EFFECT OF STREPTOCOCCAL LIPIDS ON EHRlich
ASCITES TUMOR CELLS

Shigeru KIGOSHI and Kosaku KITAJIMA
Department of Pharmacology, School of Medicine, Kanazawa University,
Kanazawa 920, Japan
Accepted October 21, 1980

Abstract—The lipids extracted from group A hemolytic streptococci (strain Su, Blackmore and C203U) were examined for their antitumor effect against Ehrlich ascites carcinoma in mice. Total lipids were extracted from streptococcal cells according to the method of Folch et al., and separated into 9 lipid fractions by thin-layer chromatography, using various solvent systems. Three fractions were compound lipids (diphosphatidyl glycerols, monoglucosyl diglycerides and diglucosyl diglycerides), and the remaining 6 fractions were neutral lipids such as free fatty acids, glycerides, sterols and sterol esters. For biological testing, the lipid fractions suspended in physiological saline containing Tween 20 (0.02%) were incubated with Ehrlich tumor cells at 37°C for 90 min, and the cell mixture was given intraperitoneally into mice thereafter. Among 9 lipid fractions, free fatty acids and monoglycerides from the streptococci examined were highly active in suppressing the development of ascites carcinoma in mice. Diphosphatidyl glycerols from two strains of streptococci (Blackmore and C203U) were also effective in suppressing the tumor growth in mice. However, the other lipid fractions had little effect on the tumor growth.

Although the antitumor activity of group A hemolytic streptococci has been studied extensively (1, 2), little is known of the possible role of lipids in the antitumor activity of the streptococci. Many studies have indicated that the lipids extracted from fungi or mammalian cells are cytotoxic to tumor cells (3–8). Thus it seems likely that the streptococcal lipids exhibit antitumor activity. The present study deals with the antitumor effect of lipids extracted from group A hemolytic streptococci (strain Su, Blackmore and C203U) against Ehrlich ascites carcinoma in mice.

MATERIALS AND METHODS

Extraction of streptococcal lipids: The following streptococci were used in this study: St. hemolyticus, strain Su (type 3), Blackmore (type 11) and C203U (a mutant of C203S, type 3). Streptococci grown in 10-liter lots of Wood and Gunsalus medium (9) at 37°C for 12 hr were harvested by centrifugation and washed thoroughly in physiological saline (yields: 20–25 g of wet cells/10 l culture). The packed cells were extracted with 20 vol. of chloroform-methanol (CM, 2:1, v/v) at room temperature, and the CM extracts were evaporated at 40°C under a stream of nitrogen (10). The crude lipid extracts were dissolved in CM and washed with saline to remove non-lipid materials, as described by Folch et al. (11). After evaporation of the CM layer, the residual
oily mass (total lipids) was dissolved in a small volume of CM, and stored at -20°C before use (yields of total lipids: about 100 mg/10 g wet cells).

**Fractionation of total lipids by thin-layer chromatography:** The total lipids were fractionated by one-dimensional thin-layer chromatography on plates (20x20 cm) covered with silica gel H (E. Merck AG, Germany; 1 mm thick) using chloroform-methanol-water (65:25:4, by vol.) (12) as an ascending solvent. The lipids were applied as a narrow band on the start line of the plates and allowed to develop until the solvent front had migrated about 16 cm. The chromatographed total lipids yielded with iodine reagent (10) four bands, referred to as Fraction I, II, III and IV in the order of their Rf values (Table 1). Each of these lipid fractions was eluated with CM from silica gel and condensed. To purify Fraction II and III, each of these fractions was further fractionated one-dimensionally using chloroform-acetone-methanol-acetic acid-water (65:35:11:4:1.5, by vol.) (13). For qualitative analysis of the 4 lipid fractions, a small portion of each fraction was chromatographed on silica gel H plates (0.25 mm thick) using the above solvent systems, and lipids were detected with the following spray reagents (10): phosphomolybdate for lipids, Dittmer-Lester reagent for phospholipids, anthrone-sulfuric acid for glycolipids and sterols, and antimony trichloride for sterols.

**Fraction IV, a mixture of neutral lipids, was one-dimensionally chromatographed on silica gel H plates (1 mm thick) using hexane-ethyl ether-acetic acid (70:30:2, by vol.) (15), thereby yielding 6 subfractions (referred to as Fraction N1, N2 and so on) (Table 2).** In this solvent system, the Rf value of Fraction N2 was similar to that of Fraction N3. Thus, the two subfractions were further chromatographed one-dimensionally with ethylene chloride-methanol (98:2, v/v) (16). To identify each subfraction, the Rf values of these subfractions were compared with those of the following substances: cholesterol (E. Merck AG, Germany), cholesterol stearate, palmitic acid, oleic acid, and mono-, di- and tri-palmitin (Wako Pure Chem. Indust., Japan).

**Analysis of phospholipids and glycolipids by paper chromatography:** Phospholipids were deacylated with 0.1 N KOH in methanol at 37°C for 15 min (17), and their watersoluble deacylated products were chromatographed two-dimensionally on paper (Toyo Roshi No. 50, 40x40 cm) using phenol saturated water-acetic acid-ethanol (100:10:12, by vol.) and methanol-formic acid-water (80:13:7, by vol.) (18). The phosphate esters on the paper were detected with

| Fraction no. | Rf values in solvent* | Color reaction by spray reagents** | Possible identification |
|--------------|-----------------------|-----------------------------------|------------------------|
|              | I                     | II                                |                        |
| I            | 0.56                  | 0.18                              | +                      | -                      | +                      | -                      | Diglucosyl diglycerides |
| II           | 0.70                  | 0.23                              | +                      | +                      | -                      | -                      | Diphosphatidyl glycerols |
| III          | 0.73                  | 0.56                              | +                      | -                      | +                      | -                      | Monoglycosyl diglycerides |
| IV           | 0.93                  | 0.88                              | +                      | +                      | +                      | +                      | Neutral lipids |

*Solvent I, chloroform-methanol-water (65:25:4, by vol.); solvent II, chloroform-acetone-methanol-acetic acid-water (56:35:11:4:1.5, by vol.). **Spray reagents: R1, phosphomolybdate for lipids; R2, Dittmer-Lester reagent for phospholipids; R3, anhydride-sulfuric acid reagent for glycolipids and sterols; R4, antimony trichloride for sterols.
Table 2. Chromatographic characterization of neutral lipids from hemolytic streptococci

| Fraction no. | Rf values in solvent* | Color reaction by spray reagents** | Possible identification  |
|--------------|-----------------------|------------------------------------|-------------------------|
| N1           | 0.07                  | +                                  | Monoglycerides          |
| N2           | 0.27                  | +                                  | Sterols                 |
| N3           | 0.29                  | -                                  | Diglycerides            |
| N4           | 0.40                  | -                                  | Free fatty acids        |
| N5           | 0.70                  | +                                  | Triglycerides           |
| N6           | 0.84                  | +                                  | Sterol esters           |

*Solvent III, hexane-ethyl ether-acetic acid (70:30:2, by vol.); solvent IV, ethylene chloride-methanol (98:2, v/v). **Spray reagents: R1, phosphomolybdate for lipids; R4, antimony trichloride for sterols and sterol esters.

Hanes-Isherwood reagent (19), and their Rf values were compared with those of the water-soluble deacylated products of phosphatidyl-1-serine or phosphatidyl-1-ethanolamine (Nb Co., USA) (18).

Glycolipids were deacylated with 0.1 N methanolic KOH as described above. The water-soluble deacylated products of glycolipids and glucose, a standard substance, were chromatographed one-dimensionally on paper (Toyo Roshi No. 50) using the following solvent systems (20): propanol-ammonia-water (16:3:1, by vol.), butanol-propionic acid-water (142:71:100, by vol.), butanol-pyridine-water (6:4:3, by vol.) and ethyl acetic acid-pyridine-acetic acid-water (5:5:1:3, by vol.). After development, the carbohydrates on the paper were detected with silver nitrate reagent (21), and their Rf values were compared with those reported in the literature (20). In addition, glycolipids were hydrolysed with 0.1 N HCl at 100°C for 40 min or with 3 N HCl at 100°C for 90 min (22). The water-soluble hydrolysates of glycolipids were examined by one-dimensional paper chromatography using butanol-acetic acid-water (6:4:3, by vol.) or ethyl acetic acid-pyridine-water (12:5:4, by vol.) (22).

Gas-chromatographic analysis of fatty acid composition of lipids: Methyl esters of fatty acids were prepared by methanolation of each lipid fraction with 20% H₂SO₄ in methanol at 65-70°C for 15 hr (23). The methyl esters were analysed quantitatively using a Hitachi model 063 gas chromatograph fitted with a flame ionization detector. Separation of the methyl esters was carried out on a glass column (200 cm×3 mm internal diameter) containing 20% NPGS on Chromasorb W (60-80 mesh) at 210°C. Dual hydrogen detectors and a flash heater were maintained at 280°C and 230°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 20 ml/min. The chart peaks were identified by the standard method of comparing the relative retention times with those of known methyl esters (Analytical reference standard kits, Gasukuro Indus., Japan). Quantitative estimations were based on measurements of the chart peak areas, which had been shown to be proportional to the amounts of the components present (24).

Examination of antitumor activity of lipids: The antitumor effect of streptococcal lipids against Ehrlich ascites tumor cells was examined by the method of Koshimura et al. (25), except that phosphate-buffered Ringer solution was replaced with physiological saline. Female mice of ddN strain, 20–22 g, were used throughout. Ehrlich tumor cells were obtained from mice at 10 days after i.p.
inoculation of the cells, and suspended in saline containing 0.02% Tween 20. The tumor cell suspension was mixed with the lipid suspension in saline containing Tween 20 (2×10^7 cells/ml), and incubated at 37°C for 90 min. After incubation, the cell mixture was injected i.p. into mice (10^7 cells/mouse), and the tumor growth in mice was observed for 60 days. Control animals were given the same dose of the tumor cells incubated without an admixture of lipids.

In addition, the number of viable Ehrlich tumor cells was examined using the trypan blue test (26) after incubation for 90 min in the presence or absence of streptococcal lipids, and the proportion of viable tumor cells was calculated from the number of viable and dead cells.

RESULTS

Identification of lipid components: The total lipids obtained from hemolytic streptococci were separated into 4 fractions (Fraction I, II, III and IV) by thin-layer chromatography using chloroform-methanol-water (65:25:4) and chloroform-acetone-methanol-acetic acid-water (65:35:11:4:1.5), irrespective of the original organisms (Table 1). Staining studies with different spray reagents indicated that Fractions I and III were glycolipids, and Fraction II was phospholipids. To identify these lipid fractions, each of the fractions was deacylated with KOH or hydrolysed with HCl, and their water-soluble products were examined by paper chromatography using various solvent systems. 1,3-diacylglycerophosphoryl glycerol, glycerophosphoryl glycerol and glycerophosphate were found in the water-soluble products of Fraction II, and this fraction was identified as diphosphatidyl glycerols. The water-soluble products of glycolipids were diglucosyl glycerol, monoglucosyl glycerol and glucose for Fraction I, and monoglucosyl glycerol and glucose for Fraction III. The gas chromatographic analysis of methylated carbohydrates obtained from glycolipids indicated the presence of glucose in Fraction I and III (20). Therefore, Fraction I and III were identified as diglucosyl diglycerides and monoglucosyl diglycerides, respectively.

The staining behavior of Fraction IV was different from that of the other lipid fractions, and this fraction was considered to be neutral lipids. Fraction IV was further separated into 6 subfractions by thin-layer chromatography using hexane-ethyl ether-acetic acid (70:30:2) and ethylene chloride-methanol (98:2). The RF values and color reaction of these subfractions were then compared with those of the authentic materials, and each of the subfractions was identified as follows: monoglycerides, sterols, diglycerides, free fatty acids, triglycerides and sterol esters (Table 2).

Antitumor activity of lipids: Table 3 shows the effect of the total lipids obtained from three strains of hemolytic streptococci against Ehrlich ascites carcinoma in mice. Mice given the tumor cells premixed with the total lipids at a concentration of 5 mg per ml of cell suspension showed no signs of tumors at the end of the 60-day period. The control animals invariably died from ascites carcinoma in less than 20 days (Fig. 1). Four lipid fractions obtained from the total lipids of hemolytic streptococci were then examined for their antitumor activity. At a concentration of 2 mg per ml, neutral lipids from three strains of hemolytic streptococci completely prevented the development of ascites carcinoma in mice, and diphosphatidyl glycerols from two strains of streptococci (Blackmore and C203U) were fully effective (Table 4). However, glycolipids from three strains of streptococci gave incomplete protection at a concentration of 2 mg per ml, and diphosphatidyl glycerols (2 mg per ml) from another strain of streptococci (Su) failed to suppress the tumor growth in mice.
Table 3. Effect of total lipids from hemolytic streptococci against Ehrlich ascites carcinoma in mice

| Hemolytic streptococci | No. of survivors at 60 days | Lipid concentration in test mixture (mg/ml of cell suspension) |
|------------------------|-----------------------------|---------------------------------------------------------------|
|                        |                             | 5.0  | 2.0  | 0    |
| Su                     | 10/10                       | 6/10 | 0/10 |
| Blackmore              | 10/10                       | 4/10 | 0/10 |
| C203U                  | 10/10                       | 6/10 | 0/10 |

Lipids suspended in physiological saline containing Tween 20 (0.02%) were mixed with Ehrlich tumor cells (2×10^7 cells/ml), and incubated at 37°C for 90 min. After incubation, the cell mixture was injected i.p. into mice (10^7 cells/mouse), and the tumor growth in mice was observed for 60 days.

In the following, the subfractions of neutral lipids from three strains of hemolytic streptococci were examined for their biological activity. At a concentration of 2 mg per ml, monoglycerides and free fatty acids were suppressed completely the tumor growth in mice, and sterol esters were partially protective, irrespective of the original organisms (Table 5). However, no antitumor activity was found in the other subfractions of neutral lipids at the same concentration. The antitumor activity of free fatty acids, monoglycerides and sterol esters from three strains of hemolytic streptococci were then tested at concentrations of 0.05 to 2.0 mg per ml of cell suspension. Among these neutral lipids, free fatty acids were the most effective, followed by monoglycerides, whereas sterol esters showed a weak effect (Table 6). The growth of tumor cells in mice was completely inhibited by 0.5 mg of free fatty acids per ml or by 1 mg of monoglycerides per ml. In contrast, more than 4 mg of sterol esters per ml were required for the complete protection.

Concerning these results, the proportion of viable tumor cells was examined after incubation for 90 min in the presence or absence of streptococcal lipids. When Ehrlich tumor cells had been incubated in the
Table 4. Effect of compounding lipids from hemolytic streptococci against Ehrlich ascites carcinoma in mice

| Hemolytic streptococci | Lipid components* | No. of survivors at 60 days |
|------------------------|-------------------|-----------------------------|
|                        | Lipid concn. in test mixture (mg/ml of cell suspension) | 2.0 | 1.0 | 0.5 |
| Su                     | DPG               | 0/10 | 0/10 |    |
|                        | MGD               | 0/10 |    |    |
|                        | DGD               | 0/10 |    |    |
|                        | NL                | 10/10|    |    |
| Blackmore              | DPG               | 10/10| 5/10| 0/10|
|                        | MGD               | 4/10 | 0/10|    |
|                        | DGD               | 2/10 |    |    |
|                        | NL                | 10/10|    |    |
| C203U                  | DPG               | 9/10 | 4/10| 0/10|
|                        | MGD               | 4/10 | 0/10|    |
|                        | DGD               | 0/10 |    |    |
|                        | NL                | 10/10|    |    |

*DPG, diphosphatidyl glycerols; MGD, monoglucosyl diglycerides; DGD, diglucosyl diglycerides; NL, neutral lipids.

Table 5. Effect of neutral lipids from hemolytic streptococci against Ehrlich carcinoma in mice

| Hemolytic streptococci | Lipid components* | No. of survivors at 60 days |
|------------------------|-------------------|-----------------------------|
|                        | Lipid concn. in test mixture (mg/ml of cell suspension) | FA | MG | DG | TG | S | SE |
| Su                     | 2.0               | 10/10 | 10/10 | 0/10 | 0/10 | 0/10 | 8/10 |
| Blackmore              | 2.0               | 5/5  | 5/5  | 0/5  | 0/5  | 0/5  | 4/5  |
| C203U                  | 2.0               | 5/5  | 5/5  | 0/5  | 0/5  | 0/5  | 2/5  |

*FA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; S, sterols; SE, sterol esters.

The presence of free fatty acids or monoglycerides from hemolytic streptococci at concentrations of 1–2 mg per ml, the proportion of the viable tumor cells was less than 2%. Whereas, the proportion of the viable tumor cells was about 81% (73–86%) after incubation with diglycerides or triglycerides from streptococci at a concentration of 2 mg per ml. The proportion of viable Ehrlich tumor cells before and after incubation without streptococcal lipids was about 85% (78–89%) and 82% (74–87%), respectively. Therefore, Ehrlich tumor cells appear to be seriously injured by free fatty acids and monoglycerides from hemolytic streptococci during the 90-min incubation. Similarly, the incubation of Ehrlich tumor cells with diphosphatidyl glycerols (2 mg per ml) from two strains of streptococci (Blackmore and C203U) resulted in a significant decrease of the viable tumor cells (proportion of viable tumor cells: less than 4%).
Table 6. Effect of free fatty acids, monoglycerides and sterol esters from hemolytic streptococci against Ehrlich carcinoma in mice

| Hemolytic streptococci | Lipid components* | No. of survivors at 60 days | Lipid concn in test mixture (mg/ml of cell suspension) |
|------------------------|-------------------|----------------------------|-----------------------------------------------------|
|                        |                   |                            | 2.0  | 1.0  | 0.5  | 0.2  | 0.1  | 0.05 |
| Su                     | FA                | 10/10                      | 10/10| 10/10| 8/10 | 4/10 | 0/10 |
|                        | MG                | 10/10                      | 10/10| 8/10 | 4/10 | 0/10 |
|                        | SE                | 8/10                       | 4/10 | 0/10 |
| Blackmore              | FA                | 5/5                        | 5/5  | 5/5  | 4/5  | 3/5  | 0/5  |
|                        | MG                | 5/5                        | 5/5  | 4/5  | 2/5  | 0/5  |
|                        | SE                | 4/5                        | 2/5  | 0/5  |
| C203U                  | FA                | 5/5                        | 5/5  | 5/5  | 3/5  | 2/5  | 0/5  |
|                        | MG                | 5/5                        | 5/5  | 4/5  | 2/5  | 0/5  |
|                        | SE                | 2/5                        | 0/5  |

*FA, free fatty acids; MG, monoglycerides; SE, sterol esters.

Table 7. Fatty acid composition of antitumor lipids from hemolytic streptococci

| Hemolytic streptococci | Lipid components* | Fatty acid composition (%)** |
|------------------------|-------------------|-------------------------------|
|                        |                   | C14:0 | C16:0 | C18:1 | C17:0 | C18:0 | C18:1 | C18:2 |
| Su                     | DPG               | 8.2   | 76.7  | —     | —     | 15.1  | —     | —     |
|                        | FA                | 7.0   | 51.0  | 4.8   | 2.6   | 21.3  | 10.5  | tr.   |
|                        | MG                | 1.6   | 44.7  | tr.   | —     | 44.7  | 8.0   | —     |
| Blackmore              | DPG               | 8.3   | 69.3  | 9.6   | —     | 5.6   | 7.1   | —     |
|                        | FA                | 4.5   | 41.5  | 12.6  | tr.   | 16.9  | 18.9  | 3.2   |
|                        | MG                | 3.0   | 28.7  | 6.4   | tr.   | 21.5  | 20.9  | 5.5   |
| C203U                  | DPG               | 2.3   | 35.2  | 14.0  | —     | 9.3   | 39.2  | —     |
|                        | FA                | 3.0   | 40.8  | tr.   | 1.7   | 51.0  | 3.0   | tr.   |
|                        | MG                | 3.2   | 39.2  | tr.   | —     | 46.3  | 10.7  | —     |

*DPG, diphosphatidyl glycerols; FA, free fatty acids; MG, monoglycerides. **tr: trace amounts (less than 1%).

Fatty acid composition of active lipid components: The biological activity of diphosphatidyl glycerols from one strain of hemolytic streptococci (Su) was quite different from that of the other strains of streptococci (Blackmore and C203U), as described above. The fatty acid composition of diphosphatidyl glycerols from three strains of streptococci was then examined by gas-liquid chromatography. The gas chromatographic analysis of fatty acid methyl esters obtained from phospholipids indicated that the fatty acid composition of diphosphatidyl glycerols markedly differed with the original organisms (Table 7). The most pronounced difference was found in the composition of unsaturated fatty acids of the phospholipids among three strains of streptococci. The amounts of unsaturated fatty acids in diphosphatidyl glycerols from two strains of streptococci (Blackmore and C203U) were about 17% and 53%, respectively. Unsaturated
fatty acids were not detected in the phospholipids from the other streptococcus (Su).

The fatty acid composition of free fatty acids and monoglycerides from three strains of hemolytic streptococci was also examined by gas-liquid chromatography. A significant difference was found in the fatty acid composition of these neutral lipids among three strains of streptococci (Table 7). However, the unsaturated fatty acids were detected in all the biologically active neutral lipids, although the composition of these acids varied from 3% to 35%.

**DISCUSSION**

The present results clearly indicate that free fatty acids and monoglycerides obtained from three strains of group A hemolytic streptococci (Su, Blackmore and C203U) are highly active in the suppression of development of Ehrlich ascites carcinoma in mice when preincubated with tumor cells before inoculation, and diphosphatidyl glycerols from two strains of the streptococci (Blackmore and C203U) are effective in suppressing the tumor growth in mice. Concerning this, the incubation of Ehrlich tumor cells with these active streptococcal lipids resulted in a significant decrease of viable tumor cells. The proportion of viable Ehrlich tumor cells was about 85% before incubation and less than 4% after incubation for 90 min with the active streptococcal lipids at a concentration of 2 mg per ml of cell suspension. At the same concentration, the biologically active lipids from hemolytic streptococci completely inhibited the growth of Ehrlich tumor cells in mice. Thus, Ehrlich tumor cells may lose their viability when incubated with free fatty acids and monoglycerides from three strains of hemolytic streptococci or with diphosphatidyl glycerols from two strains of the streptococci (Blackmore and C203U). There are many studies indicating that free fatty acids and their esters are highly cytotoxic to tumor cells, and these cytotoxic lipids are the factors responsible for the antitumor activity of fungi or mammalian cells (3–8). Therefore, it is likely that the cytotoxic lipids, such as free fatty acids or monoglycerides, are involved in the antitumor activity of the group A streptococci.

The mechanism by which fatty acids and their derivatives inhibit tumor growth is not clear. However, several investigations have established that the antitumor activity of fatty acids depends upon the number of the carbon chain and the degree and position of unsaturation, and the long chain unsaturated fatty acids, such as oleic acid (18:1) and linoleic acid (18:2), are involved in the tumor cell lysis by fatty acids in vitro (6, 27, 28). The gas chromatographic analysis of fatty acid composition of the cytotoxic lipids from hemolytic streptococci indicated that the main components of free fatty acids and monoglycerides are palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1), and oleic acid (18:1). These saturated and unsaturated fatty acids were also found in the cytotoxic phospholipids from hemolytic streptococci. Therefore, the long chain fatty acids, in particular the unsaturated acids, may play an important role in the cytotoxic lipids from group A hemolytic streptococci. Interestingly, unsaturated fatty acids have been reported to influence the properties of mammalian cell membrane in vitro (29).

**REFERENCES**

1) Okamoto, H., Shoin, S., Koshimura, S. and Shimizu, R.: Studies on the anticancer and streptolysin S-forming abilities of hemolytic streptococci. Japan. J. Microbiol. 11, 323-336 (1967)

2) Okamoto, H.: Mechanisms in Bacterial Toxinology, Edited by Bernheimer, A. D., 1st ed., p. 238-257, John Wiley and Sons Inc., New York (1976)

3) Williams, R.H., Lively, P.H., Delong, D.C., Cline, J.C., Sweeney, M.J., Poore, G.A. and
Larson, S.H.: Mycophenolic acid: antiviral and antitumor properties. J. Antibiotics 21, 463–464 (1968)

4) Ando, K., Suzuki, S., Suzuki, K., Kodama, K., Kato, A., Tamura, G. and Arima, K.: Isolation of fatty acids with antitumor activity from fungal mycelia. J. Antibiotics 28, 18–22 (1969)

5) Kato, A., Ando, K., Kodama, K., Suzuki, S., Suzuki, K., Tamura, G. and Arima, K.: Production, isolation and purification of antitumor monoglycerides and other antibiotics from *Sepe-donum ampullosporum*. J. Antibiotics 22, 71–76 (1969)

6) Seno, S. and Yamamoto, M.: Chemical and biological activities of fatty acids from the liver of X-irradiated rabbit, the antitumor agent so-called OX. Acta Med. Okayama 19, 59–72 (1965)

7) Okudaira, H., Kataoka, T., Okada, H., Furuse, Irie, K., Kawachi, S., Nojima, S. and Nishioka, K.: Cytotoxic factor demonstrated in lymph node extract. J. Biochem., Tokyo 68, 379–394 (1970)

8) Kigoshi, S. and Ito, R.: High levels of free fatty acids in lymphoid cells, with special reference to their cytotoxicity. Experientia 29, 1408–1409 (1973)

9) Wood, A.J. and Gunsalus, I.G.: The production of active resting cells of streptococci. J. Bact. 44, 333–341 (1942)

20) Koshimura, S., Murasawa, K., Nakagawa, E., Ueda, N., Bando, Y. and Hirata, R.: Experimental anticancer studies. Part III. On the influence of living hemolytic streptococci upon the invasion power of Ehrlich ascites carcinoma in mice. Japan. J. exp. Med. 25, 93–102 (1955)

21) Trevelyan, W.E., Procter, D.P. and Harrison, J.S.: Detection of sugars on paper chromatogram. Nature 166, 444–445 (1950)

22) Brundish, D.E., Shaw, N. and Baddiley, J.: The occurrence of glycolipids in gram-positive bacteria. Biochem. J. 95, 21c–22c (1965)

23) Christophe, A. and Matthijs, F.: New method for the determination of the fatty acid pattern of serum lipid classes. Clin. Chim. Acta 16, 39–43 (1967)

24) Horning, M.G.: Lipid Pharmacology. Edited by Paoletti, R., p. 1–62, Academic Press, New York and London (1964)

25) Kosower, E.M., Kosower, N.S., Faltin, Z., Diver, A., Saltoun, G. and Frendorf, A.: Membrane mobility agents. A new class of biological active molecules. Biochim. Biophys. Acta 363, 261–266 (1974)