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Effect of unsaturated fatty acids on aspartate transport in *Staphylococcus aureus* and on staphylococcal lipid monolayers

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SUMMARY

Unsaturated fatty acids, ranging from $C_{16:1}$ to $C_{22:6}$, have been shown to stimulate the uptake and accumulation of aspartate by *Staphylococcus aureus*. Unsaturated, but not saturated, fatty acids prevent the thermal expansion of monolayers of staphylococcal lipid spread on a water–air or acid–air interface; the weight of fatty acid required to prevent expansion of a given amount of lipid can be correlated with the weight producing a given stimulation of aspartate accumulation by intact cells containing that same amount of lipid.

Staphylococcus aureus accumulates certain amino acids, *e.g.* glutamate, aspartate, lysine in the free stage within the cell so that the internal concentration may be 2–3 orders of magnitude greater than the external concentration[1]. Gale and Folkes[1] found that transport of aspartate and glutamate but not lysine by washed suspensions of *S. aureus* was stimulated by staphylococcal lipid; hydrolysis and fractionation of the lipid showed that activity resided in the unsaturated fatty acid fraction which could be replaced by $C_{18:1}$ and $C_{18:2}$ acids. Osmotic shock decreases the activity of the same transport system, and normal activity can be restored by addition of lipid, found to be enhanced in unsaturated fatty acids, prepared from concentrated "shock supernatant"[2]. A range of unsaturated fatty acids has now been tested under the conditions described by Gale and Folkes[1]; Table I shows that all had some stimulatory effect on aspartate accumulation, the response varying in the degree of stimulation obtained and the amount of fatty acid required to produce a given stimulation. The rate of aspartate accumulation was approximately doubled by $C_{22:6}$, $C_{20:5}$, $C_{18:1}$, $C_{18:2}$, $C_{16:1}$ and $C_{18:3}$ in order of decreasing effectiveness as judged by the amount of acid necessary to produce 25% stimulation. The other fatty acids tested, and the methyl esters of $C_{18:1}$ and $C_{18:2}$, were less effective. Of the positional isomers of $C_{18:1}$ tested, $\Delta 9$ and $\Delta 12$ were equally active while $\Delta 11$ and $\Delta 6$ were markedly less active. In all cases stimulation increased with fatty acid concentration to an optimum and then decreased, higher concentrations becoming

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TABLE I

THE ACTION OF FATTY ACIDS ON THE ACCUMULATION OF ASPARTATE BY *S. aureus*

S. aureus was harvested, washed once in a buffered salts solution[1], and 0.6 mg dry weight suspended at 20° in 1.5 ml buffered salts solution containing fatty acid as below; after 15 min the cells were centrifuged down and resuspended in 1.5 ml buffered salts solution containing 20 μM aspartate (uniformly ^{14}C-labelled, specific activity 10 mC/mmole), 1% (w/v) glucose and 50 μg chloramphenicol per ml. Tubes were incubated at 15° for experiments in columns (a) and (b) and at 10, 15, 20 and 25° for columns (c) and (d); tubes were harvested at appropriate times, reaction stopped by rapid cooling and addition of 0.1 ml 30 mM 2,4-dinitrophenol, and ratio of radioactivity in the hot water extractable pool to that in the external medium determined[1]. Q_{10} for control without fatty acid = 3.6, S.D. 0.35, n = 17, range 3.2–3.8.

Identification of fatty acid added	*Max. stimulation obtained (%)*	*μg fatty acid/1.5 ml giving*		*Effect of temperature*	
		Max. stimulation (a)	*25% stimulation (b)*	*μg fatty acid/1.5 ml (c)*	$Q_{10}^{15\text{-}25°}$ *(d)*
None	–	–	–	–	3.6 ± 0.35
14:1	50	5	2	–	–
16:0	0	–	–	10–20	3.4
16:1	100	5	2	–	–
18:0	0	–	–	10–20	3.4–3.5
18:1 Δ9	85	2	0.6	–	–
18:1 Δ12	85	2	0.7	–	–
18:1 Δ6	70	11	5	–	–
18:1 Δ11	70	6	3	–	–
18:2	90	3	1.3	3	3.0
18:3	65	9	4.5	10	2.9
20:0	0	–	–	10–20	3.6
20:1 Δ11	30	10	8	–	–
20:5	105	2	0.5	1	2.45
22:0	0	–	–	10–20	3.6
22:6	95	1.5	0.3	1.5	2.8
24:0	0	–	–	20	3.75
24:1	30	10	6	20	3.35
18:1 Δ9 Me	35	20	17.5	–	–
18:2 Me	25	15	15	–	–

inhibitory as previously reported[1,2]. The fatty acids are equally effective whether they are present during the amino acid uptake or taken up by the cells in the course of previous treatment in buffer containing known amounts of the fatty acids.

The rate of aspartate uptake was determined at temperatures ranging from 10 to 30° and the Q_{10} for 15 to 25° estimated for cells treated with various fatty acids. Table I shows that unsaturated fatty acids which gave a marked stimulation of aspartate accumulation also gave a significant decrease in the temperature coefficient for that process. Aspartate transport therefore occurs with a lower activation energy in the presence of unsaturated fatty acid. Later experiments were carried out at 15°. In the earlier investigations[1], small stimulatory effects were reported for saturated fatty acids, C_{16}–C_{22}, but these effects were very variable; working at 15° saturated fatty acids (C_{12}–C_{24}) have been consistently without effect and it may be that the earlier results were due to metabolic modification of the acids occurring during the experimental period.

The question is whether unsaturated fatty acids mediate aspartate transport by forming metabolic intermediates or act in an indirect fashion by altering the properties or

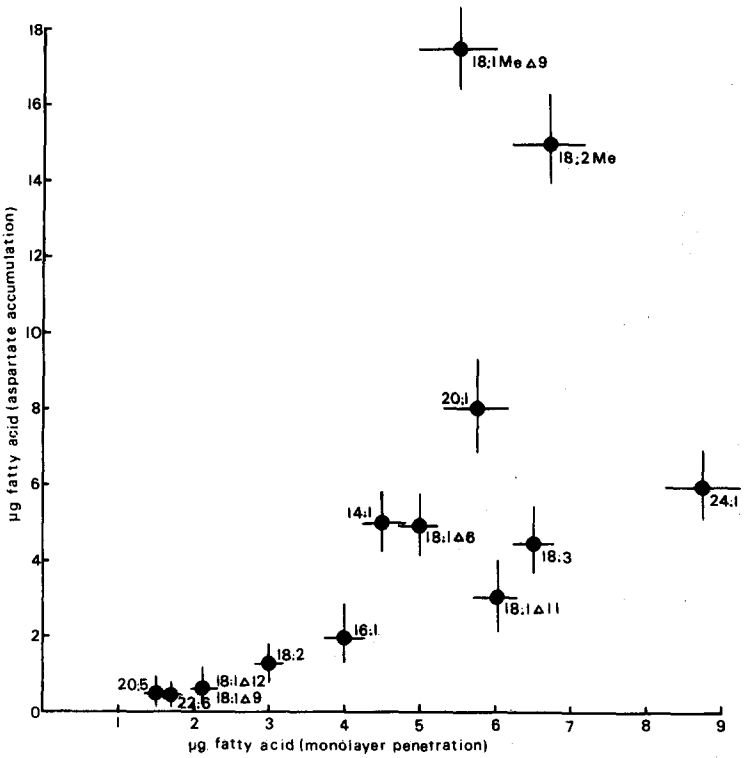

Fig. 1. Relationship between stimulation of aspartate accumulation in *S. aureus* and penetration of a monolayer of staphylococcal lipid by unsaturated fatty acids. Ordinate = μg fatty acid/0.6 mg dry weight of cells in 1.5 ml giving 25% stimulation of aspartate accumulation measured as in Table I. Abscissa = μg fatty acid added to monolayer of staphylococcal lipid, extracted from 0.6 mg dry weight of cells, to produce an increase in surface pressure of 7.5 dynes/cm when the area of the film is maintained at 165 cm^2.

organisation of the cell membrane. If the effect were due simply to alterations of the "melting point" or mobility of the hydrophobic core of the membrane, there should be a correlation between the melting point of the acid and its effect on aspartate accumulation; no clear-cut correlation of this nature is obtainable and such an explanation would, in any case, require some effect of short-chain saturated fatty acids. Accordingly the action of fatty acids on the properties of monolayers of staphylococcal lipid has been examined. Lipid was extracted and prepared as previously described[3] and applied to the surface of water or 0.1 M HCl in a Langmuir trough; lipid (approx. 16 μg) extracted from 0.6 mg dry weight of cells occupied an area of 165 cm^2 at a pressure of 5 dynes/cm which is in reasonable agreement with the measurements of Few[4]. No significant differences in pressure area curves or in the effects of fatty acids were observed whether experiments were carried out with distilled water or 0.1 M HCl as the aqueous phase, and acid has been used for all results quoted.

In one series of experiments, the ability of fatty acids to penetrate the monolayer was examined by measuring the increase in surface pressure when increasing amounts of fatty acids were added to the film at constant area[4,5]. Fig.1 shows the relationship between

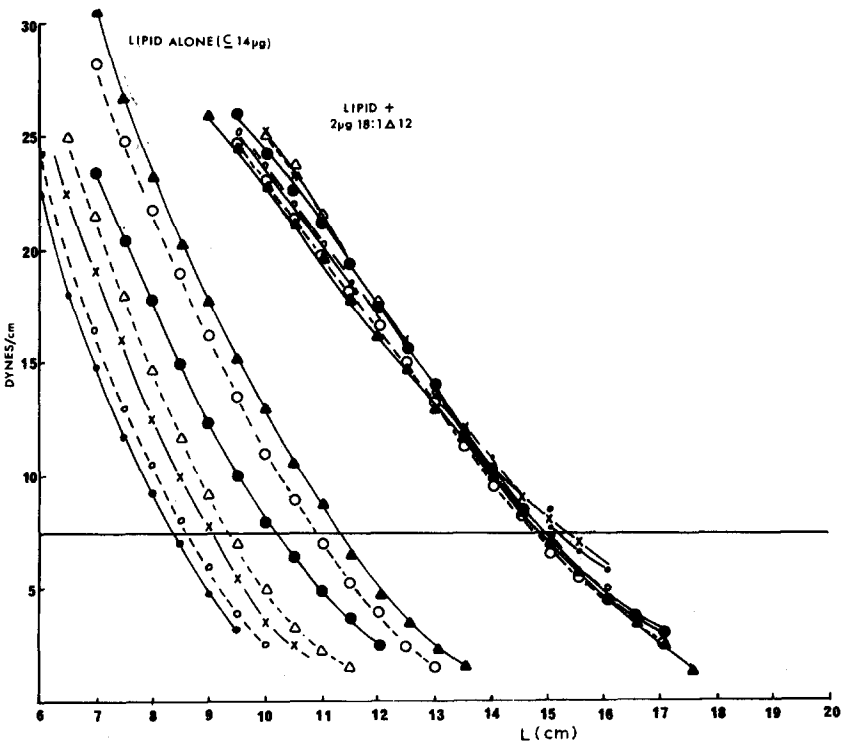

Fig.2. Effect of temperature on pressure–area curves obtained with monolayers of approx. 14 μg staphylococcal lipid (a) alone or (b) with addition of 2 μg octadecenoic acid (18:1). Staphylococcal lipid spread on 0.1 M HCl–air interface and pressure–area curves determined at 5° intervals from 5 to 35°. L = distance in cm between spacers limiting film; area = 15L cm^2. ●, 5°; o, 10°; X, 15°; Δ, 20°; ●, 25°; ○, 30°; ▲, 35°.

the amount of fatty acid giving 25% stimulation of aspartate accumulation in cells and the amount giving an increase of 7.5 dynes/cm in the standard film held at 165 cm^2. Any correlation that holds for the unsaturated fatty acids does not hold for their methyl esters; also, saturated C_{12}–C_{16} fatty acids penetrate the film at least as readily as some of the less active unsaturated fatty acids.

In a second series of experiments, the temperature of the monolayer was varied by passing temperature-controlled water through coils immersed in the aqueous layer. Area–pressure curves were then determined at 5° intervals over the range 5–35°. Fig.2 shows a family of such curves. Since the width of the film is constant, the distance (L) between the spacers enclosing the film can be taken as a measure of the area and it can be seen that, for the example shown in Fig.2, with a film containing approximately 14 μg staphylococcal lipid and maintained at a pressure of 7.5 dynes/cm, L increased from 8.3 cm at 5° to 11.4 cm at 35°. Fig.2 also shows that thermal expansion of this nature did not take place when 2 μg $C_{18:1}$ fatty acid was added to the film. The effect on thermal expansion depends upon the amount of fatty acid added; in the experiment illustrated in Fig.2, amounts of $C_{18:1}$ less than 2 μg decreased but did not prevent expansion whereas 2.5–3.0 μg gave a slight

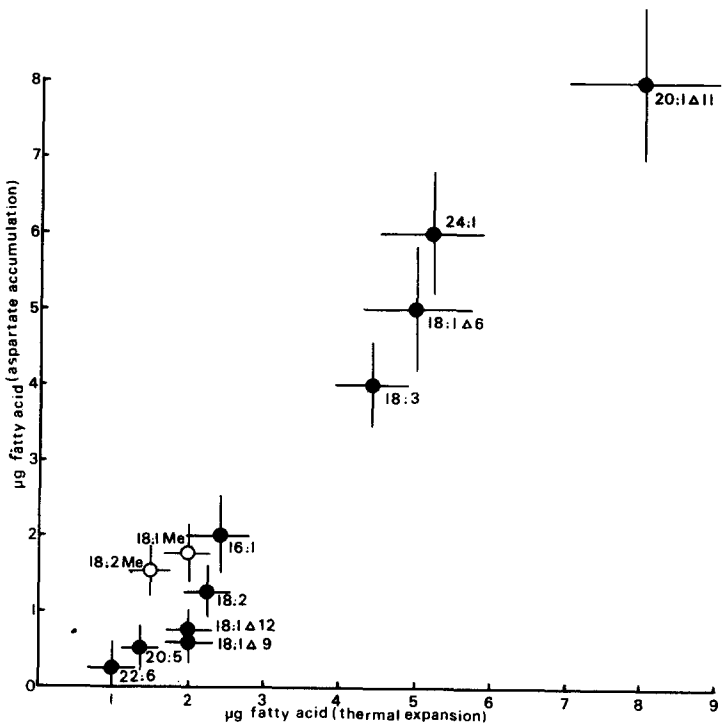

Fig.3. Relationship between stimulation of aspartate accumulation in *S. aureus* and inhibition of thermal expansion in a monolayer of staphylococcal lipid by unsaturated fatty acids. Ordinate = μg fatty acid/0.6 mg dry weight of cells in 1.5 ml giving 25% stimulation of aspartate accumulation measured as in Table I. Abscissa = μg fatty acid required to stop thermal expansion of monolayer of staphylococcal lipid as in Fig.2. For methyl esters of 18:1 and 18:2 values in both ordinate and abscissa are x 10^{-1}.

contraction of the film with rising temperature. More than 3 μg gave a film showing some degree of expansion again. This type of response was obtained with all the unsaturated fatty acids tested and with the methyl esters of $C_{18:1}$ and $C_{18:2}$; no inhibition of expansion was observed with up to 12 μg of any saturated fatty acid in the range C_{12}–C_{24}. Fig.3 shows the relationship between the amount of fatty acid producing 25% stimulation of aspartate accumulation in cells and the amount preventing thermal expansion of a monolayer of lipid extracted from an equivalent amount of cells; linear correlation is obtained between the two quantities for all the unsaturated fatty acids and the two methyl esters tested.

It is difficult to interpret these data in terms of our present knowledge of membrane structure and permeability. The action of unsaturated fatty acids on aspartate accumulation can be correlated with their effect on certain physical properties of the membrane lipids. The effect on thermal expansion suggests that unsaturated fatty acids bind lipid molecules together and it may be that this would affect the formation of pores through the membrane. In this connection, it is interesting that the permeability of liposomes towards small organic molecules and also the valinomycin-induced leak of cations increases with increasing unsaturation of the fatty acids of the lipids[6,7] while Fox[8] has reported that unsaturated fatty acids are necessary for the induction of β-galactosidase in *Escherichia coli*.

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