the percentage of palmitate recovered under an unesterified form was higher than 97% and no aqueous metabolites were detectable.

A different picture emerges when oleate is taken up by the cells, as shown in Fig. 5. The rate of uptake is significantly decreased in the presence of albumin, with a significant decrease in the recovery of unesterified labelled oleate. It is clear that the difference in the uptake rate with and without albumin becomes more important at high concentrations of oleate. The increasing contribution of the passive diffusion-like component [1] and/or the increased rate of fatty

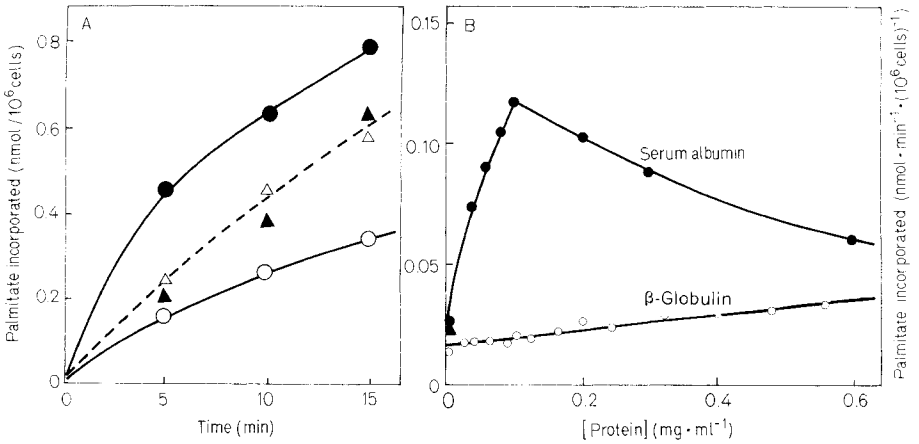

Fig. 3. *Effect of sequential addition of serum albumin on the initial rate of palmitate uptake and the lack of effect of β-globulin.* (A) The concentration of palmitate was 8 μM in all assays. (○) No serum albumin; (△) 1.15 μM serum albumin and [1^{14}C]palmitate at zero time; (▲) 1.15 μM serum albumin added to the cells in the uptake medium 5 min before starting the experiment by addition of [1-^{14}C]palmitate; (●) a solution containing 1 mM palmitate and 0.143 mM serum albumin was equilibrated beforehand and used directly after dilution in the uptake medium. (B) The concentrations of palmitate was 5 μM in all assays. (●) Albumin; (○) β-globulin

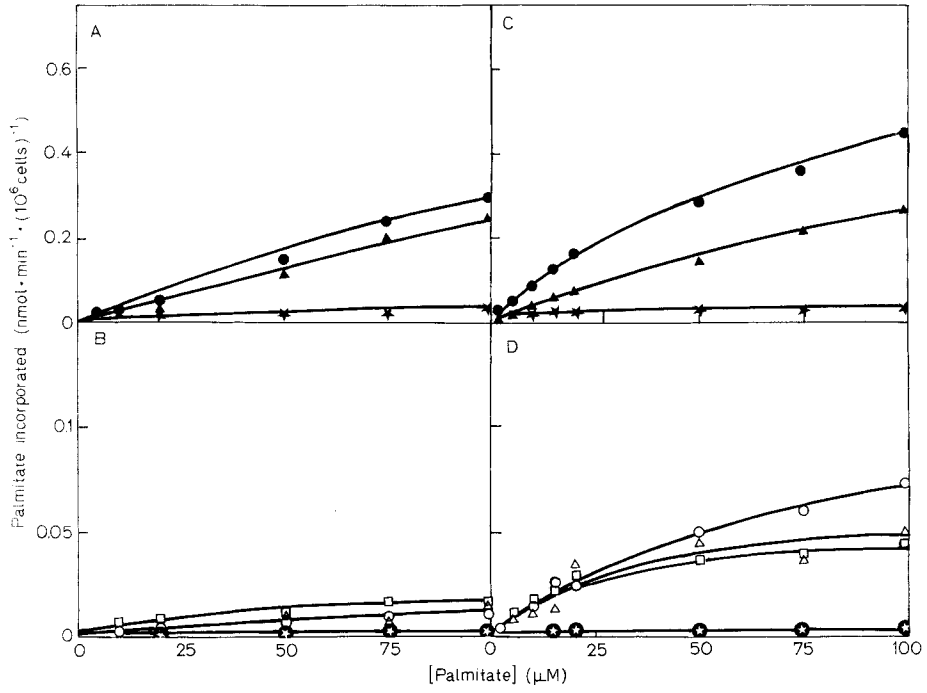

Fig. 4. *Nature of the labelled compounds recovered in the presence or in the absence of serum albumin at increasing concentrations of [1-^{14}C]palmitate.* The palmitate/albumin molar ratio was 6.0 in all experiments. The other assay conditions are described in Methods. The aqueous phase was counted after total extraction of the lipids (esterified plus unesterified fatty acids); (A, B) no albumin; (C, D) albumin present. (●) Total uptake; (▲) unesterified fatty acid; (✱) aqueous phase; (□) polar lipids; (△) diglycerides; (○) triglycerides; (✪) monoglycerides

acid efflux observed in the presence of albumin (see Discussion) could be involved in this observation. In the second place the curves of Fig. 4 indicate that, at all concentrations of palmitate, the presence of serum albumin increases the rate of incorporation (and the percentage) of the labelled fatty acid recovered into polar and neutral lipids. With oleate the curves of Fig. 5 indicate that at equal rates of uptake firstly, a rather higher proportion of oleate incorporated into esters can be recovered when albumin is present and secondly, the incorporation rate of oleate into triglycerides is increased and that into polar lipids and diglycerides decreased.

The effects of serum albumin were extended to another saturated fatty acid, laurate, and to another unsaturated one, linoleate. The curves of Fig. 6 are in agreement with the results of Fig. 4 and 5: when present, albumin increases the incorporation rate of laurate

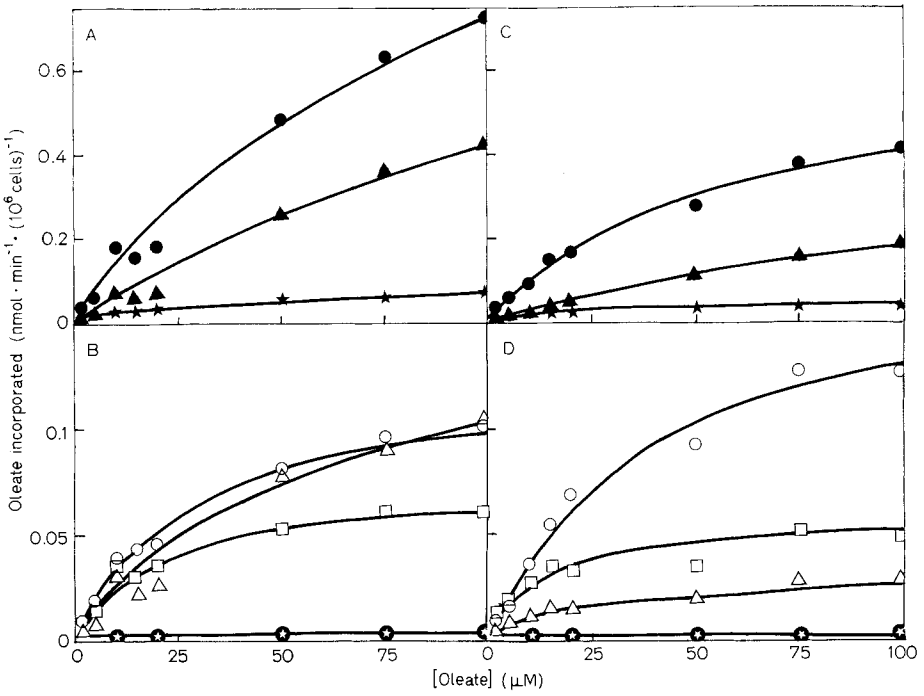

Fig. 5. *Nature of the labelled compounds recovered in the presence or in the absence of serum albumin at increasing concentrations of oleate.* The oleate/albumin molar ratio was 6.0 in all experiments. The other conditions and the legends are identical to those of Fig. 4; (A, B) no albumin, (C, D) albumin present

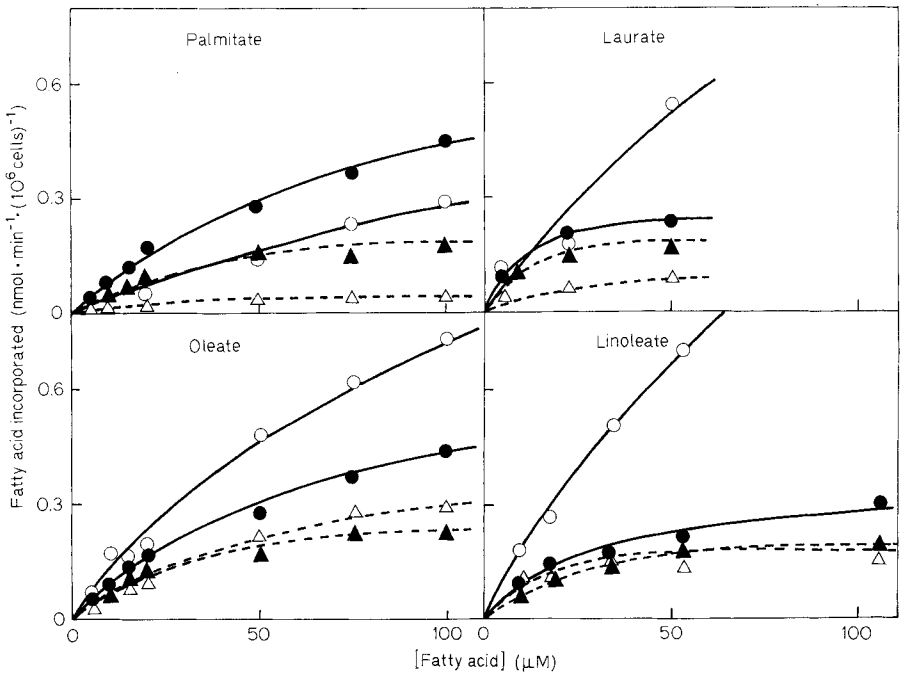

Fig. 6. *Effect of serum albumin upon the nature of the labelled compounds recovered after incorporation of palmitate, laurate, oleate or linoleate.* The fatty acid/albumin molar ratios were respectively 6 for palmitate and for oleate, 4.7 for laurate and 6.6 for linoleate. (●) Total lipids, albumin present; (○) total lipids, no albumin; (▲) total esters, albumin present; (△) total esters, no albumin. The curves obtained for palmitate and for oleate were redrawn from the curves of Fig. 4 and 5 respectively

into esters and increases, at equal rates of uptake, the percentage of incorporation of linoleate into esters. In both cases the presence of albumin lowers the proportion of labelled unesterified fatty acids recovered into the cell.

Effect of Serum Albumin on the Uptake of Palmitate in Other Cultured Animal Cells

Experiments were conducted with other cultured animal cells in order to see whether the effect of serum

Table 1. *Stimulatory effect of serum albumin on the uptake of palmitate by different animal cells*
The palmitate concentration was 15 μM in all assays. The palmitate/albumin molar ratio was 6.0 with the cardiac cells and 5.3 with the other animal cells. The other assay conditions are those described in Fig. 1. The aqueous phase was counted after total extraction of the lipids as previously described [1]. The numbers in parentheses correspond to the percentage of labelled palmitate recovered into esters (polar plus neutral lipids) versus labelled palmitate recovered into total lipids (polar plus neutral lipids plus unesterified fatty acids)

Addition	Cells	[^{14}C]Palmitate recovered in			
		total	esterified fatty acid fraction	unesterified fatty acid fraction	aqueous phase
		pmol × min^{-1} × mg^{-1} (%)			
None	cardiac	345	95 (32)	200	50
	hepatocytes	135	15 (12)	112	8
	3T3 fibroblasts	218	42 (21)	156	20
	3T6 fibroblasts	94	44 (49.5)	45	5
Serum albumin	cardiac	934	396 (53)	354	184
	hepatocytes	292	117 (47)	131	44
	3T3 fibroblasts	243	76 (40)	114	43
	3T6 fibroblasts	353	292 (86)	48	13

albumin was specific or not to the chick embryo cardiac cells. Although the capacity of the different lines to synthesize esters varied largely, low in hepatocytes and high in the transformed 3T6 cell line, the stimulation factor (as defined above) was, under conditions used for cardiac cells, approximately two-fold in all cases, as shown by the data of Table 1. Therefore, serum albumin presents a general ability to stimulate the formation of esters in all cells when palmitate is the substrate by facilitating its entry into the cells.

Fatty Acid Uptake in the Presence of Tween 40

To summarize the above results, it is clear that serum albumin does specifically stimulate the uptake of long-chain saturated fatty acids and their incorporation into esters in all animal cells and it does lower the proportions of radioactive unesterified saturated and unsaturated fatty acids recovered in the cultured cardiac cells after uptake, and it also presents no effect upon the uptake of other solutes.

Since the fatty acid has first to be bound to albumin before being delivered in order to enter the cell (Fig. 1 – 3), an explanation would be that this binding favors in some way the presentation of the saturated fatty acids as monomers. This increase in the formation of monomers should correspond to the possible but controversial existence of dimers (or higher aggregates) of fatty acids in solution [11]. Such an effect of serum albumin favoring the formation of monomers should be mimicked by detergents since these compounds, which associate to fatty acids mainly above their critical micellar concentration, should be capable of disrupting dimers and/or higher aggregates of fatty acids.

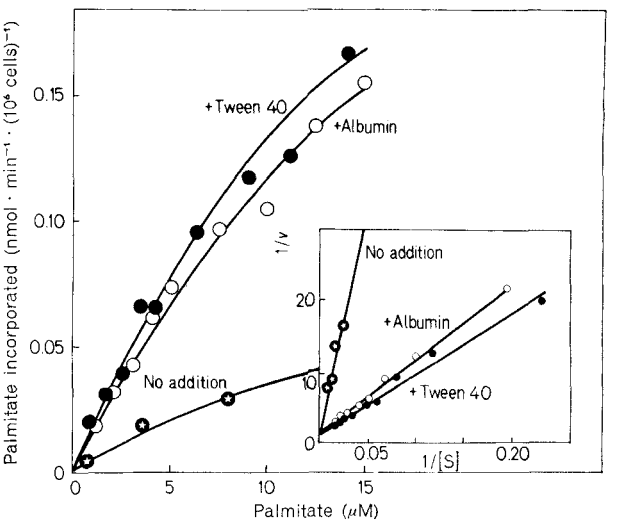

Fig. 7. *Saturation curves for palmitate in the absence or in the presence either of Tween 40 or of albumin.* The palmitate/albumin molar ratio was equal to 7 and Tween 40 was present at a concentration of 0.05% in all experiments. (○) Palmitate alone; (○) palmitate plus albumin; (●) palmitate plus Tween 40

Among the different neutral detergents tested (Brij 35, Triton X-100, Triton X-305, Tween 40 and Lubrol) Tween 40 was found to be the least harmful to the cardiac cell:cell viability was unaffected by 0.1% Tween 40 during 30 min at 37 °C under the uptake conditions. As presented in Fig. 7, the comparison of the saturation curves for palmitate alone and for palmitate plus Tween 40 clearly shows that the stimulatory effect of the detergent is, as for albumin, an effect on the K_m value (20 μM against 70 μM) and not on the V value [0.33 nmol · min^{-1} (10^6 cells)$^{-1}$ in both cases].

The results of Table 2 indicate that the stimulation by Tween 40 of palmitate uptake is maximal when the detergent is present above its critical micellar concentration, whereas an inhibition of oleate uptake is observed under these conditions. It is not known whether polyoxyethylene sorbitol monopalmitate enters the cell as palmitate does, or does not enter as it was previously shown for albumin [1]. In any event the data of Table 3 indicate that both Tween 40 and albumin stimulate the rate of palmitate uptake and the rate of its incorporation into polar and neutral lipids.

DISCUSSION

The observations reported in this paper show that albumin, which presents among the different serum proteins the specific ability to bind fatty acids, stimulated the uptake of saturated fatty acids by cultured cardiac cells. This phenomenon, mainly studied with palmitate, was extended to hepatocytes as well as to untransformed 3T3-M cell line and to its transformed 3T6 counterpart. The stimulation occurs providing that palmitate binds to albumin with formation of the different complexes which include a majority of macroscopic species of stoichiometry 8:1 to 4:1. It can be assumed, and calculated with some approximations (not shown), that under these conditions the majority of palmitate molecules binds to the low and medium affinity sites of serum albumin. Therefore the addition of the protein, which can be replaced by Tween 40 above its critical micellar concentration, should favor through binding of the fatty acid the equilibrium

dimers of palmitate (or higher aggregates) $\rightleftharpoons$ monomers of palmitate (bound and unbound to albumin)

to the right. If one assumes that dimers (or aggregates) cannot penetrate the cell, such an equilibrium would be compatible with the change observed (when albumin is present) for palmitate in the K_m value with no change in the V value; the formation of dimers (or aggregates) could be related to the diminished rotational freedom of the saturated fatty acyl chains. The effect of serum albumin would be a stimulatory one until the majority of bound palmitate is associated to the high affinity sites. The latter sites would then behave as competitors of the cardiac cells for palmitate. This hypothesis would imply that unsaturated fatty acids with increased fluidity would not extensively form dimers (or aggregates) under the same conditions; although not exceeding a 3-fold difference, the degree of dimerization was calculated to be lower for oleate than palmitate and this result would be in favour of our hypothesis [13]. If it is so albumin, when present, would simply behave as a competitor of cardiac cells for unsaturated fatty acids and this would lead to a decrease observed in the uptake rate of oleate under all conditions.

As shown in Fig. 1, the stimulatory effect of albumin upon the uptake rate of palmitate is maximal

Table 2. *Effect of different concentrations of Tween 40 below and above its critical micellar concentration*
The initial rates of uptake were measured as described in Methods in the presence of 10 μM palmitate or of 9 μM oleate. The critical micellar concentration of Tween 40, determined with iodine at 345 nm [12] in the absence or in the presence of 10 μM palmitate under conditions identical to those used for uptake studies, was found to be around 0.012% (range 0.008 – 0.016%)

Tween 40	[^{14}C]Palmitate uptake	[^{14}C]Oleate uptake
	nmol × min^{-1} × (10^6 cells)$^{-1}$ (%)	
0	0.063 (100)	0.12 (100)
0.005	0.077 (122)	0.12 (100)
0.009	0.096 (152)	0.10 (83)
0.05	0.14 (220)	0.07 (58)
0.1	0.123 (195)	0.06 (50)

Table 3. *Nature of the labelled compounds recovered in the absence or in the presence of Tween 40*
The conditions of uptake and the analysis of the labelled products were performed as described in Methods. Experiment 1: 8 μM palmitate; experiment 2: 50 μM palmitate; experiment 3: 10 μM palmitate plus 0.05% Tween 40; experiment 4: 10 μM palmitate plus 1.5 μM serum albumin

Experiment number	[^{14}C]Palmitate recovered in					
	Total	esterified fatty acid fraction			unesterified fatty acid fraction	aqueous phase
		total	neutral	polar		
	nmol × min^{-1} × (10^6 cells)$^{-1}$					
1	0.057	0.025	0.012	0.013	0.013	0.017
2	0.164	0.049	0.023	0.026	0.073	0.032
3	0.215	0.107	0.070	0.037	0.089	0.015
4	0.189	0.114	0.058	0.056	0.025	0.043

at molar ratios between 7 and 10. The physiological significance, if any, of this observation remains to be evaluated since *in vivo* the molar ratios usually vary between 0.3 and 1.6 in man and in neonatal chick [11] and since the concentration of albumin is approximately 0.6 mM and that of unesterified fatty acids is between 0.2 and 1 mM. However it must be pointed out that *in vivo* the fatty acid/albumin molar ratio can attain much higher values in the serum [14] and that the concentrations of albumin and of fatty acids in the immediate vicinity of the cells are not precisely known.

At equal rates of uptake the presence of albumin leads to a significant increase in the proportion of labelled fatty acids recovered into esters (Table 3, experiments 2 and 4). Two possible explanations were excluded. First it could be that either in the absence or in the presence of low concentrations of serum albumin as compared to that of the fatty substrate—that is at high concentration of unbound fatty acid—these compounds (or products derived from them such as acyl-CoA) would act as uncouplers of oxidative phosphorylation [15]. This uncoupling effect would decrease the intracellular concentration of ATP and lead to a decrease in the proportion of esterified fatty acids. Control experiments clearly indicated that it is not the case: under conditions similar to those used in the uptake studies (3 min at 37 °C, 20 μM palmitate) no significant change in the ATP content of the cells (approximately 9 mM) can be observed as compared to cells maintained in the absence of palmitate and albumin or in the presence of albumin alone.

A second possible explanation would be that the serum-albumin-saturated fatty acid complex is a better substrate than the unbound fatty acid, through the specific recognition of the protein by some components of the outer surface of the cardiac cell. Extensive studies were conducted to analyze the binding to the cardiac cells of ^{125}I-labelled serum albumin (either free or bound to [^{14}C]palmitate with a molar ratio of 2.5). The results are not in favour of the recognition of albumin since we did not observe any saturation curve for albumin from 1 nM to 0.1 mM, any protection of ^{125}I-labelled albumin binding by prior incubation with unlabelled albumin, or any displacement of labelled albumin first bound to the cells by subsequent addition of unlabelled albumin. Therefore a possible explanation regarding the increase in the proportion of labelled fatty acids recovered into esters would be that albumin favors in some way the orientation of the fatty acid entering the cell or more likely that it favors the efflux of the fatty acid superficially but firmly bound to the plasma membrane of the cell (it must be recalled that a simple adsorption process of fatty acids into the membranes has been previously excluded [1]). It is known that the fatty acid bound to the sarcolemma cannot become

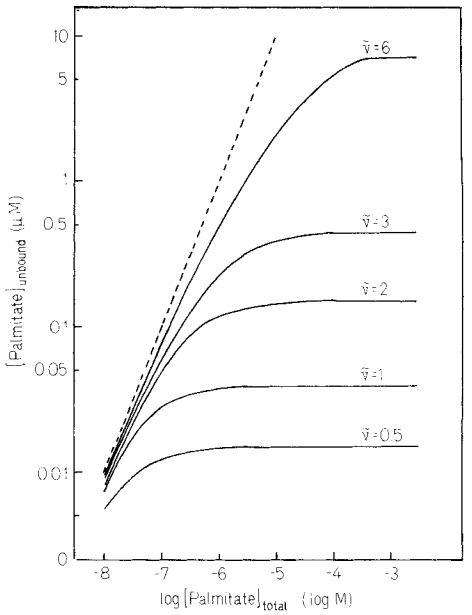

Fig. 8. *Concentrations of unbound palmitate as a function of increasing concentrations of total palmitate and at different palmitate/albumin molar ratios ($\bar{\nu}$).* The concentration of unbound palmitate was calculated as described in Fig. 1. The curve with dashed line would be obtained if the concentration of total palmitate would equal that of unbound palmitate, that is in the absence of albumin

esterified since no fatty-acid-activating enzymes are present at this level [16]. The hypothesis of an increased efflux rate is supported by the fact that, after preloading of the cells in the absence of albumin, the rate of discharge is very low if albumin is absent from the efflux medium (not shown); in other words the presence of albumin would prevent the accumulation of unesterified fatty acids at the plasma membrane level. Although it is hazardous to extrapolate to the situation *in vivo*, this 'normalizing' role of serum albumin could be of importance if one recalls the low proportion of unesterified fatty acids present in chick embryo and mamalian cardiac cells [17].

The curves of Fig. 8 show on the one hand that, at any given molar ratio between 0.5 and 3, the concentration of unbound palmitate becomes nearly independent of the total concentration of the fatty acid between 3 μM and 1 mM; on the contrary the concentration of unbound palmitate increases significantly at a molar ratio of 6 under the same range of concentration. The curves of Fig. 8 clearly show on the other hand that, if one assumes a constant physiological concentration of 0.6 mM albumin, increasing the total fatty acid concentration from 0.3–3.6 mM (and above) will lead to an increase of unbound palmitate from 15 nM to 7.8 μM. Therefore under almost any physiological condition, that is at molar ratios between 0.2 and 4, the fatty acids dissociating from the different sites of albumin should enter normally the cells while the concentration of unbound fatty acid is negligible. However, at the highest molar ratios the increase in

the micromolar range (and above) of the unbound fatty acids might eventually lead in the case of the saturated substrates to the formation of dimers or higher aggregates in the blood plasma.

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REFERENCES

1. Samuel, D., Paris, S. & Ailhaud, G. (1976) *Eur. J. Biochem. 64*, 583–596.
2. Lecam, A., Guillenzo, A. & Freychet, P. (1976) *Exp. Cell Res. 98*, 382–395.
3. Todaro, G. J. & Green, H. (1963) *J. Mol. Biol. 17*, 299–313.
4. Todaro, G. J., Green, H. & Goldberg, B. D. (1964) *Proc. Natl Acad. Sci. U.S.A. 51*, 66–73.
5. Stanley, P. E. & Williams, S. G. (1969) *Anal. Biochem. 29*, 381–392.
6. Chen, R. F. (1967) *J. Biol. Chem. 242*, 173–181.
7. Adair, G. S. (1925) *J. Biol. Chem. 63*, 529–545.
8. Spector, A. A., Fletcher, H. E. & Ashbrook, J. D. (1971) *Biochemistry, 10*, 3229–3232.
9. Wosilait, W. D., Soler-Argilaga, C. & Nagy, P. (1976) *Biochem. Biophys. Res. Commun. 71*, 419–426.
10. McMenamy, R. H. & Oncley, J. L. (1959) *J. Biol. Chem. 233*, 1436–1447.
11. Spector, A. A. (1975) *J. Lipid Res. 16*, 165–179.
12. Ross, S. & Olivier, J. P. (1959) *J. Phys. Chem. 63*, 1671–1675.
13. Mukerjee, P. (1965) *J. Phys. Chem. 69*, 2821–2827.
14. Rodahl, K., Miller, H. I. & Issekutz, B., Jr (1964) *J. Appl. Physiol. 19*, 489–492.
15. Pressman, B. C. & Lardy, H. A. (1956) *Biochim. Biophys. Acta, 21*, 458–466.
16. Paris, S., Fosset, M., Samuel, D. & Ailhaud, G. (1977) *J. Mol. Cell Cardiol. 9*, 161–174.
17. Oram, J. F., Bennetch, S. L. & Neely, J. R. (1973) *J. Biol. Chem. 248*, 5299–5309.

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