72 h. Mortality rate differences for the 2 experiments are presumably due to variation in number of organisms in the diluted inocula since in all other aspects the trials were similar. There was no mortality or morbidity in control mice.

In each experiment, males died more rapidly than females, with the sex difference already evident between 48 and 60 h. Chi Square analysis (2×2 contigency test) of the dead/alive ratio for males vs females showed that the sex difference was statistically significant in Experiment I after 54 h. In Experiment II, significance was seen primarily in the early (54–60 h) and later periods (120–144 h). When the 2 trials were combined, the sex difference was significant at all points between 48 and 144 h.

Subgroups of approximately 10 males and 10 females were exposed from time of inoculation to altered O_2 environments which ranged from 12% O_2 to 100% O_2 at sea level[8] and to an altitude of 13,000 ft in air. One additional group had food removed. In general, male mortality was higher than female mortality in all subgroups, indicating that the sex difference persists during stress situations.

In agreement with FRIEDMAN et al.[1], however, the sex difference tended to be obscured in those conditions which increased the overall rate of mortality (i.e., high O_2, starvation). This observation may also explain VON HAMM's and ROSENFELD's[6] failure to observe a sex difference in the absence of estrogen, since their control animals were all dead in 26–36 h. However, their experiments differed also in that they used a type 1 pneumococcus and injected subcutaneously, whereas we used type 12 and inoculated i.p.

The marked sex effect we observed indicates that considerable caution should be exercized in selection of animals in mouse-pneumococcus studies if experimental variability is to be kept low. The rapidity with which a significant male-female difference in mortality is seen, as early as 2 days post inoculation, and its persistance over a considerable range in overall rate of mortality suggests that *D. pneumoniae* in mice may serve as a useful model for exploring the mechanism of the sex difference in resistance to infection[9].

Résumé. L'injection de *Diplococcus pneumoniae* (type 12) par voie intrapéritonéale à des souris albinos provoque un taux de mortalité significativement plus élevé chez les mâles que chez les femelles. Ce fait pourrait servir de modèle à l'étude des différences sexuelles dans la résistance à l'infection.

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Chromatographic Characterization of Antitumor Lipids from a Group A *Streptococcus*

In the previous paper[1], it was reported that lipids extracted from a group A *Streptococcus*, when preincubated with tumor cells before inoculation, completely suppressed the development of Ehrlich ascites tumor in mice. Fractionation studies established that this antitumor activity was associated exclusively with nonpolar lipid fraction. The present study is concerned with further characterization of active lipid components from a hemolytic streptococcus, strain Su, by two-dimensional thin-layer chromatographic analysis.

Total lipids (2.5 g/100 l culture) isolated from streptococci as described previously[1] were extracted with acetone to separate an acetone-soluble lipid fraction, containing all of active nonpolar lipids and a portion of inactive glycolipids, from an acetone-insoluble phospholipids. The acetone-soluble lipids were chromatographed two- dimensionally on plates covered with silica gel H (0.25 mm thick) using ethylene chloride-methanol (98:2)[2] and *n*-hexane-diethyl ether-acetic acid (70:30:2)[3] as the solvent systems. Lipids were detected with iodine, phosphomolybdate or antimony trichloride. The Rf values of individual lipid components were compared with those of the following authentic materials: fatty acids (palmitic, stearic, oleic, linoleic and linolenic acid), monoglycerides (glycerol-α-monopalmitate and glycerol-α-monostearate), diglycerides (dipalmitin and distearin), triglycerides (tripalmitin and tristearin), cholesterol and cholesterol stearate.

For biological testing, lipids were isolated by preparative one-dimensional thin-layer chromatography as follows: acetone-soluble lipids were streched on a silica gel plate (1 mm thick), and developed with ethylene chloride-methanol (98:2), thereby yielding 4 fractions (referred to as Fraction A, B, C and D in the increasing order of the migrating rates). Each of these fractions were further resolved into several subfractions (A1, A2, B1 and so on) by chromatography with *n*-hexane-diethyl ether-acetic acid (70:30:2) or (80:20:1)[4], the latter mixture being more effectively employed for Fraction D.

The in vitro antitumor effect of lipids was examined by the method described previously[1]: admixture of lipids and tumor cell suspension (2×10^7 cells/ml in 0.85% NaCl) was preincubated at 37 °C for 90 min, at the end of which 0.5 ml of the mixture was implanted i.p. into groups of *dd*N mice weighing 20–22 g. Control mice received the same dose of tumor cell suspension incubated without admixture of lipid. The survival time of mice was observed for a period of 60 days. Usually, control mice died of ascitic tumor in less than 20 days.

As shown in the Figure, two-dimensional chromatography of acetone-soluble lipid fraction resulted in separation of 11 distinct lipid classes, leaving the contaminating glycolipids at the start point. All these components were

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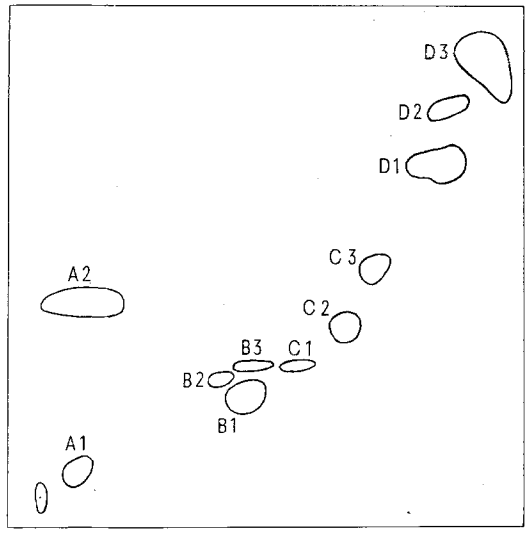
Effect of nonpolar lipids from a group A *Streptococcus hemolyticus*, strain Su, against Ehrlich ascites tumor in mice

Nonpolar lipids from *St. hemolyticus*, Su						In vitro antitumor activity						
						No. of survivors at 60 days						
Fraction No.	Yields: mg/100 l culture (%)	Average Rf values in solvent [a]		Colour reaction with $SbCl_3$ [b]	Possible identification [c]	Concentration in test mixture (mg/ml of cell suspension)						Cell control
		I	II			2.0	1.0	0.5	0.2	0.1	0.05	
A1	30 (1.2)	0.07	0.07	—	Monoglycerides	10/10	10/10	8/10	4/10	0/10	0/10	0/10
A2	89 (3.6)	0.10	0.40	—	Fatty acids	10/10	10/10	10/10	8/10	4/10	0/10	0/10
B1	46 (1.8)	0.39	0.24	—		0/10	0/10	0/10				0/10
B2	29 (1.2)	0.34	0.27	+	Sterols	0/10	0/10	0/10				0/10
B3	34 (1.4)	0.41	0.30	—		0/10	0/10	0/10				0/10
C1	18 (0.7)	0.52	0.29	—	Diglycerides	0/10	0/10	0/10				0/10
C2	21 (0.8)	0.53	0.41	—		0/10	0/10	0/10				0/10
C3	22 (0.9)	0.71	0.54	—		0/10	0/10	0/10				0/10
D1	24 (1.0)	0.86	0.70	—	Triglycerides	0/10	0/10	0/10				0/10
D2	18 (0.7)	0.88	0.84	+	Sterol esters	8/10	4/10	0/10	0/10			0/10
D3	40 (1.6)	0.93	0.94	—	Hydrocarbons	0/10	0/10	0/10				0/10

[a] Solvent I, ethylene chloride-methanol (98:2); solvent II, *n*-hexane-diethyl ether-acetic acid (70:30:2). [b] $SbCl_3$: antimony trichloride for sterols and sterol esters[8]. [c] Rf values for authentic compounds in solvent I and II, respectively were found to be: monoglycerides, 0.05, 0.04; fatty acids, 0.10, 0.40; cholesterol, 0.34, 0.24; diglycerides, 0.49, 0.28; triglycerides, 0.87, 0.70; cholesterol ester, 0.87, 0.84.

detected with iodine[5] and phosphomolybdate[6] but were negative to reagents for phosphorus[7], amino groups and carbohydrates. From the chromatographic behavior presented in the Table, fractions A1, A2, C1 and D1 were possibly identified as monoglycerides, free fatty acids, diglycerides and triglycerides, respectively. The Rf values of 2 antimony trichloride-positive[8] fractions, B2 and D2, corresponded to that of cholesterol and cholesterol stearate, respectively.



Tracing of a two-dimensional thin-layer chromatogram of nonpolar lipids from a group A *Streptococcus hemolyticus*, strain Su. The chromatogram was developed in ethylene chloride-methanol (98:2) (solvent I) in the *x*-direction and then in *n*-hexane-diethyl ether-acetic acid (70:30:2) (solvent II) in the *y*-direction. The spots were detected with iodine, followed by staining with phosphomolybdate. Numbers refer to those in the Table.

The results of biological tests with graded levels (2.0–0.05 mg per ml of cell suspension) of the individual lipid fractions revealed that the in vitro antitumor activity was confined exclusively to the fractions A1, A2 and D2. Of these 3 fractions, A2 was shown to be the most effective to prevent the development of ascitic tumor, followed by A1, whereas D2 had a weak effect (Table). Concentrations higher than 0.5 mg per ml of cell suspension of A1 and A2 were found to inhibit completely the development of ascitic tumor, while 2.0 mg of D2 was only partially protective. The minimal effective doses of these active lipids, which gave definite protection in mice, were 0.2 mg of A1, 0.1 mg of A2 and 1.0 mg of D2. All other subfractions were shown to have no antitumor effect in a concentration of 2.0 mg per ml of cell suspension.

From the foregoing results, it is reasonably presumed that free fatty acids and monoglycerides may account for all, or nearly all of the in vitro antitumor effect of lipids extracted from hemolytic streptococci against Ehrlich ascites tumor in mice. Needless to say, the isolated components comprise a homologous lipid class of wide molecular

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weight range that varies in chain length and degree of unsaturation. Although precise chemical constitutions of these active acids obtained remained as yet undetermined, preliminary data of gas chromatographic analysis now in progress suggested that major components of Fraction A2 are saturated and unsaturated acids with the carbon numbers of 16 and 18, concomitant with minor components with shorter and longer chain lengths.

Since the first report of NAKAHARA[9] in 1922, evidence has been accumulating to suggest the possible significance of fatty acids and their esters as antitumor agents[10]. The present results appear to offer the first demonstration of an in vitro antitumor activity of fatty acids and their esters isolated from hemolytic streptococci. The mechanism by which fatty acids and their derivatives inhibit tumor growth is not yet clear. However, it is well known that the antitumor activity of fatty acids is dependent on a variety of factors, i.e., pH of medium, types of tumor etc.[11]. KATO et al.[10] emphasized that the antitumor effect of fatty acids cannot be simply attributed to their physical attack on cell surface as surface-active agents, as indicated by the absence of parallelism between hemolytic activity and antitumor effect. In this connection, it is worthy of note that the active lipids from hemolytic streptococci were incapable of lysing the rabbit erythrocytes in vitro[12].

Résumé. Par la chromatographie en couches minces, l'activité antitumorale de l'extrait lipidique obtenu d'une souche de *Streptococcus hemolyticus* a été reconnue dans les trois composants: acides gras, monoglycérides et stérols estérifiés.

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PRO LABORATORIO

Efficiency of Anthracene as a Suspended Scintillator for Counting Aqueous Nickel-63 Samples

Nickel-63, a 67 keV beta-emitting radionuclide, has found application in tracer techniques for biological and metallurgical studies. It has also been used to determine the concentrations of dust and aerosol. The liquid scintillation method for counting ^{63}Ni activity has been well developed[1], but no measurement using a suspended scintillator has been reported. This paper evaluates the performance of anthracene, which was used as a suspended scintillator, for assaying ^{63}Ni activity in aqueous medium.

Anthracene crystals of the blue-fluorescence grade were screened in dry state through standard sieves. The size of the crystals used were between 150 and 250 μm[2]. The crystals had not been coated with detergent prior to assay because the addition of detergent does not improve setting fully or prevent completely the undesired adsorption of small air bubbles[3]. Imperfect coating may introduce unpredicted counting errors. A ^{63}Ni stock solution of 1×10^3 dpm was prepared by diluting an appropriate amount of a standard $^{63}NiCl_2$ solution with water. Since the aqueous Ni^{++} solution is green, some absorption of the fluorescent emission should occur during the scintillation process. A Beckman DB-GT UV-Visible Spectrophotometer was used to determine the absorbance of the

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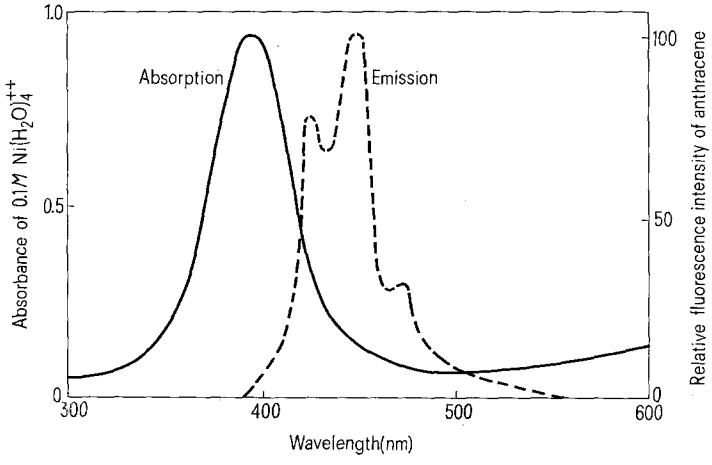

Fig. 1. Absorption spectrum for $Ni(H_2O)_4^{++}$ and fluorescence spectrum for anthracene.