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# EFFECT OF AGE ON THE MEMBRANE LIPID COMPOSITION OF *STREPTOCOCCUS SANGUIS*

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## Summary

The cell membrane of *Streptococcus sanguis* contains three classes of lipid: neutral lipid, glycolipid and phospholipid. A striking difference in membrane lipid composition between cells in the exponential and in the stationary phases of growth was observed. During the exponential phase, approx. 37—45%, 14—19% and 37—45% of the lipids synthesized were found to be neutral lipid, glycolipid and phospholipid, respectively. The amount of lipid synthesized reached a maximum at the early stationary phase. The amount of phospholipid drastically declined thereafter and that of neutral lipid slightly declined. In contrast, the amount of glycolipid markedly increased and exceeded the amount of phospholipid. The phospholipid present during the exponential phase was found to be mainly phosphatidylglycerol (82—88%) and a small amount of cardiolipin (12—18%). At the stationary phase, the amount of phosphatidylglycerol greatly decreased and reached approx. 16% of that in the early stationary phase, while cardiolipin steadily increased and became the major phospholipid in the late stationary phase. The glycolipid was found to be composed of mainly mono- and diglucosyldiglycerides. At the end of the experiment (after 8 h incubation), the distribution of lipids was found to be: neutral lipid, 46%; glycolipid (monoglucosyldiglyceride, 28%; diglucosyldiglyceride, 13%) 41%; and phospholipid (phosphatidylglycerol, 3%, cardiolipin, 8%) 13%.

## Introduction

The lipid of the cytoplasmic membrane of Gram positive bacteria consists of three classes of lipid: neutral lipid, glycolipid and phospholipid [1]. The cellular phospholipids are found mainly in the cytoplasmic membrane. The active turnover of phosphatidylglycerol has been well documented in *Escherichia*

*coli* [2,3]. In this organism the major phospholipid was found to be phosphatidylethanolamine which constitutes 70—80% of the cellular phospholipid. Phosphatidylglycerol and cardiolipin, on the other hand, are present only in the amount of 5—15% of the cellular phospholipid [4]. Therefore, the turnover of phosphatidylglycerol does not greatly affect the total cellular phospholipid content. The phospholipid composition in streptococci has not been well established. The major phospholipid in *Streptococcus faecalis* was reported to be either phosphatidic acid [5], a derivative of diphosphatidylglycerol [6], or various aminoacyl derivatives of phosphatidylglycerol [6,7]. In *Streptococcus sanguis*, phosphatidylglycerol was found to be the major phospholipid and was engaged in active turnover during the exponential phase of growth [8]. We were interested in determining if the active turnover of the major phospholipid continues when cells reach the stationary phase in which little synthesis of lipid occurs.

## Materials and Methods

The sources of *S. sanguis* and chemicals were the same as previously reported [8,10]. Thin-layer chromatography was performed on Anasil H plates (0.25 mm or 0.5 mm thick) obtained from Analabs, North Haven, CT. The plates were developed in solvent (a), chloroform/methanol/water (65 : 25 : 4) or in solvent (b), 2,6-dimethylheptan-4-one/acetic acid/water (60 : 45 : 6). Paper chromatography was carried out on Whatman 1MM paper and developed in solvent (c), pyridine/water/ethylacetate/acetic acid (5 : 3 : 5 : 1), or solvent (d), butanone/acetic acid/water (75 : 25 : 10). Radiolabeled and reference compounds on chromatograms were detected as described before [8,10]. The growth of *S. sanguis* was determined with a Beckman DBGT spectrophotometer at a wavelength of 520 nm. The determination of extracellular lipoteichoic acid and the fractionation of crude lipid by silicic acid column chromatography into neutral lipid, glycolipid and phospholipid have been described [8]. Mono- and diglucosyldiglycerides were prepared in our laboratory. These lipids exhibited the same thin-layer chromatographic mobilities in solvents a and b as those of the authentic compounds kindly provided by Dr. R.A. Pieringer, Temple University.

Glycerol uptake activity was measured by adding 0.025 $\mu$Ci of [2-$^3$H]-glycerol (200 $\mu$Ci/$\mu$mol) (25 $\mu$l) to 1 ml of culture which was shaken at room temperature for 1 min. The cells were collected on a Millipore filter (25 mm in diameter) and washed with 2 ml of culture medium. The filter was removed and the radioactivity was determined in Triton-Scintillation fluid [8].

## Results and Discussion

The synthesis of lipid as measured by the incorporation of [2-$^3$H]glycerol was found to parallel that of the growth of *S. sanguis* (Fig. 1). The total radiolabeled lipid and the extracellular lipoteichoic acid reached the maximum when the cells reached the stationary phase. Fractionation of the lipid by silicic acid column chromatography revealed that the rates of synthesis of neutral lipid and phospholipid are similar, but that of glycolipid was approximately one-half that

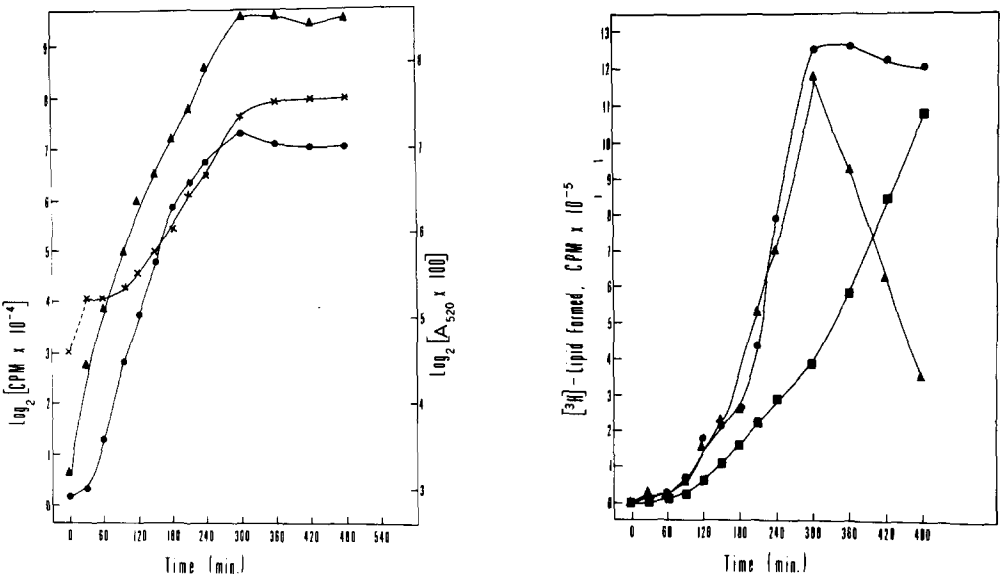


Fig. 1. Growth and lipid synthesis of *Streptococcus sanguis* culture. A 125-ml Erlenmeyer flask with 50 ml of Todd-Hewitt broth containing 2 mCi of [$^3$H]glycerol was inoculated with 2 ml of overnight culture of *S. sanguis* and incubated at 35°C with shaking (50 strokes/min). Samples of 2 ml were removed at the times indicated and centrifuged at 5900 $\times g$ for 10 min. The pellet obtained was washed once with 1 ml of saline, suspended in 0.1 ml of saline and extracted twice with 2 ml of chloroform/methanol (1 : 1) (CM) for 1 h at room temperature. The CM extracts were separately washed twice with saline and the radioactivity in the chloroform phase was determined. •, $A_{520}$; X, extracellular lipoteichoic acid; ▲, total lipid.

Fig. 2. Lipid composition of *S. sanguis* at various stages of growth. Samples of one-half of the crude lipid extracts in Fig. 1 were fractionated into neutral lipid, glycolipid and phospholipid as described in the text. Aliquots of 10% were removed for radioactivity determination. •, neutral lipid; ■, glycolipid, ▲, phospholipid.

of the other two lipids. A sharp decline of the phospholipid content after it reached its maximum at the early stationary phase was observed. The amount of neutral lipid remained fairly constant but that of glycolipid increased continuously, and eventually surpassed the phospholipid in quantity (Fig. 2).

The phospholipid and glycolipid were further fractionated by thin-layer chromatography in solvents (a) and (b). Two major lipids were found in each group of lipids: phosphatidylglycerol and cardiolipin in phospholipid, and mono- and diglucosyldiglycerides in glycolipid. A typical profile of the thin-layer chromatography is shown in Fig. 3. The identity of each lipid was further confirmed by paper chromatography in solvents (c) and (d) of mild alkaline hydrolyzates of each lipid isolated by preparative thin-layer chromatography in solvent (a). Upon mild alkaline hydrolysis of phosphatidylglycerol and cardiolipin, a compound with the paper chromatographic mobility of glycerylphosphorylglycerol was released from phosphatidylglycerol and glycerylphosphorylglycerylphosphorylglycerol was obtained from cardiolipin. The same treatment resulted in the release of compounds exhibiting the paper chromatographic mobility of glucosylglycerol from monoglucosyldiglyceride and that of diglucosylglycerol from diglucosyldiglyceride. The quantitative change of each lipid after 180 min of incubation is shown in Fig. 4. Cardiolipin and both

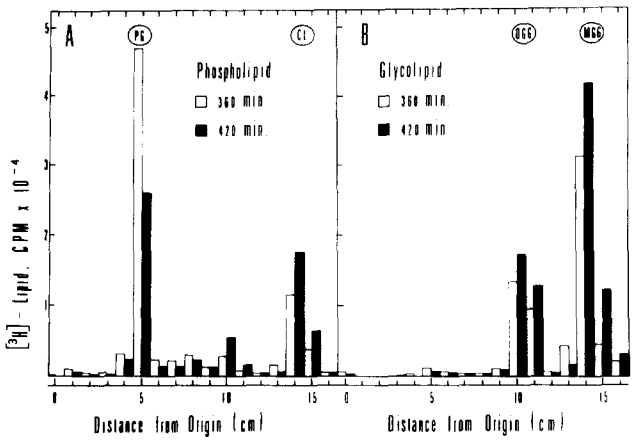


Fig. 3. Thin-layer chromatography of phospholipid and glycolipid. Samples of 5% phospholipid and glycolipid from 360 and 420 min cultures were evaporated to dryness, dissolved in 20 µl of chloroform, chromatographed on Anasil H plates (0.25 mm thick) in solvent (a). PG, phosphatidylglycerol; CL, cardiolipin; DGG, diglucosyldiglyceride; MGG, Monoglucosyldiglyceride.

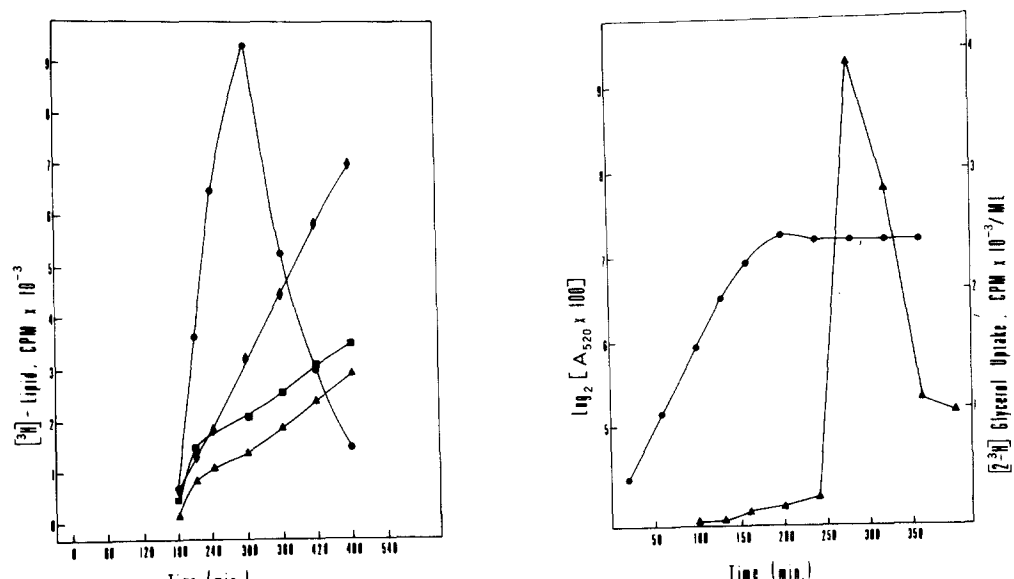


Fig. 4. Kinetics of various glycolipid and phospholipid syntheses in *S. sanguis* culture. Aliquots of 5% of glycolipid and phospholipid in Fig. 2, were chromatographed in Anasil H thin-layer plates in solvent (a) and the radioactivity of various lipids was determined as previously reported [8]. ●, phosphatidylglycerol; ▲, cardiolipin; ◆, monoglucosyldiglyceride; ■, diglucosyldiglyceride.

Fig. 5. Glycerol transport activity of *S. sanguis* culture. Todd-Hewitt broth, 50 ml in a 125 ml Erlenmeyer flask, was inoculated with 5 ml of overnight culture of *S. sanguis* and incubated as described in Fig. 1. Two 1-ml samples were removed at time indicated and used for the determination of growth and transport activity as described in Materials and Methods.

glycolipids, the mono- and diglucosyldiglycerides, were found to continuously increase, even after the cells reached the stationary phase. Phosphatidylglycerol, on the other hand, started to decline drastically at the onset of stationary phase.

In order to examine whether the radioactivity of the lipids determined represents the relative amount of each lipid after three generations (150 min incubation) of growth, the residual [$^3$H]glycerol and glycerol uptake activity were determined. More than 80% of the [2-$^3$H]glycerol added was found in the culture medium at the end of the experiment (480 min incubation). The glycerol uptake activity was found to be proportional to the cell density until 60—80 min after the onset of stationary phase. A sharp increase, approximately 15-fold, in glycerol uptake activity was observed. The uptake activity declined drastically after it reached the maximum (Fig. 5). The majority (60—70%) of [2-$^3$H]glycerol uptaken were found in the lipid fraction when the cells were subjected to lipid extraction as previously described [8]. This result indicates that [2-$^3$H]glycerol uptake as well as lipid synthesis from exogeneous glycerol was not impaired during the stationary phase. The cause of the sharp increase in the glycerol uptake 60 min after the onset of the stationary phase is not known.

With the assumption that newly synthesized glyceride lipids were homogeneously labeled with [2-$^3$H]glycerol, [2-$^3$H]glycerol labeled lipids would represent at least 75, 87 and 94% of the total lipid after two (120 min), three (165 min) and four (270 min) generations of growth, respectively. Based on the distribution of the radiolabeled lipid, the cells in the exponential phase of growth consisted of the similar lipid composition, 37—45% of neutral lipid, 39—45% of phospholipid and 14—19% of glycolipid. When the cells reached the stationary phase, the amount of phospholipid steadily decreased and reached only 13% of the total lipid at the end of the experiment (Fig. 2). The turnover of phosphatidylglycerol was found to be responsible for the decrease in phospholipid content (Fig. 4). At the end of the experiment, only 16% of phosphatidylglycerol remained associated with the cells when compared to the phosphatidylglycerol content of the early stationary phase cells. Glycolipids, on the other hand, continuously increased throughout the experimental period, and eventually represented more than 40% of the total lipid. The content of neutral lipid remained fairly constant. Streptococci are notorious for their short lives in most common culture media, including the well buffered media. This may be due to their abnormal membrane lipid distribution in the late stationary phase.

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