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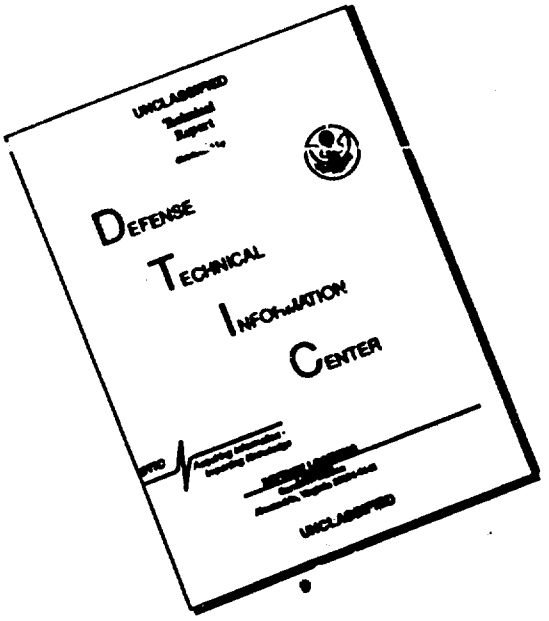

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Resistance to Bacterial Infection Induced by Statolon and Pyran

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The antibacterial properties of statolon and pyran were compared with those of bacterial endotoxin and found to be similar. Although high resistance was transient, it was followed by immunity of the same order as in mice infected without pretreatment with the interferon inducers.

Various bacterial endotoxins, statolon, and a random copolymer of maleic acid and divinyl ether (pyran) are well known interferon inducers (2, 4, 8). An intraperitoneal injection of any of these substances, given approximately 1 day before infection with many unrelated viruses, greatly enhances the survival of challenged animals. The principle of the interferon-mediated type of increased resistance has been described (3). Apart from induction of interferon, bacterial endotoxins are known to also induce properdin (5), which was reported to play a role in nonspecific increased resistance to bacterial infection. The basic mechanisms of action of interferon and properdin are thought to be unrelated.

During the course of an investigation designed to study the common properties of various interferon inducers, it was observed that statolon and pyran exert a properdin-like effect, manifested by increased survival of animals subjected to bacterial challenge. Some of the findings are presented in this paper. The antibacterial properties of statolon and pyran were compared with those of bacterial endotoxin. Bacterial rechallenge has shown that the survivors once protected by the interferon inducers had a high degree of immunity.

Adult male Swiss mice were treated with 100 μg of *Escherichia coli* endotoxin (Difco), or 8 mg of statolon (The Lilly Research Laboratories, Indianapolis, Ind.) or 200 μg of pyran (Hercules Laboratories). These substances, suspended in 0.2-ml amounts of Hanks' balanced salt solution, were given by the intraperitoneal route at 24 hr before infection with *Klebsiella pneumoniae*. The challenge organism was grown overnight at 30 C in Brain Heart Infusion broth (Difco). After a thorough mixing, 0.2 ml of the undiluted culture was inoculated intraperitoneally.

Table 1 shows that the treatment with any of the three interferon inducers was followed by a markedly increased survival of the challenged animals. The differences from the untreated control group are based on the chi-square determinations. The treatments with statolon or pyran appeared to be as effective as the treatment with endotoxin.

An earlier report has shown that induction of interferon by statolon did not diminish immune response of mice to foreign red-blood cells, and that a measurable degree of specific immunity was achieved by repeated inoculations of virulent virus into animals suitably protected by interferon (6). Since other investigators reported that administration of some interferon inducers may interfere with antibody response to certain antigens (1, 7), it was of interest to determine the immune status of mice once protected by the inducers.

Survivors which were challenged with *K. pneumoniae* 1 day after the treatment with the interferon inducers were challenged once more at 21 days after treatment. The dose for the first challenge consisted of 0.2 ml of the *K. pneumoniae* culture (approximately 2×10^8 of viable cells), whereas, in the second challenge, it was increased to 0.3 ml. The survival rates after both inoculations are compared in Table 2. Group 6 served as a control for the first challenge, and group 7 for the rechallenge. After both the first and second challenge, the survival of animals once treated with endotoxin, pyran, or statolon (groups 1, 2, and 3) was significantly higher ($P < 0.005$) than in the corresponding control groups. In contrast, when the challenge of similarly treated mice was delayed until the 21st day (groups 1a, 2a, and 3a), the survival rate did not differ from that of the control. Group 4 was treated with 0.2 ml and

TABLE 1. *Comparison of protection against Klebsiella pneumoniae infection by endotoxin, pyran, and statolon*

Treated with	No. of animals	Per cent survived	Difference from control	
			x^2	P
Pyran	20	80.0	12.13	<0.001
Statolon	18	88.9	15.64	<0.001
Endotoxin	20	100.0	24.00	<0.001
Control	20	25.0		

TABLE 2. *Effect of interferon inducers on immunity of survivors to rechallenge with Klebsiella pneumoniae*

Group	Treated with	Challenged 1 day after treatment		Rechallenged 21 days after treatment	
		Survival rate	P[a]	Survival rate	P[a]
1	Endotoxin	29/30	<0.001	26/29	<0.001
1a	Endotoxin	ND[b]	ND	9/28	NS[c]
2	Pyran	26/30	<0.005	26/26	<0.001
2a	Pyran	ND	ND	10/30	NS
3	Statolon	30/30	<0.001	30/30	<0.001
3a	Statolon	ND	ND	7/27	NS
4	*Klebsiella* filtrate	20/20	<0.001	20/20	<0.001
5	*Klebsiella* filtrate	ND	ND	4/10	NS
6	Control for first challenge	15/30		13/14	<0.001
7	Control for second challenge			4/20	

[a] Level of significance of the difference from control based on χ^2 determinations.
[b] Not done.
[c] Not significant.

group 5 with 0.5 ml of centrifuged and filter-sterilized culture fluid of *K. pneumoniae*. Group 4 received both challenges, and each time all animals survived. Group 5 received only the second challenge, and its survival did not differ from the control (group 7). Among the animals which served as control for the first challenge (group 6), the refractoriness of the second inoculation equalled that seen in groups 1, 2, 3, and 4.

These experiments have shown that statolon and pyran exerted an antibacterial effect closely resembling that of bacterial endotoxin. The high resistance elicited by these substances was transient and did not last 21 days. However, it was followed by development of immunity, which was of the same order as seen in animals infected without the pretreatment with interferon inducers. Since immune response to certain killed bacterial vaccines is not as good as that seen after natural infection, vaccinations with live organisms after a treatment with interferon inducers are worth further investigations. More detailed related studies concerning the nature of the resistance here described will be reported elsewhere.

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13. ABSTRACT

Intraperitoneal (i.p.) infection of mice with Klebsiella pneumoniae was markedly suppressed by i.p. treatment with statolon or random copolymer of maleic acid and divinyl ether (pyran) given one day before infection. These two substances are known mostly for their capability to induce interferon and with it associated protection against viral infections. Their antibacterial effect, not reported previously, resembled that of bacterial endotoxin administered by the i.p. route one day before infection. The treatment with statolon, pyran or endotoxin did not impair immune response to the challenge organism, since the treated animals which survived one challenge were refractory to a later rechallenge.

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