Sensitivity of Mice to *Neisseria gonorrhoeae* Lipopolysaccharide After Injection of Ehrlich Ascites Tumor Cells. (24977)

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This report is part of studies(1,2) concerning properties of protein and nucleic acid-free lipopolysaccharide phosphoric acid ester endotoxin (LPSP) obtained from *N. gonorrhoeae*. Investigators have found that lipopolysaccharides of certain gram negative bacteria induce hemorrhage and necrosis in certain types of tumors of animals and man(3). The malignant tumors responded to a single injection of lipopolysaccharide in the above fashion. In this study we were interested to know whether LPSP of *N. gonorrhoeae* would offer protection against or enhancement of the toxic nature of Ehrlich mouse ascites carcinoma cells.

Materials and methods. Ehrlich ascites tumor was maintained in male CF1 mice by transferring 10^6 tumor cells, suspended in 0.1 ml sterile saline, into the peritoneal cavity. The mice were sacrificed by chloroforming 8 or 9 days after inoculation. LPSP was injected intraperitoneally 18 hours after injection of 0.1 ml tumor cell-suspension (10^6 cells). Five different LPSP concentrations, increasing logarithmically, with 5 mice for each concentration were used. Preparation and chemical nature of LPSP-endotoxin has been described(4). The LPSP powder was dissolved in a small volume of 0.05 N sodium hydroxide. The resulting solution was adjusted to pH 7.4 with 0.1 N acetic acid, diluted to desired concentration with sterile distilled water and administered intraperitoneally. Karber's method was employed for calculating LD_{50} values(5). Experiments were carried out as follows: each group of mice was sub-divided into LPSP-control, ascites-control, and ascites-LPSP groups. The LD_{50} value for the LPSP varied between 300-500 μg.

TABLE I. Effect of LPSP on Mouse Ascites Tumor.

Exp.	Range of LPSP (μg)	Mortality (%) in days* 5	10	15
1	300	78	88	100
1 C	"	36	40	40
2	200	70	80	100
2 C	"	12	12	12
3	50–150	50	78	100
3 C	"	4	8	8
4 AsC	none	0	16	32

Exp. 1-3 represent mice which received both ascites tumor cells and LPSP. Exp. 1C-3C represent LPSP controls. Exp. 4 AsC represents mortality rate of mice receiving ascites tumor alone.

Total No. of mice employed were 175.

* Mortality figures represent death rates after No. of days given.

Results are summarized in Table I. Three different LPSP ranges varying from 50-300 μg LPSP have been studied. Within these ranges most mice (50-78%) which received ascites tumor prior to LPSP died within 5 days. Death rate in controls which received LPSP was 4-36% within 5 days. In a typical experiment (not listed in Table I) 18 hours after mice were inoculated with 10^6 ascites tumor cells, the groups received 5 different concentrations of LPSP varying from 10.5-40 μg. These quantities of LPSP did not increase the sensitivity of mice to ascites tumor or influence its effect on mice bearing tumors. Our results definitely indicate that mortality rate of mice infected with Ehrlich ascites tumor cells was increased by sublethal doses of LPSP from *N. gonorrhoeae*. This increase in mortality rate commenced 24 hours after injection of LPSP. Mortality rates of mice which received ascites tumor alone was 32% between 10-15 days after ascites inoculation.

Summary. 1. The mortality rate of mice infected with Ehrlich ascites tumor cells was increased considerably by sublethal doses of LPSP. 2. Very small quantities of LPSP had no effect on such mice.

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Effect of Quinidine on Ventricular Fibrillation in Hypothermic Dogs.* (24978)

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Ventricular fibrillation occurs regularly during profound hypothermia in dogs(1). Gollan(2) has prevented this arrhythmia by a combination of quinidine, slow rewarming period, prevention of left heart distention, and control of rate of coronary perfusion. In experiments reported below we tested the efficacy of quinidine alone as a means of preventing V. F. in hypothermic dogs.

Methods. Hypothermia was induced in healthy, mongrel dogs anesthetized with pentobarbital. Extracorporeal circulation was carried out with a pump-oxygenator-heat exchanger device as described elsewhere(3). A thermistor was placed in the esophagus and flow through the extracorporeal circuit set at 50 ml/min/kg until esophageal temperature fell to 20°C. Flow was then reduced to 25 ml/min/kg. E. K. G., E. E. G. and femoral blood pressure were recorded. The chest was opened only when electrical defibrillation was needed or when other direct procedures on the heart were carried out.

Methods. Eleven dogs (Group I) were cooled to temperatures of 6-10°C without receiving quinidine. Fifteen dogs (Group II) were similarly cooled but received 28-30 mg/kg quinidine: HCl 10-15 minutes before start of cooling period. The quinidine was given i.v. in doses of 100 mg every 5 minutes until QRS interval doubled or until PR interval increased to 40-60 mS.

Result. All 11 dogs in Group I developed ventricular fibrillation either during cooling, rewarming, or both periods. In all dogs, electrical defibrillation was successfully accomplished at temperatures of approximately 30°C. Dog 4, placed on a pacemaker, fibrillated at 16° on rewarming, even though heart continued to respond up to point of fibrillation. In 5 runs where cooling fibrillation did not occur, the heart spontaneously stopped. When it fibrillated on rewarming, there were usually 3 or 4 slow rhythmical beats before ventricular fibrillation began. Acetylcholine (3 mg/kg) Mecholyl (3.5 mg/kg) and TEA (3 mg/kg) were ineffective in preventing onset of ventricular fibrillation in single experiments. Maintenance of left auricular pressure below 15 mm Hg was similarly ineffective in one experiment.

In 15 dogs in Group II, there were only 2 instances (dogs 6 and 7) of fibrillation. One was due to inadequate initial dose of quinidine and second was probably due to too low quinidine blood levels on the fourth hypothermic episode. Complete cardiac standstill occurred at about 8.5°, though in one instance this was at 13°C. On rewarming, cardiac action returned at about 16° varying from 11° to 19°. Following rewarming, there were no EKG abnormalities to suggest residual quinidine toxicity; 11 dogs were allowed to survive.

Discussion. Large doses of quinidine have proven effective in preventing ventricular fibrillation during profound hypothermia of 10°C or lower, when this is accomplished by core cooling with pump-oxygenator-heat exchanger system. Quinidine, to achieve this effect, was administered intravenously until suppressive effect was noted in the EKG as shown by changes in QRS and PR intervals (4). This occurred well before any signifi-

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