EFFECT OF PENTAZOCINE AND RELATED COMPOUNDS ON THE LIPID COMPOSITION OF EHRlich ASCITES TUMOR CELLS

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Abstract—The effect of pentazocine and its related compounds on the lipid composition of Ehrlich ascites tumor cells was examined. Ehrlich tumor cells were suspended in Hanks balanced salt solution (pH 7.4) supplemented with 2% bovine albumin (2×10^6 cells/ml) and incubated at 37°C for 120 min with and without 10^{-3} M of pentazocine, morphine or dihydrocodeine. After incubation, the tumor cell lipids were extracted with chloroform-methanol (2:1, v/v), and they were analyzed quantitatively by the dichromate reduction procedure of Amenta. The tumor cells treated with pentazocine contained lower levels of triglycerides and cholesterol esters as compared with the tumor cells incubated alone. The amounts of triglycerides and cholesterol esters in the tumor cells treated with and without pentazocine were about 82 and 23 mg/10^10 cells and 182 and 55 mg/10^10 cells, respectively (P<0.01), whereas there was only a slight difference in the contents of these neutral lipids between the tumor cells treated with and without 10^{-3} M morphine or dihydrocodeine. In addition, the fatty acid pattern of triglycerides and cholesterol esters from the pentazocine-treated tumor cells differed markedly from that of the corresponding lipids from the tumor cells incubated alone.

Pentazocine, a non-narcotic analgesic, has recently been shown to be cytotoxic to Ehrlich ascites tumor cells in vitro and exhibit a weak antitumor activity against Ehrlich ascites carcinoma in mice (1). This suggests that pentazocine might affect the cellular components of the tumor cells. In the present study, pentazocine and its related compounds, such as morphine and dihydrocodeine, were examined for their effect on the lipid composition of Ehrlich tumor cells. The quantitative analysis of the tumor cell lipids indicated that pentazocine causes a significant decrease of triglycerides and cholesterol esters in Ehrlich tumor cells, whereas morphine and dihydrocodeine have little effect on the contents of these neutral lipids in the tumor cells.

MATERIALS AND METHODS

Treatment of tumor cells with drugs: Ehrlich ascites tumor cells were inoculated intraperitoneally into female mice of the ddY strain, 7–8 weeks of age. At 10 days after inoculation, the tumor cells were obtained from the mice and were suspended in Hanks balanced salt solution (BSS, pH 7.4) containing penicillin G (100 IU/ml) and streptomycin (100 μg/ml). The tumor cells used were prepared by velocity sedimentation as described previously (1), and they were suspended in BSS (pH 7.4) supplemented...
with 2% bovine albumin fraction V (Wako Pure Chem. Indust.).

Drugs used were as follows: pentazocine (Sankyo Co., 15 mg/ml solution), and morphine hydrochloride and dihydrocodeine phosphate (Takeda Chem. Indust). Morphine hydrochloride and dihydrocodeine phosphate were dissolved in physiological saline (5 x 10^{-2} M) and stored at 4°C until use.

About 20 ml of the tumor cell suspension (2 x 10^6 cells/ml) in 50-ml test tubes were incubated with drugs at 37°C for 15 to 120 min in a shaking water bath. The tumor cells were also incubated in the absence of the drugs. After incubation, the tumor cells collected by centrifugation were washed twice with BSS and used for lipid extraction.

**Extraction of tumor cell lipids:** The total lipids of Ehrlich tumor cells were extracted with chloroform-methanol (2:1, v/v) at room temperature overnight, and they were purified by the method of Folch et al. (2). The yields of the total lipids from 10^10 cells were 400-550 mg.

**Qualitative analysis of tumor cell lipids:** The total lipids of Ehrlich tumor cells were separated into phospholipid classes and neutral lipids on a plate covered with silica gel H (E. Merck, Germany; 0.25 mm thick) by one-dimensional thin-layer chromatography using chloroform-methanol-water (65:25:4, by vol.) (3) as an ascending solvent (4). To identify individual phospholipids, lipids were detected with the following spray reagents (4): phosphomolybdate, ninhydrin reagent, Dragendorff reagent, Dittmer-Lester reagent, anthrone-sulfuric acid and antimony trichloride. The Rf values of individual phospholipids were then compared with those of phosphatidyl choline, phosphatidyl-l-ethanolamine or sphingomyelin (Nutritional Biochemicals, USA).

In addition, the total lipids were fractionated into neutral lipid classes and phospholipids on a silica gel H plate (0.25 mm thick) by one-dimensional thin-layer chromatography using hexane-ethyl ether-acetic acid (70:30:2, by vol.) (5). Identification of individual neutral lipids was achieved by spraying with phosphomolybdate and antimony trichloride and by comparing Rf values with those of known lipid substances (4).

**Quantitation of tumor cell lipids:** The tumor cell lipids were analyzed quantitatively according to the dichromate reduction procedure of Amenta (6). For the quantitation of neutral lipids, the total lipids were developed on silica gel H plates (0.25 mm thick) using hexane-ethyl ether-acetic acid (70:30:2). The total lipids were chromatographed with chloroform-methanol-water (65:25:4) to examine the phospholipid contents. After development, lipids were detected with iodine reagent, and the silica gel containing lipids were transferred into test tubes. Each lipid fraction was treated with 0.25% potassium dichromate in sulfuric acid for 45 min in a boiling water bath. After treatment, the supernatant obtained from the reaction mixture by centrifugation was examined at 350 nm with a Spectronic 20 (Bausch & Lomb). Phosphatidyl choline (Nutritional Biochemicals, USA), cholesterol (E. Merck, Germany) and palmitic acid (PI-Biochemicals, USA) were used as standard substances.

**Gas chromatographic analysis of fatty acid pattern of lipid classes:** To examine the fatty acid pattern of lipid components, each component was prepared by preparative thin-layer chromatography using the above solvent systems (4) and was methylated with 20% sulfuric acid in methanol at 65-70°C for 15 hr (7). The methyl esters of fatty acids were analyzed 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 x 3 mm internal diameter) containing 20% NPGS on
Chromosorb W (60–80 mesh) at 210°C. Dual hydrogen detectors and a flame 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 comparing of relative retention times with those of known methyl esters (Analytical reference standard kits, Gasukuro Indust.). Quantitative estimations were based on direct measurements of the chart peak areas which had been shown to be proportional to the amounts of the components present (4).

RESULTS

Lipid composition of tumor cells: Qualitative analysis of the total lipids from Ehrlich tumor cells indicated that the main lipid components of the tumor cells were phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, free cholesterol, free fatty acids, triglycerides and cholesterol esters (8–10). Thus, the contents of these lipid components in the tumor cells were examined after treatment with and without drugs.

Table 1 shows the contents of neutral lipids in the tumor cells that had been incubated for 120 min with and without 10^{-3} M concentration of drugs. The tumor cells treated with 10^{-3} M pentazocine contained lower levels of triglycerides and cholesterol esters as compared with the tumor cells incubated alone. The quantities of triglycerides and cholesterol esters in the tumor cells treated with and without the drug were about 82 and 23 mg/10^{10} cells and 182 and 55 mg/10^{10} cells, respectively (P<0.01). The contents of free fatty acids in the pentazocine-treated tumor cells also differed from those in the tumor cells incubated alone (about 11 and 20 mg/10^{10} cells, P<0.01). In contrast, only a slight difference was found in the quantities of these neutral lipids between the tumor cells incubated with and without 10^{-3} M morphine or dihydrocodeine for 120 min. On the other hand, the amounts of individual phospholipids in the tumor cells treated with the drugs for 120 min were similar to those in the tumor cells incubated alone (Table 2).

The contents of neutral lipids in Ehrlich tumor cells were then examined after incubation with 10^{-3} M pentazocine for 15 to 120 min. The levels of triglycerides and cholesterol esters in the tumor cells were reduced significantly during the 60-min

| Drugs used for treatment of tumor cells | Incubation time (min) | Contents of neutral lipids in tumor cells (mg/10^{10} cells) |
|----------------------------------------|-----------------------|----------------------------------------------------------|
|                                        |                       | Triglycerides   | Cholesterol esters | Free fatty acids | Free cholesterol |
| Pentazocine                            | 120                   | 81.9± 6.8*     | 23.3±1.9*         | 11.0±1.4*       | 55.2±1.9       |
| Morphine                               | 120                   | 160.3± 3.2     | 55.6±1.1          | 19.5±2.6        | 56.1±0.5       |
| Dihydrocodeine                         | 120                   | 176.2±11.9     | 50.4±4.0          | 20.6±1.6        | 56.7±0.8       |
| (−)                                    | 120                   | 182.3± 8.1     | 54.9±2.1          | 19.9±2.1        | 55.7±1.1       |
| (−)                                    | 0                     | 185.7± 4.4     | 53.4±3.2          | 19.5±1.2        | 55.3±1.6       |

Ehrlich tumor cells were suspended in Hanks balanced salt solution (pH 7.4) supplemented with 2% bovine albumin (2×10^6 cells/ml) and incubated at 37°C for 120 min with and without 10^{-3} M pentazocine morphine or dihydrocodeine. After incubation, the tumor cell lipids were extracted with chloroform-methanol (2:1, v/v) and analyzed quantitatively thereafter. Each value represents the mean±S.E. of 6 experiments. *Significantly different from the corresponding value of tumor cells incubated alone (P<0.01).
incubation with the drug (Fig. 1). The amounts of triglycerides and cholesterol esters in the tumor cells treated with $10^{-3}$ M pentazocine for 30 and 60 min were about 60% and 50% of those of the corresponding lipids in the untreated tumor cells, respectively ($P<0.01$). The contents of these neutral lipids in the tumor cells treated with $10^{-3}$ M pentazocine for 120 min were about 45% of those in the untreated tumor cells.

In the following experiments, the effect of pentazocine on the lipid composition of Ehrlich tumor cells was examined at concentrations of $10^{-6}$–$10^{-3}$ M. In the tumor cells treated with pentazocine, the levels of triglycerides and cholesterol esters were decreased with an increase of the drug concentration (Fig. 2). The amounts of triglycerides and cholesterol esters in the tumor cells treated with $10^{-4}$ M pentazocine for 120 min were about 60% of those in the tumor cells incubated alone ($P<0.01$). The contents of free fatty acids in the tumor cells treated with $10^{-4}$ M pentazocine for 120 min were also smaller than those in the tumor cells incubated alone (about 13 and 20 mg/10$^{10}$ cells, $P<0.01$). On the other hand, no significant difference was found in the quantities of these neutral lipids between the tumor cells treated with and without $10^{-6}$ M pentazocine for 120 min. The contents of free cholesterol and phospholipids in the tumor cells treated with pentazocine for 120 min were nearly equal to those in the tumor cells incubated alone, irrespective of
the drug concentrations.

**Fatty acid pattern of lipid classes of tumor cells**: As described above, a marked difference was found in the lipid composition between Ehrlich tumor cells treated with and without pentazocine. The fatty acid pattern of neutral lipids and phospholipids from Ehrlich tumor cells was then examined after incubation with and without $10^{-3}$ M pentazocine for 120 min. The fatty acid pattern of triglycerides and cholesterol esters from the pentazocine-treated tumor cells markedly differed from that of the corresponding lipids from the tumor cells incubated alone (Table 3) (9, 11). The proportions of some fatty acids in the triglycerides from the tumor cells treated with and without the drug were as follows: about 28% and 22% for palmitic acid (16:0), 4% and 2% for palmitoleic acid (16:1), 28% and 22% for oleic acid (18:1), 7% and 21% for linoleic acid (18:2). The proportion of stearic acid (18:0), oleic acid (18:1) and decosatetraenoic acid (22:4) in the cholesterol esters was about 14%, 14% and 16% for the pentazocine-treated tumor cells, respectively, and about 21%, 23% and 2% for the tumor cells incubated alone, respectively. The fatty acid pattern of free fatty acids from the pentazocine-treated tumor cells also differed from that of the corresponding lipids from the tumor cells incubated alone [about 2% and 5% for lauric acid (12:0), 1% and 3% for palmitoleic acid (16:1), 27% and 17% for stearic acid (18:0), 4% and less than 0.5% for decosatetraenoic acid (22:4)].

In addition, a significant difference was found in the proportion of some fatty acids in the individual phospholipids between Ehrlich tumor cells incubated with and without $10^{-3}$ M pentazocine for 120 min (Table 4). The proportion of oleic acid (18:1) in the phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin was about 14%, 11% and 11% for the pentazocine-treated tumor cells, respectively, and about 18%, 17% and 16% for the tumor cells incubated alone, respectively. The proportion of linoleic acid (18:2) in these phospholipids from the tumor cells treated with and without pentazocine was as follows: about 2% and 9% for phosphatidyl choline, 9% and 15% for phosphatidyl ethanolamine, 2% and 5% for sphingomyelin. The proportion of decosatetraenoic acid (22:4) in the phospholipids from the pentazocine-treated tumor cells also differed from that of the tumor cells incubated alone (about 2% and 1% for phosphatidyl choline, 6% and 0.5% for phosphatidyl ethanolamine, 3% and 1% for sphingomyelin).
Table 3.  Fatty acid pattern of neutral lipids from Ehrlich tumor cells treated with and without 10^{-3} M pentazocine (PZ)

| Tumor cells  | Lipid classes* | 12:0 | 14:0 | 16:0 | 16:1 | 17:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 22:0 | 22:4 |
|--------------|----------------|------|------|------|------|------|------|------|------|------|------|------|------|
| Tumor cells treated with PZ | TG | 1.1 | 3.3 | 27.5 | 3.5 | 0.8 | 16.5 | 27.9 | 7.0 | 0.5 | 4.4 | 1.9 | 1.3 |
|               | ChE          | tr.  | 5.3 | 34.2 | 1.5 | 1.3 | 13.9 | 13.6 | 5.4 | 1.5 | 2.1 | —   | 15.6 |
|               | FA           | 1.9 | 3.8 | 29.1 | 1.0 | 1.4 | 27.1 | 13.9 | 4.2 | 1.9 | 2.9 | 2.0 | 4.0 |
| Tumor cells incubated alone | TG | 0.8 | 3.2 | 21.9 | 1.9 | tr. | 14.6 | 22.3 | 20.5 | 0.7 | 2.9 | 1.2 | 1.4 |
|               | ChE          | 0.8 | 4.8 | 32.9 | 1.8 | 1.0 | 21.4 | 23.2 | 6.6 | 1.3 | 2.0 | 0.6 | 1.5 |
|               | FA           | 4.9 | 4.0 | 32.2 | 3.0 | 2.2 | 16.8 | 17.1 | 5.9 | 1.2 | 2.9 | 0.7 | tr. |
| Tumor cells without incubation | TG | 0.8 | 3.1 | 28.5 | 1.4 | 0.5 | 17.2 | 15.3 | 16.2 | 0.7 | 2.0 | 1.1 | 4.9 |
|                | ChE          | 2.0 | 6.7 | 31.5 | 2.1 | 2.2 | 17.4 | 16.4 | 2.9 | 1.3 | 1.7 | 2.3 | 5.5 |
|                | FA           | 1.5 | 4.1 | 33.8 | 1.1 | 2.3 | 24.1 | 9.6 | 6.6 | 1.2 | 0.7 | 0.9 | 3.6 |

Neutral lipids were obtained from Ehrlich tumor cells incubated with and without 10^{-3} M pentazocine at 37°C for 120 min. and methylated with methanolic sulfuric acid at 65-70°C for 15 hr. The fatty acid methyl esters were then analyzed quantitatively by gas-liquid chromatography. *TG, triglycerides; ChE, cholesterol esters; FA, free fatty acids. **Each value represents the mean of 2 determinations. Fatty acids are presented as carbon length and number of double bonds. Tr.: trace amount (less than 0.5%).

Table 4. Fatty acid pattern of phospholipids from Ehrlich tumor cells treated with and without 10^{-3} M pentazocine (PZ)

| Tumor cells  | Lipid classes* | 12:0 | 14:0 | 16:0 | 17:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1 | 20:2 | 22:0 | 22:4 |
|--------------|----------------|------|------|------|------|------|------|------|------|------|------|------|------|
| Tumor cells treated with PZ | PC | 1.2 | 1.4 | 25.0 | 0.8 | 39.6 | 13.9 | 7.3 | tr. | 0.8 | 3.8 | 0.7 | 2.1 |
|                | PE | 1.6 | 1.7 | 22.1 | 1.8 | 33.6 | 10.8 | 8.5 | 1.4 | 2.3 | 2.0 | 1.4 | 6.4 |
|                | SM | 1.6 | 1.6 | 25.5 | 1.0 | 44.2 | 11.3 | 2.0 | 0.6 | tr. | 1.0 | 1.0 | 3.3 |
| Tumor cells incubated alone | PC | 2.1 | 2.4 | 25.0 | 0.8 | 35.2 | 17.6 | 8.7 | 0.9 | 1.3 | tr. | 0.7 | 0.7 |
|                | PE | 1.3 | 2.4 | 21.5 | 0.8 | 29.5 | 17.4 | 14.6 | 1.2 | 2.7 | 0.9 | tr. | 0.5 |
|                | SM | 1.8 | 2.8 | 29.5 | 0.7 | 32.2 | 16.0 | 5.1 | 1.0 | 1.8 | tr. | 0.7 | 1.2 |
| Tumor cells without incubation | PC | 2.4 | 1.7 | 24.3 | 1.1 | 31.5 | 12.3 | 12.1 | 1.2 | 1.2 | 3.9 | 2.0 | 2.4 |
|                | PE | 2.4 | 2.3 | 25.7 | 1.0 | 29.4 | 11.3 | 10.8 | 1.0 | 0.7 | 2.9 | 0.8 | 5.3 |
|                | SM | 2.6 | 3.1 | 30.6 | 1.4 | 29.0 | 12.0 | 11.8 | 0.6 | 0.6 | 5.0 | —   | —   |

*PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; SM, sphingomyelin. **Each value represents the mean of 2 determinations. Fatty acids are presented as carbon length and number of double bonds. Tr.: trace amount (less than 0.5%).
On the other hand, there was only a slight difference in the fatty acid pattern of individual lipid fractions between Ehrlich tumor cells incubated with and without $10^{-3}$ M morphine or dihydrocodeine for 120 min.

**DISCUSSION**

The present results indicate that the incubation of Ehrlich ascites tumor cells with $10^{-3}$ or $10^{-4}$ M pentazocine results in a marked decrease of triglycerides and cholesterol esters in the tumor cells, while no significant change is observed in the contents of free cholesterol and phospholipids in the tumor cells during the incubation. In addition, the fatty acid pattern of triglycerides and cholesterol esters from the tumor cells treated with $10^{-3}$ M pentazocine for 120 min markedly differed from that of the corresponding lipids from the tumor cells incubated alone.

The pharmacological action of pentazocine is similar to that of morphine or dihydrocodeine (12, 13). However, morphine and dihydrocodeine caused no significant change in the lipid composition of Ehrlich tumor cells *in vitro*. Recently, pentazocine has been reported to be cytotoxic to Ehrlich tumor cells *in vitro* (1). When Ehrlich tumor cells were incubated with $10^{-3}$ M pentazocine, the proportion of the viable cells were reduced markedly within 120 min (proportions of the viable cells before and after incubation for 120 min were about 85% and 0.9%, respectively). On the other hand, morphine and dihydrocodeine have been shown to have little effect on the viability of the tumor cells *in vitro* (1). Therefore, it may be concluded that pentazocine markedly differs from morphine or dihydrocodeine in the *in vitro* effect on the viability and lipid composition of Ehrlich tumor cells. Concerning this, dextromethorphan (13) and dimemorfan ($d$-3-methyl-N-methylmorphi-}

nan) (14), the centrally acting antitussives, were similar to pentazocine in the *in vitro* effect on Ehrlich tumor cells. The incubation of Ehrlich tumor cells with $10^{-3}$ M dextromethorphan or dimemorfan at 37°C resulted in a decrease of the viable cells (proportion of the viable tumor cells after incubation for 120 min: about 0.5% for dextromethorphan and 20% for dimemorfan). Further, the tumor cells treated with $10^{-3}$ M concentration of these drugs for 120 min contained lower levels of triglycerides and cholesterol esters as compared with the tumor cells incubated alone. The contents of triglycerides and cholesterol esters in the tumor cells treated with and without the drugs were about 85 and 30 mg/10^10^ cells and 182 and 55 mg/10^10^ cells, respectively. Thus, the decrease in the proportion of viable Ehrlich tumor cells may be accompanied with the decrease of triglycerides and cholesterol esters in the tumor cells when the tumor cells are incubated with pentazocine or other cytotoxic drugs.

Recently, lipids have been reported to be involved in the lysis of mammalian cells including tumor cells (15). Turnell et al. have indicated that in corticosteroid-induced lymphocytolysis, corticosteroids cause a depletion of triglycerides in lymphocytes, and the free fatty acids released from the triglycerides affect the viability of the cells (16). Schlager et al. have demonstrated that in the complement-dependant cytolysis by antibody, changes in the contents of triglycerides and free fatty acids in the target cells are intimately connected with target cell lysis (17, 18). Therefore, it is likely that triglycerides and cholesterol esters may be involved in the damage of Ehrlich tumor cells by pentazocine.

There are several reports suggesting that the fatty acid pattern of phospholipids in tumor cells or other cells is closely related to the properties of the cell membrane of these cells (19–23). Phospholipids are
known to be the main constituent of the cell membrane lipid. As described above, the fatty acid pattern of phospholipids from Ehrlich tumor cells treated with 10⁻³ M pentazocine for 120 min differed from that of the corresponding lipids from the untreated tumor cells. Thus, Ehrlich tumor cells treated with 10⁻³ M pentazocine might differ from the untreated tumor cells in the properties of the cell membrane.

REFERENCES

1) Kigoshi, S.: Effect of pentazocine on Ehrlich ascites tumor cells. Japan. J. Pharmacol. 31, 781–785 (1981)
2) Folch, J., Lees, M. and Sloan Stanley, G.H.: A simple method for isolation and purification of total lipids from animal tissues. J. biol. Chem. 226, 487–509 (1957)
3) Wagner, H., Horhammer, C. and Wolf, R.: Dünnschichtchromatographie von Phosphatiden und Glykolipiden. Biochem. Z. 334, 175–184 (1961)
4) Kigoshi, S. and Kitajima, K.: Effect of streptococcal lipids on Ehrlich ascites tumor cells. Japan. J. Pharmacol. 31, 201–209 (1981)
5) Jatzkewitz, H. and Mehl, E.: Dünn schichtchromatographie der Gehirn-Lipoide, ihrer Umbau- und Abbauprodukte. Hoppe-Seyler's Z. physiol. Chem. 320, 251–257 (1960)
6) Amenta, J.S.: A rapid chemical method for quantitation of lipids separated by thin-layer chromatography. J. Lipid Res. 5, 270–272 (1964)
7) Christoph, 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)
8) Yamakawa, T., Ueta, N. and Irie, R.: Biochemistry of lipids of neoplastic tissue. I. Lipid composition of ascitic tumor cells of mice. Japan. J. Exp. Med. 32, 289–296 (1962)
9) Yamakawa, T. and Ueta, N.: Biochemistry of lipids of neoplastic tissue. II. Variability of the fatty acid composition of mouse ascites tumor cells. Japan. J. Exp. Med. 32, 591–598 (1962)
10) Ligard, P.H. and Greenberg, D.M.: The phosphatides of some ascites tumor and hepatoma. Cancer Res. 22, 361–367 (1962)
11) Wood, R.: Tumor lipids: Identification of sterol ester species in Ehrlich ascites cells. Cancer Res. 30, 151–154 (1970)
12) Brogden, R.N., Speight, T.M. and Avery, G.S.: Pentazocine: a review of its pharmacological properties, therapeutic efficacy and dependence liability. Drugs 5, 6–91 (1973)
13) Jaffe, J.H. and Martin, W.R.: Opioid analgesics and antagonists. In The Pharmacological Basis of Therapeutics, Edited by Gilman, A.G., Goodman, L.S. and Gilman, A., 6th edition, p. 494–534, Macmillan Publishing Co., New York (1980)
14) Kase, Y., Kito, G., Miyata, T., Takahama, K., Uno, T. and Ida, H.: On the site of antitussive action of d-3-methyl-N-methylmorphinan (AT-17). Arzneim.-Forsch. 26, 361–366 (1976)
15) Meade, C.J. and Mertin, J.: Fatty acids and immunity. Adv. Lipid Res. 16, 127–165 (1978)
16) Turnell, R.W., Clarke, L.H. and Burton, A.F.: Studies of mechanism of corticosteroid-induced lymphocytolysis. Cancer Res. 33, 203–212 (1973)
17) Schlager, S.I., Ohanian, S.H. and Boros, T.: Stimulation of the synthesis and release of lipids in tumor cells under attack by antibody and C. J. Immunol. 120, 895–901 (1978)
18) Schlager, S.I., Ohanian, S.H. and Boros, T.: Identification of lipids synthesized and released by tumor cells under attack by antibody and complement. J. Immunol. 120, 1644–1650 (1978)
19) Ferber, E., de Pasquale, G.G. and Resch, K.: Phospholipid metabolism of stimulated lymphocytes: composition of phospholipid fatty acid. Biochim. Biophys. Acta 389, 384–378 (1975)
20) Resch, K. and Ferber, E.: The role of phospholipids in lymphocyte activation. In Immune Recognition, Edited by Rosenthal, A. S., p. 281–312, Academic Press Inc., New York (1975)
21) Mandel, G., Shimizu, S., Gill, R. and Clark, W.: Alteration of the fatty acid composition of membrane phospholipids in mouse lymphoid cells. J. Immunol. 120, 1631–1636 (1978)
22) Mandel, G. and Clark, W.: Functional properties of EL-4 tumor cells with lipid-altered membrane. J. Immunol. 120, 1637–1643 (1978)
23) Schlager, S.I. and Ohanian, S.H.: Tumor cell lipid composition and sensitivity to humoral killing. I. Modification of cellular lipid and fatty acid content by metabolism inhibitor and hormones. J. Immunol. 124, 626–634 (1980)