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ON THE AMINO ACID ESTERS OF PHOSPHATIDYL GLYCEROL FROM BACTERIA

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SUMMARY

1. The phospholipids of *Staphylococcus aureus* were fractionated on silicic acid columns. The major compounds all appeared to be polyglycerol lipids. Diphosphatidyl glycerol and phosphatidyl glycerol were identified by comparison with the synthetic phospholipids.

2. An amino acid derivative of phosphatidyl glycerol was isolated in pure form and shown to be identical with an L-lysine ester of 1,2-diacyl-glycero-3-phosphoryl-1'-glycerol.

3. The content and composition of phospholipids of *Staphylococcus aureus* were found to depend on the pH of the medium. In cells harvested at pH 7.0 phosphatidyl glycerol was the major phospholipid, whereas the lysine ester of phosphatidyl glycerol appeared to prevail below pH 5. Under certain conditions a relative increase in diphosphatidyl glycerol was observed. These alterations in the phospholipid pattern of the bacterial culture were reversible.

4. Cells of *Streptococcus faecalis* manifested a comparable shift in the ratio of phosphatidyl glycerol and its lysine ester, but not all bacterial species studied responded in a similar way upon exposure to an acidic environment.

The phospholipid composition of *Eubacteriaceae* is known to differ significantly from that of other organisms. With a few exceptions choline containing phospholipids are lacking, while a number of bacterial species exhibit a rather low content of other types of amphiphatic phospholipids, certain negatively charged components being predominant (for references see 1). On the other hand some bacteria appear to produce more complex phospholipids which may compensate for the absence of simple phosphoglycerides containing a nitrogenous moiety. Of particular interest are the amino acid esters of phosphatidyl glycerol, since these compounds have been found to contain substantial quantities of basic amino acids[2,3]. *Staphylococcus aureus* has been demonstrated to contain as major phospholipids phosphatidyl glycerol (PG) and its *O*-lysine ester (lys-PG)[2,4]. It appeared possible to govern the ratio between both differently charged phospholipids in this bacterium[4] which gave opportunities

to study possible relations between structure and functions of the lipids concerned. The present paper gives further information on the isolation and chemical characteristics of lys-PG and the effect of the pH of the culture medium on the phospholipid composition of *S. aureus* and some other bacteria.

EXPERIMENTAL

Cells of *Staphylococcus aureus* PS 187 (NCTC 9754) were cultured in a broth containing 10 g of pepton, 10 g of yeast extract, 5 g of sodium chloride, 400 mg of sodium phosphate and 300 μC of [^{32}P]orthophosphate per l of water (pH 7.2). When mentioned, 10 g of glucose was added to this medium. After incubation for a given period at 37° the cells were harvested by centrifuging, washed with adequately buffered distilled water and extracted according to a modification of the procedure of BLIGH AND DYER[5]. The cells from 2 l of medium were made up to 30 ml with acetate or borate buffer of a given pH (mostly identical with that of the medium at the time of harvesting), then 30 ml of chloroform and 65 ml of methanol were added so as to give a one phase system. After storage for several hours at 2° the cells were collected by centrifugation and treated several times with the same solvent mixture until no more phospholipid could be extracted. The combined extracts were mixed with a quarter of the volume each of water and chloroform. The aqueous phase not containing any appreciable quantity of phospholipid was separated, and the chloroform layer was evaporated under reduced pressure.

To permit the determination of the content of total lipids and phospholipids on a dry weight basis in a number of experiments the packed cells were lyophilized, weighed and extracted according to the method described above. The lipids were dried over phosphorus pentoxide *in vacuo*; determinations of the phosphorus content were carried out according to established procedures[6], and the amount of phospholipid was calculated, the assumption being made that the bacterial phospholipids contained 4% of phosphorus.

Paper chromatography of the lipids was performed on silica impregnated paper according to the method of MARINETTI *et al.*[7]. The phospholipid composition was derived from the radioactivity of the separated spots. Individual phospholipids were isolated by fractionation of the lipid mixtures on silicic acid columns (MALLINCKRODT, 140–200 mesh) discontinuously eluted with mixtures of chloroform–methanol. The radioactivity was measured by running the eluate through a spirally wound capillary placed in front of an end-window G.M. tube. The radioactivity was recorded automatically thus making it possible to combine the fractions without taking any samples.

Mild alkaline hydrolysis of the phospholipids was carried out according to the method of BENSON AND MARUO[8]. The water-soluble products formed were identified by paper chromatography, the hydrolysates of various synthetic phosphoglycerides, being used as references.

Analyses of total nitrogen[9], amino nitrogen[10] and glycerol[11] were carried out according to established methods.

RESULTS AND DISCUSSION

In agreement with our previous observations[4] the phospholipid composition

of *S. aureus* was found to depend on the acidity of the medium. The conspicuous differences in the phospholipid pattern of cells harvested at pH 7.2 and pH 4.8 are illustrated by autoradiograms reproduced in Fig. 1. In this type of experiment cells

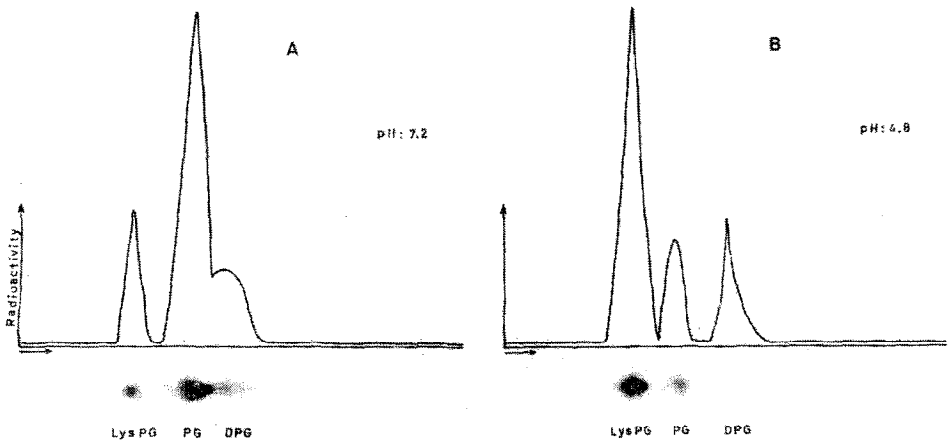


Fig. 1. Autoradiograms and radioactivity tracings of paper chromatograms showing the influence of pH of the culture medium on the phospholipid pattern of *S. aureus*. After reaching the stationary phase at pH 7.2 part of the cells was harvested and analysed (A). The pH of the remaining culture was brought to 4.8 and incubated for another 2 h (B). DPG, diphosphatidyl glycerol or cardiolipin; PG, phosphatidyl glycerol; Lys PG, lysyl-phosphatidyl glycerol.

were grown at pH 7.2 until the stationary phase was reached, whereafter a part of the cells was analysed. The pH of the remaining culture was adjusted to 4.8, and after subsequent incubation for 2 h at this pH the cells were extracted and analysed. Apparently phosphatidyl glycerol was the prevailing phospholipid of *S. aureus* when harvested at pH 7.2, whereas the amino acid derivative (lys-PG) dominated in the cells exposed to the acidic medium. Furthermore the relative amount of lys-PG recovered in the lipid extract from cells cultured at this pH was found to depend most significantly on the acidity of the aqueous phase used during extraction of the bacteria. As shown in Table I it was essential to maintain an acidic pH of the extraction

TABLE I

THE INFLUENCE OF PH DURING EXTRACTION OF THE LIPIDS FROM *S. aureus* ON THE PHOSPHOLIPID COMPOSITION

Cells harvested at pH 4.7 (A) and 7.2 (B) were extracted according to the procedure of BLIGH AND DYER[1], using as aqueous phase 0.1 M acetate buffer (pH 4.7), 0.1 M borate buffer (pH 7.0) or distilled water (pH 6.3). Only the percentage of phosphatidylglycerol and its lysine ester are given since the amount of DPG remained fairly constant.

	A		*B*	
pH	*Phosphatidyl glycerol* (%)	*Lysyl-phosphatidyl glycerol* (%)	*Phosphatidyl glycerol* (%)	*Lysyl-phosphatidyl glycerol* (%)
4.7	30.5	67.5	89	9
6.3	44	54	90	8
7.0	72.5	25.5	94	4

medium in order to prevent degradation of lys-PG into phosphatidyl glycerol and lysine. A similar effect was, of course, noted when bacteria cultured at pH 7.2 were extracted under different conditions, but it is clear that the actual content of lys-PG in these cells was indeed of a very low order (Table I).

In addition to the phospholipids mentioned, the autoradiograms (Fig. 1) revealed the presence of a compound having an R_F value similar to that of diphosphatidyl glycerol (cardiolipin). Furthermore the paper chromatograms showed the presence of a glycolipid and other lipids devoid of phosphorus, which have not been the subject of the present investigation.

Fractionation of phospholipids

In order to facilitate the isolation of lys-PG we explored the effect of pH on the phospholipid composition. To this end cells of *S. aureus* were cultured in a medium containing glucose[4], and care was taken to extract the phospholipids under suitable conditions to prevent degradation of lys-PG. The quantity of cells harvested at pH 4.8 amounted to about 500 mg (dry weight) per l of culture giving, after extraction (at the same pH), a content of 2.7% lipids and 1.2% phospholipids on a dry weight basis. The lipid extract was subjected to chromatography on a silica column with mixtures of chloroform and methanol as eluent. As shown by a representative recording of the radioactivity of the eluate (Fig. 2), the separation of the phospholipids

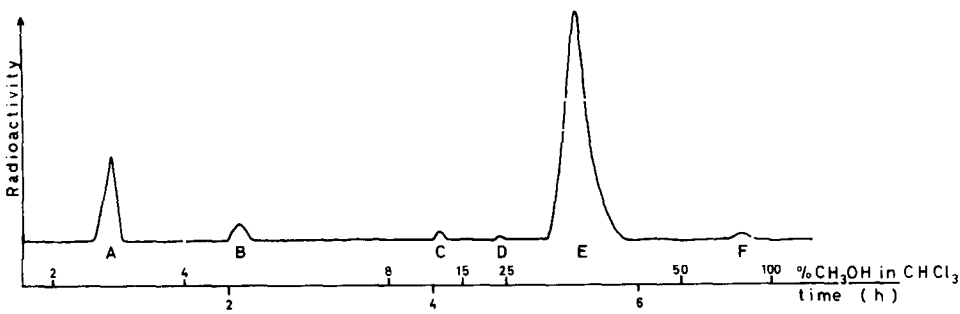


Fig. 2. Fractionation of phospholipids of *S. aureus* on a silica column as indicated by the radioactivity-time curve. An amount of 100 mg of lipid, containing 43 mg of phospholipid was applied to a column containing 7 g of silica (MALLINCKRODT, 140–200 mesh). For the composition of fractions A–F see text.

from *S. aureus* yielded six phosphorus containing fractions.

Fraction A eluted by chloroform–methanol (98:2, v/v) was found to contain a trace of a compound behaving similarly on paper chromatograms to synthetic phosphatidic acid. The major component present in this fraction exhibited chromatographic properties identical with those of synthetic diphosphatidyl glycerol[12] (Fig. 3). Cochromatography of the isolated and synthetic products yielded one spot only. Furthermore the product obtained after mild alkaline hydrolysis of both substances were indistinguishable on paper chromatograms developed in propanol–ammonia–water (6:3:1, v/v). Hence it may be concluded that this phospholipid from *S. aureus* is identical with diphosphatidyl glycerol (cardiolipin).

Fraction B (Fig. 2) recovered with chloroform–methanol (96:4, v/v) was found to contain phosphatidyl glycerol exclusively. The identification was performed by a

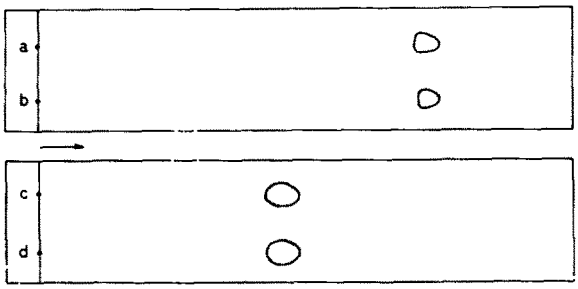


Fig. 3. Chromatographic characterization of lipid fraction A from *S. aureus*. Chromatograms on silica impregnated paper: (a) Fraction A; (b) synthetic diphosphatidyl glycerol. Chromatograms of the alkaline hydrolysis products developed on Whatman paper 1 with propanol–ammonia–water (6:3:1, v/v); (c) Fraction A; (d) synthetic diphosphatidyl glycerol.

comparison of the chromatographic properties and staining reactions with those of a synthetic reference substance. Furthermore alkaline hydrolysis was found to yield glycerophosphoryl glycerol only.

Fraction C [chloroform–methanol (92:8, v/v)] appeared to contain PG and glycolipid while in a number of experiments traces of a ninhydrin positive compound were found. The minor fraction D [chloroform–methanol (85:15, v/v)] also contained this latter component which was found to behave on paper chromatograms similarly to *O*-alanyl phosphatidyl glycerol synthesized recently by BONSEN *et al.*[13]. However, the amounts isolated did not allow any detailed investigation, only the presence of alanine being established.

Fraction E eluted by chloroform–methanol (75:25, v/v) was found to consist of pure lys-PG. The analyses carried out on this compound will be described in the next section.

Fraction F eluted with chloroform–methanol (1:1, v/v) and methanol was found to contain residual quantities of lys-PG together with a compound which behaved on paper chromatograms similarly to the hydrolysis product obtained by the action of snake venom phospholipase A (EC 3.1.1.4) on lys-PG. Probably the lyso product had been formed by deacylation during the chromatographic procedure.

Characterization of lysyl-phosphatidyl glycerol

The identity of the amino acid containing phospholipids obtained in a chromatographically pure state (20 mg from a 100 l culture) was established along different routes. Mild alkaline hydrolysis gave rise to the production of one water-soluble radioactive component, which was found to display a chromatographic behaviour identical with that of glycerophosphoryl glycerol. Chromatography of the amino acid freed after acid hydrolysis revealed only one ninhydrin-positive spot coinciding with that of lysine-HCl in the following solvent systems: phenol–water (5:2, v/v), butanol–acetic acid–water (4:1:5, v/v, upper phase) and propanol–ammonia–water (6:3:1, v/v). Chemical analysis of the phospholipid (Table II) gave a ratio glycerol: $P:N:NH_2 = 1.85:1:2.15:2.1$. Thus it can be concluded that one lysine molecule is esterified to one molecule of phosphatidyl glycerol and that both amino groups of the amino acid are free.

Further support for this structure was gained from hydrolysis experiments

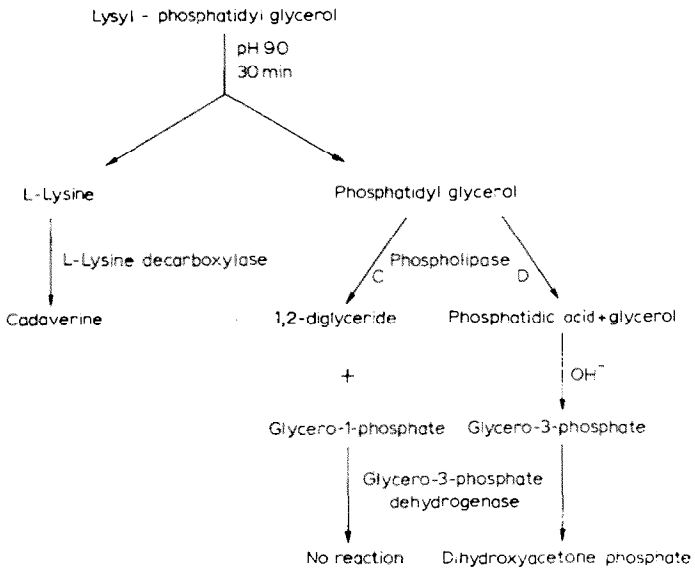
TABLE II

ANALYTICAL DATA OF LYSYL-PHOSPHATIDYL GLYCEROL ISOLATED FROM *S. aureus*

	Total nitrogen	*NH_2-nitrogen*	*Phosphorus*	*Glycerol*
μmole	1.30	1.25	0.60	1.11
Proportions	2.15	2.1	1	1.85

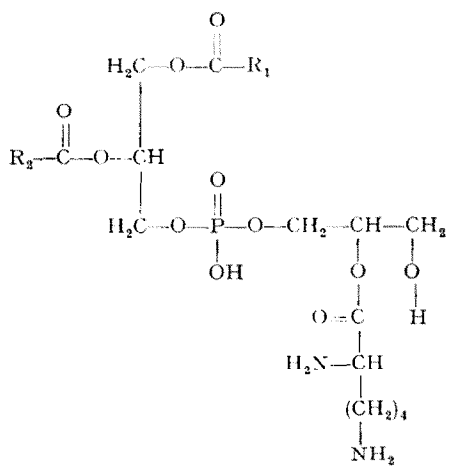
designed to elucidate the stereochemical configuration of the three asymmetric carbon atoms present in lys-PG (scheme 1). For this purpose use was made of the lability of the amino acid ester bond of this type of phospholipid which resembles the instability of similar bonds present in teichoic acids[14]. Pure lys-PG was emulsified ultrasonically in a borate buffer (pH 9.0), and incubated for 30 min at room temperature followed by a separation of the water-soluble and lipid-soluble hydrolysis products[5]. Chromatography of both fractions indicated that this treatment caused a practically complete hydrolysis of the phospholipid into one free amino acid and phosphatidyl glycerol without any detectable cleavage of the fatty acid ester linkages. In order to obtain confirmative evidence about the nature of the amino acid, and to determine its stereochemical configuration, the water-soluble fraction was incubated with a preparation of L-lysine decarboxylase (EC 4.1.1.18) obtained from *Escherichia coli*[15]. This enzyme acts on the L-isomer of lysine only, whereas ornithine is not converted at all. The amino acid derived from the phospholipid under discussion was completely decarboxylated by the enzyme, cadaverine being the only product detectable on paper chromatograms developed in phenol–water (5:2, v/v) and propanol–ammonia–water (6:3:1, v/v). In our opinion this result proves the amino acid constituent of the phospholipid to be L-lysine.

The chloroform phase consisting of phosphatidyl glycerol and a trace of the lysine derivative was subjected to chromatography on a silica column in order to remove the latter phospholipid. The pure phosphatidyl glycerol thus obtained was subjected to the degradation procedure outlined by HAVERKATE AND VAN DEENEN[16] in order to establish the stereochemical configuration of both glycerol entities. Hydrolysis with phospholipase C (EC 3.1.4.3) from *Bacillus cereus* in Tris buffer (pH 7.1) yielded 1,2-diglyceride and glycerophosphate (scheme 1), which gave a quantitative reaction on vicinal hydroxyl groups. This hydrolysis product completely resisted the action of L-α-glycerophosphate dehydrogenase which showed it to be identical with D-α-glycerophosphate or glycero-1-phosphate. Thus in the parent molecule the glycerol moiety carrying the lysine residue appears to have the D-configuration. Hydrolysis of phosphatidyl glycerol with phospholipase D (EC 3.1.4.4) in acetate buffer (pH 5.6; 0.02 M $CaCl_2$) mixed with an equal volume of ether gave a quantitative conversion of PG to phosphatidic acid (PA) and glycerol (scheme 1). Mild alkaline hydrolysis of PA furnished glycerophosphate giving a positive reaction on vicinal hydroxyl groups. Determination of its configuration with L-α-glycerophosphate dehydrogenase showed the presence of 0.93 equivalents of L-α-glycerophosphate or glycero-3-phosphate per μmole of phosphorus. Thus the phosphatidyl moiety of lys-PG is derived from glycero-3-phosphate, and both glycerol entities in this molecule have an opposite configuration, in agreement with previous observations of HAVERKATE AND VAN DEENEN[16] on phosphatidyl glycerol from *B. cereus* and chloroplasts.



Scheme 1. Determination of the stereochemical configuration of L-lysyl-L-α-phosphatidyl-D-glycerol.

Based on these analyses the following structure can be assigned to lys-PG, leaving unsettled the position of the linkage between lysine and PG:



As regards the nature of the fatty acid constituents of lys-PG it may be noted that the isolated compound was found to contain mainly branched saturated C_{15} fatty acids of the iso and anteiso types. The fatty acid patterns of PG and lys-PG from *S. aureus* were very similar and our results were in good agreement with the findings of MACFARLANE [17].

Action of phospholipases

O-amino acid esters of phosphatidyl glycerol were found in this laboratory to be susceptible to the action of phospholipases [3], and model experiments on a synthetic

substance confirmed the suitability of this approach for the molecular characterization of these structures[13]. According to expectation lys-PG was hydrolysed by phospholipase A from *Crotalus adamanteus* so as to give the corresponding monoacyl derivative. Enzymic hydrolysis in a collidine buffer at pH 6 (ref. 13) did not give rise to the formation of lysophosphatidyl glycerol, by contrast to the results obtained when the incubation was carried out at pH 7 (see ref. 3). Apparently, under the latter conditions the amino acid ester linkages of the phospholipid are cleaved, followed by or following an enzymic hydrolysis of phosphatidyl glycerol. Since phospholipase A from *Crotalus adamanteus* exhibits a strong stereochemical specificity[18] the complete breakdown of lys-PG by this enzyme confirms that its phosphatidyl moiety is derived from glycero-3-phosphate.

Phospholipase C (from *B. cereus*) has been shown to catalyse the hydrolysis of this class of phosphoglycerides into a diglyceride and an amino acid ester of glycerophosphate[13]. Since a non-enzymic conversion of lys-PG into PG had to be prevented, it was advisable to carry out the incubation at a rather low pH[13], but in the case of the lysine analogue, the action of phospholipase C was found not to commence below pH 7. An emulsion of lys-PG in Tris buffer (pH 7.0) containing phospholipase C was vigorously shaken in the presence of ether for 2 h at room temperature. The ether layer was found to contain diglyceride, lys-PG and PG. Chromatography of the water-soluble fraction revealed the presence of glycerophosphate which had been formed by enzymic cleavage of PG. However, a second spot having considerable radioactivity was found to be present; this compound showed no reaction on vicinal hydroxylgroups, but gave a positive ninhydrin reaction. This hydrolysis product is believed to be identical with lysyl-glycerophosphate, and a comparison with synthetic reference substances may perhaps furnish information about the position of the amino acid residue, provided that a migration of this ester linkage can be prevented. In theory such information can also be expected to be obtained after an enzymic hydrolysis of lys-PG by phospholipase D (EC 3.1.4.4). This enzyme has been shown to catalyse the hydrolysis of synthetic alanyl-phosphatidyl glycerol into phosphatidic acid and alanyl-glycerol[13]. However, under conditions which were favourable for the hydrolysis of the alanine analogue no breakdown of lys-PG was obtained. Since the rapid decomposition of lys-PG into PG limits the pH range applicable a suitable "activator" has to be sought.

The influence of pH on the phospholipid composition of S. aureus

Recently MACFARLANE[19] observed that in gram-positive bacteria the formation of amino acid esters of PG is closely linked to the phase of growth. As regards the behaviour of *S. aureus* our previous investigations[4] and the experiment dealt with above (Fig. 1) strongly suggested that the pH of the medium is of primary importance for the ratio between PG and lys-PG in this bacterium. Accumulation of lys-PG was observed when the pH reached values below 5 thus inducing a stationary phase; but when cells were allowed to enter the stationary phase at pH 7 PG was found to be the prevailing phospholipid. This effect of a substitution of PG by lys-PG was most pronounced and reproducible when the low pH was attained through the production of acid from glucose. The results were more complex when the acidic pH was induced by the addition of HCl. This is demonstrated by experiments with logarithmically growing cultures which were subjected to different pH. As shown in Fig. 4, lowering

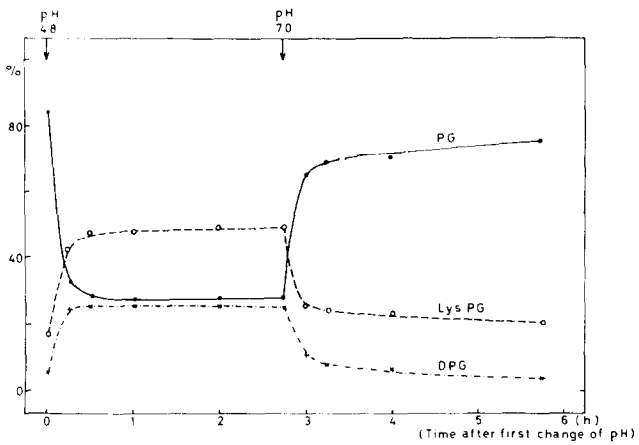


Fig. 4. Alterations in phospholipid composition of *S. aureus* induced by changes of pH. At zero time the pH of a logarithmically growing culture was brought from 7.2 to 4.8 by the addition of HCl. After 3 h the pH was raised again to 7.0. At the times indicated analyses were carried out to determine the ratios of phosphatidyl glycerol (PG), lysylphosphatidyl glycerol (Lys-PG) and diphosphatidyl glycerol; values are given as percentages of phosphorus.

of the pH from 7.0 to 4.8 gave a rapid alteration of the proportions of PG, lys-PG and DPG (diphosphatidyl glycerol). At this pH the phospholipid composition remained constant, but when the pH was restored to 7.0 the original phospholipid composition was attained again very soon. Under the conditions of this experiment the dividing of the bacteria stopped, but the viability had not been affected, so apparently the bacteria were not killed at the low pH. Under the latter conditions the relative amounts of lys-PG and DPG were found to increase at the expense of PG (Fig. 4). It appeared important to determine the absolute quantities of the bacterial phospholipids present under both conditions. Though calculations on a dry weight basis may be somewhat ambiguous it was found that at pH 7.0 the phospholipid content was about twice that of cells of *S. aureus* harvested at pH 4.8 (Table III). The decrease in

TABLE III

CONTENT AND COMPOSITION OF LIPIDS FROM *S. aureus* HARVESTED AT DIFFERENT pHs

Values are the means of six experiments and are expressed as mg lipid per g of lyophilized cells.

	pH 7.2	*pH 4.8*
Total lipid	34	27
Total phospholipid	23	12
Total diphosphatidyl glycerol	0.5	3.0
Total phosphatidyl glycerol	19.3	3.2
Total lysyl-phosphatidyl glycerol	3.8	5.8

phospholipid content was caused by a most significant disappearance of PG, which was compensated for only in part by an increase of lys-PG and DPG. Even when the permissible assumption is made that the increase in lys-PG and DPG involves a biosynthesis from PG the data obtained indicate that an alternative mechanism must be involved to account for the significant decrease in the amount of PG extractable by the solvent system used. It may be relevant to note that KANFER AND KENNEDY[20]

observed that in *E. coli* PG was in a highly dynamic state. The shift in phospholipid content noted in *S. aureus* after a given change in pH of the medium theoretically may involve the activation of a phospholipase acting on PG or eventually on its derivatives. Secondly the phenomenon may involve an in corporation of phospholipid, perhaps particularly of PG, into a more complex molecular structure, making the lipid unextractable into the solvent systems used. Detailed investigations will be required to elucidate these problems.

As mentioned before some small discrepancies in the ratio of individual phospholipids of *S. aureus* were found when cells were harvested from cultures with an acidic pH induced by means of HCl. In order to ascertain more precisely at which pH the effect under discussion occurs two different types of experiments were performed. According to the first approach (Fig. 5) the pH of one logarithmically growing

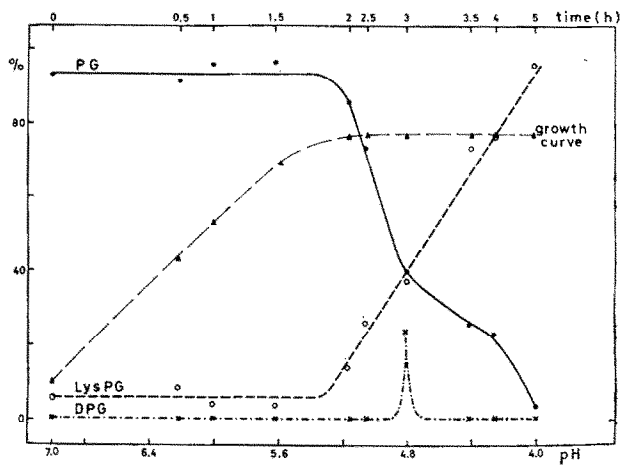


Fig. 5. The pH of a logarithmically growing culture of *S. aureus* was lowered stepwise after 30 min periods to the values indicated. At the end of each period the growth was measured nephelometrically and the phospholipid composition was determined; values are given as percentages of phosphorus. For abbreviations see legend to Fig. 4.

culture was lowered stepwise after 30-min periods and the phospholipid ratio was determined. In the range pH 7–5.6 the composition of the phospholipids did not alter. At pH 5.2 the changes in ratio between different polyglycerol phospholipids began to be noted, while simultaneously the bacterial growth stopped and, because of the pH, the culture entered the stationary phase. Further lowering of pH brought about an increase in the relative amount of lys-PG and a decrease in PG, but at about pH 4.9 a relatively large quantity of DPG was found to be present; while at other pH values only traces of this phospholipid were detected. At pH 4.0 the phospholipids from *S. aureus* appeared to consist almost exclusively of lys-PG (Fig. 5). Under normal conditions of growth a pH lower than 4.8 was not attained, but at pH 4.2 the cells were still metabolically active as was shown by the incorporation of [^{32}P]-phosphate into the bacterial phospholipids.

In the second type of experiment cells of a logarithmically growing culture of *S. aureus* were divided into ten aliquots, each of which was incubated at a given pH for 4 h (Fig. 6). In agreement with our previous observations the phospholipid com-

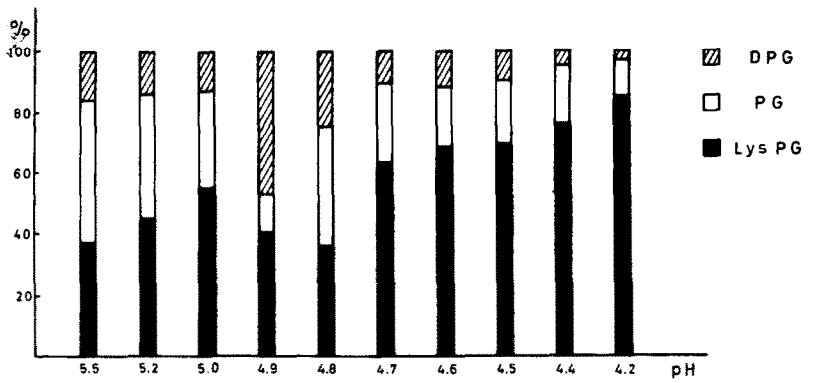


Fig. 6. One logarithmically growing culture of *S. aureus* was divided into ten equal parts, which were brought to the pH values indicated. After an additional incubation for 2 h the cells were harvested, the lipids were extracted and the phospholipid composition was determined (expressed as percentages of phosphorus). For abbreviations see legend to Fig. 4.

position appeared to differ markedly between cells exposed to media of different pH. Diphosphatidyl glycerol was found to accumulate between pH 4.9 and 4.7, whereas in the other regions of the pH range studied the decrease and increase in the percentages of PG and lys-PG respectively, produced by lowering of pH again were very conspicuous. Though the biochemical mechanism underlying these shifts in phospholipid composition and content remains obscure the influence of pH on these lipid characteristics of *S. aureus* is beyond doubt.

Phospholipid patterns of some other bacteria

The foregoing results stimulated us to investigate whether similar effects of pH on the phospholipids occur in other bacteria. Furthermore the observations on the lability of the *O*-amino acid esters of phosphatidyl glycerol and the accumulation of these compounds below a certain pH made it of interest to reinvestigate some bacteria, which so far had not been found to contain this class of phosphoglycerides.

The results briefly recorded here show that *O*-amino acid esters of phosphatidyl glycerol are not ubiquitously distributed in bacteria, while several bacterial species containing this type of phospholipid responded in a different way to the acidity of the culture medium. In accord with the experience of KANFER AND KENNEDY[20,21] amino acid esters of PG were not detected in *Escherichia coli*, even when cultivated under conditions which gave an accumulation of these compounds in *S. aureus*. A small quantity of an amino acid ester of PG was isolated from *B. cereus*[3,22], but we have been unable so far to augment its content either by the addition of glucose or by a direct alteration of the pH of the medium. *Bacillus megatherium* was found to react to the acidity of the medium, but the picture appeared to be rather different from that of *S. aureus*. Cells of *B. megatherium* harvested at pH 7 were found to contain diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl glycerol and an amino acid derivative of phosphatidyl glycerol (perhaps identical with or related to lys-PG[23]). At pH 5 the same constituents were found to be present, but in addition another phospholipid could be detected, which turned out to be a PG derivative staining with ninhydrin. A pronounced effect of pH on the

phospholipid composition occurred in *Lactobacillus acidophilus*, but the phospholipid(s) containing amino acids may be more complex and deserve further investigation. In this context it is of interest to note that SINHA AND GABY[24] recently reported that *Pseudomonas aeruginosa* may contain a polar lipid-amino acid complex structurally distinct from the *O*-amino acid esters of phosphatidyl glycerol.

The effect of pH on the phospholipids from *Streptococcus faecalis* was found strongly to resemble the events observed with *S. aureus*. The relative amount of amino acid derivatives of PG in this bacterium when cultured in the presence of glucose or by lowering of the pH in other ways, was found to increase when compared with bacteria cultured at pH 7.0, while the acidic pH caused a lowering of the content of PG and DPG (Fig. 7). VORBECK AND MARINETTI[25] found in cell membranes of

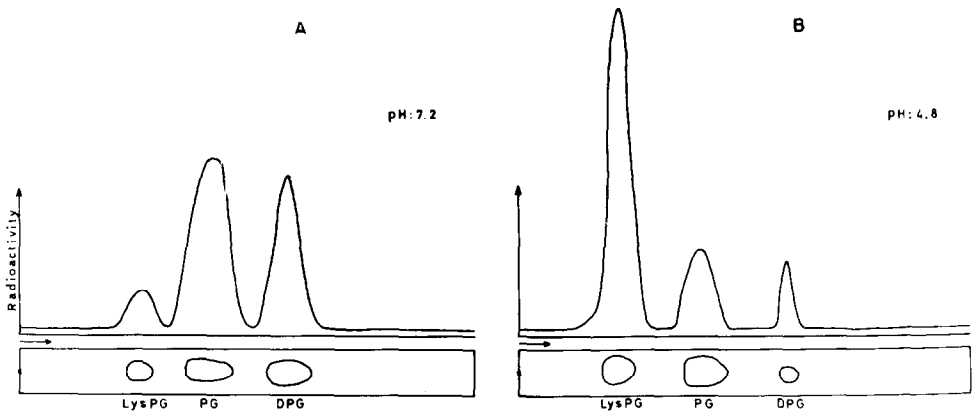


Fig. 7. The influence of pH of the medium on the phospholipid pattern of *S. faecalis*. Chromatograms on silica impregnated paper of lipids extracted from a culture at pH 7.2 (A) and pH 4.8 (B). For abbreviations see legend to Fig. 4.

S. faecalis glycine, lysine and alanyl esters of phosphatidyl glycerol. We observed that the major amino acid derivative which was present in *S. faecalis* at the acidic pH was identical with lys-PG.

The present study does not yet permit any final conclusion about the specific significance of the *O*-amino acid esters of phosphatidyl glycerol. As discussed before, these phospholipids may perhaps have a role in the selective transport of amino acids needed for cell wall structure or may display a function in regulating the charge of the lipid core of the cell membrane[4]. Further studies on their distribution in the bacterial domaine and electron microscopic resolution of the alterations occurring in cellular structure under conditions which give changes in content and composition of phospholipid may enable us to judge between these and other alternatives.

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