proportions variable ont été réalisées dans les conditions du laboratoire. Les résultats montrent que l'introduction de mâles stériles réduit fortement la viabilité des œufs; mais une réduction encore plus importante est obtenue en introduisant à la fois des mâles et des femelles stériles. Nous constatons que mélangés dans la proportion de 10:10:1:1 d'insectes (mâles, femelles stériles; mâles, femelles normaux) provoque une stérilité absolue.

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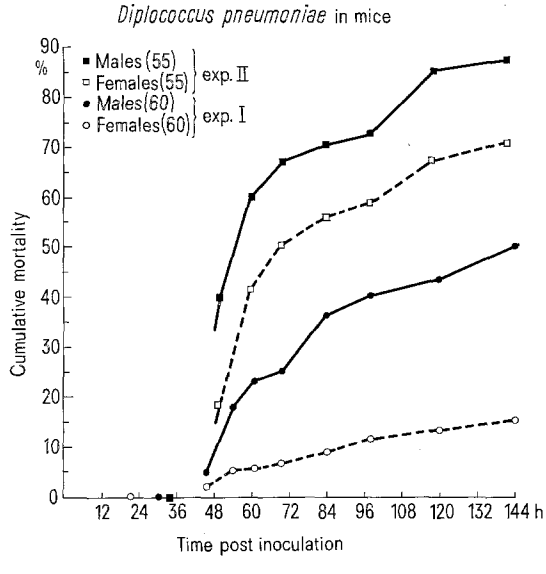
Sex Difference in Susceptibility of Mice to Pneumococcus

Clinically and experimentally, males appear to have less resistance to infectious diseases than females[1–3]. With pneumococcus, a sex difference in susceptibility has been reported for man [3,4] and rachitic rats[5]; in mice, altered resistance has been noted only after the treatment of the animals with estrogen[6]. We show here that mortality is distinctly higher in males than females following i.p. inoculation of normal mice with *Diplococcus pneumoniae*.

Materials and methods. We used a strain of *D. pneumoniae*, type 12, which had been isolated from man. Alternate broth-mouse-broth passages were made to adapt the organism for infection of mice. The organism was grown for 20–24 h in brain heart infusion (BHI) broth containing 10% horse serum. Several mice were then inoculated i.p. with 0.1–0.5 ml of the culture. When a mouse appeared to be terminally ill, heart blood was removed and 1 drop added to 10 ml of broth medium. After 20–24 h incubation, the pure culture of pneumococcus type 12 obtained was used for another round of animal inoculations. Under this procedure, the strain became highly virulent for mice and an inoculum of 70–100 microorganisms gave an LT_{50} in less than 48 h. When we diluted the inoculum, animals tended to survive the infection.



Cumulative % mortality of male and female albino mice (29 g av.) inoculated i.p. with *D. pneumoniae*, type 12. The sex difference is stastically significant by Chi Square contingency tests.

Since a rapidly attained LT_{50} could obscure any sex difference[1], we 'attenuated' the organism by 24 serial passages through BHI in the absence of horse serum. The undiluted culture from the 24th passage was divided into 0.3 ml portions and stored at –60°C. For the experiments with sex segregated animals described here, inoculum was thawed and diluted with BHI containing 10% horse serum.

Albino mice, 6–8 weeks old and averaging 29 g were used to test the effect of sex on resistance to pneumococcus. They were bred in our laboratory from Charles River CD-1 stock. At weaning, males and females were separated and held on litter in colony cages, food (Purina Laboratory Chow) and water being supplied ad libitum. Just prior to an experiment, the mice were sorted into wire bottomed cages, 15×30 cm, holding 10 each of one sex. Wire bottomed cages were used to aid in observing and removing dead animals.

Using a plastic 1 ml syringe with a 27 gauge needle, each treated mouse was injected i.p. with 0.1 ml of a diluted suspension estimated to contain about 1700 organisms by the most probable number method[7]. A total of 115 male and 115 female mice were infected in the two experiments reported here. Approximately half that number of control animals were injected i.p. with 0.1 ml of sterile broth.

Heart blood from a random sample of terminally ill animals was streaked on blood agar plates for reisolation and identification of organisms. Pneumococci were invariably recovered in pure culture. We used specific typing serum purchased from the Statens Serum Institut, Copenhagen, Denmark, to identify the isolates as type 12 pneumococci.

Results and discussion. Cumulative % mortality is plotted by sex, separately for the 2 experiments, in the Figure. In both experiments, mortality began between 36 and 48 h. In Experiment I, the LT_{50} for male mice was reached at 144 h, while females at that time showed less than 20% mortality. In Experiment II, the LT_{50} for males was reached between 48 and 60 h, and for females, at

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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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