Protection of Mice Against Gram-Positive Bacteria with *Maytenus laevis* and Other RES Stimulants. (29199)

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(Introduced by William M. Govier)
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A survey of botanical preparations revealed a number of specimens capable of inducing hyperphagocytic activity in mice(1). Judged by effect upon rate of clearance of intravascular carbon(2,3), the most potent preparation was a suspension of pulverized leaves of a Venezuelan evergreen (*Maytenus laevis*). This preparation was interesting because (a) administered intravenously, it accelerated phagocytosis without inducing hepatosplenomegaly, and (b) it showed evidence of activity following oral administration.

The present study deals with the ability of *Maytenus laevis* leaves to protect mice experimentally challenged with pathogenic bacteria. For comparison, 3 other RES stimulants (endotoxin, zymosan and live *Mycobacterium phlei* Halpern) were investigated with the same test systems.

Materials and methods. The mice employed were 18-22 g females, Swiss Ha/ICR strain supplied by K-G Farms, Parsippany, N. J. Desiccated leaves of *Maytenus laevis* were pulverized to $<$ 80 mesh, and employed as a suspension in physiological saline. Zymosan (Fleischmann Laboratories, Stamford, Conn.) and fresh *Mycobacterium phlei* Halpern were also suspended in saline for administration to mice. Endotoxin from *Salmonella abortus equi* (Difco Laboratories, Detroit, Mich.) was administered in saline solution.

Three series of protection tests were conducted. Test series 1 and 2 involved experimental infection with *Staphylococcus aureus* Smith. The challenge microorganism in test series 3 was β-hemolytic *Streptococcus pyrogenes* type 4. The virulence of these pathogenic bacteria was maintained by a series of 24 hour mouse passages. Then heart blood was collected with sterile technique and used to inoculate sterilized trypticase soy broth (Baltimore Biological Laboratory, Baltimore, Md.) The bacteria were propagated for 18 to 24 hours at 37°C. Virulence titrations rather than bacterial counts, were performed. Considering the broth culture to have unit concentration, suspensions were diluted serially for injection. Staphylococci were diluted with trypticase soy broth fortified with 5% hog gastric mucin to enhance pathogenicity. Streptococcal cultures were diluted only with soy broth.

In each test series, the virulence titration and the challenge of treated mice were conducted at the same time. In test series 1, the virulence of the *Staphylococcus aureus* Smith culture was determined by intraperitoneal injection of groups of 6 mice with 0.5 ml of culture diluted to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}. At that time, there were available groups of 30 mice which had been injected intravenously with each RES stimulant on the previous day and groups of 30 mice which had been treated similarly 5 days earlier. Appropriate groups of 6 treated mice were challenged with 5 dilutions (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) of the staphylococcal culture. The animals were caged by group (of 6) and observed for the subsequent week. Dead mice were removed as soon as possible after death. From the recorded deaths, the LD_{50} of the culture and the standard error were calculated by the statistical method of Miller and Tainter(4).

The virulence titrations of staphylococci and streptococci in test series 2 and 3 were performed with 10 dilutions (10^{-1} to 10^{-10}) of each culture. Groups of 6 mice were challenged with each level of *Staphylococcus aureus* and groups of 10 mice were challenged with *Streptococcus pyrogenes*. The evaluation of each RES stimulant for prophylactic activity was conducted with 60 mice as described for test series 1. The dilution ranges used were 10^{-3} to 10^{-7} for staphylococcal challenge and 10^{-5} to 10^{-9} for challenge with streptococci.

TABLE I. Protective Effects of RES Stimulants Against *Staphylococcus aureus* Smith and β-hemolytic *Streptococcus pyrogenes* Type 4.

Test series	Group	Dose (mg/kg)	Challenge (i.p.) Route	Bacteria	Day	LD_{50}
1	Control (saline)	–	i.v.	*Staphylococcus*	–	1.4×10^{-5}
1	*Maytenus laevis*	15	"	"	1	1.3×10^{-4}*
1	" "	15	"	"	5	3.2×10^{-4}*
1	Endotoxin	5	"	"	1	1.2×10^{-5}
1	"	5	"	"	5	2.6×10^{-4}*
1	Zymosan	50	"	"	1	8.9×10^{-5}
1	"	50	"	"	5	1.0×10^{-4}*
1	*Mycobacterium phlei*	6†	"	"	1	7.5×10^{-3}
1	" "	6†	"	"	5	$> 10^{-3}$*
2	Control (saline)	–	"	"	–	1.0×10^{-6}
2	*Maytenus laevis*	15	"	"	1	3.2×10^{-5}*
2	" "	15	"	"	5	9.0×10^{-5}*
2	Endotoxin	5	"	"	1	3.0×10^{-7}
2	"	5	"	"	5	2.4×10^{-4}*
2	Zymosan	50	"	"	1	9.0×10^{-7}
2	"	50	"	"	5	2.6×10^{-5}*
2	*Mycobacterium phlei*	6†	"	"	1	3.0×10^{-7}
2	" "	6†	"	"	5	4.6×10^{-5}*
3	Control (saline)	–	"	*Streptococcus*	–	1.0×10^{-7}
3	*Maytenus laevis*	15	"	"	1	1.0×10^{-5}*
3	" "	15	"	"	5	$> 10^{-5}$*
3	Endotoxin	5	"	"	1	1.0×10^{-6}*
3	"	5	"	"	5	2.0×10^{-7}
3	Zymosan	15	"	"	1	1.0×10^{-6}*
3	"	15	"	"	5	2.0×10^{-7}

* These values represent statistically significant protection ($p = 0.05$) derived by the method of Miller and Tainter(4).
† Dry weight basis.

TABLE II. Summary of Protection Study.

Test substance	Dose (mg/kg)	Route	Day of challenge	Protection* against Staphylococcus (i.p.) Test series 1	Staphylococcus (i.p.) Test series 2	Streptococcus (i.p.) Test series 3
Maytenus laevis	15	i.v.	1	+	+	+
" "	15	"	5	+	+	+
Endotoxin	5	"	1	—	—	+
"	5	"	5	+	+	—
Zymosan	50	"	1	—	—	+
"	50	"	5	+	+	—
Mycobacterium phlei	6	"	1	—	—	Not run
" "	6	"	5	+	+	" "

* + = protection. — = no protection.

Results. The experimental data are shown in Table I. The results obtained in the 3 test series are summarized in Table II.

Administered intravenously, leaves of *Maytenus laevis* protected mice against experimental challenge with *Staphylococcus aureus* Smith and with β-hemolytic *Streptococcus pyrogenes* type 4. Protection against both pathogens was observed when challenge was conducted 1 and 5 days after administering *Maytenus laevis*.

Zymosan, endotoxin and live *Mycobacter-*

ium phlei showed the same pattern of protection against *Staphylococcus aureus* Smith; namely, effectiveness at 5 days, but not at 1 day, post administration. Endotoxin and zymosan also gave the same results against *Streptococcus pyrogenes,* namely, protection after 1 day, but not after 5 days.

Discussion. The present study confirms previous reports. It was shown earlier that endotoxin protects mice against subsequent staphylococcal(5,6) and streptococcal(7) infections. It was also known that pretreatment of mice with zymosan induces resistance against gram-positive cocci(8).

The new findings extend our knowledge of the prophylactic spectrum of *Mycobacterium phlei* which Biozzi *et al*(9) reported to protect mice against *Salmonella enteritidis*. The observation that the recently discovered stimulant, leaves of *Maytenus laevis,* induces protection against pathogenic microorganisms supports the view that RES stimulants frequently have the capacity to augment host defense.

Summary. Intravenous administration of pulverized leaves of *Maytenus laevis* to mice induced resistance to subsequent experimental challenge with pathogenic cocci. Endotoxin, zymosan and *Mycobacterium phlei* showed more transient prophylactic activity against the same pathogens.

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Observations on the Use of Avidin in Bacteriological Media.* (29200)

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Avidin is used widely as a tool in studies of the biochemical functions of biotin because of its ability to form a biologically inactive complex with the vitamin(1). It has been reported also that avidin can be used in the purification of biotin since the complex with biotin is formed in a stoichiometric manner and can be split readily by heat treatment(2).

During our studies on the physiological control of biotin synthesis in *Escherichia coli,* an attempt was made to investigate the amount of biotin synthesized by the bacterial cells in the presence of avidin with the expectation that it would remove biotin quantitatively from the growth medium and that biotin would be released into an assayable form by subsequent treatment with heat. However, it was found that avidin could not be used for this purpose because of difficulties in releasing biotin from the avidin-biotin complex when it was allowed to form in certain media. In this paper are reported some observations on the release of biotin from avidin-biotin complexes formed under various conditions.

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Materials and methods. d-Biotin was obtained from the California Foundation for Biochemical Research, Los Angeles. Avidin