Studies on the Mechanism of Protodyne-Induced Protection against Microbial Infections (35904)

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It has been known for more than 90 years that certain substances, when given parenterally, will increase the resistance of animals not only to a single bacterial pathogen but also to a number of other immunologically unrelated pathogenic microorganisms. Among the agents that increase the "nonspecific" or general resistance of the host, endotoxin, a lipopolysaccharide extracted from the cell walls of gram-negative bacteria, is the best known, most thoroughly studied, and effective, whether given intraperitoneally or intravenously. Among other substances that increase nonspecific resistance there are many, such as broth or serum, that are effective only if given by the intraperitoneal route (1). It appears that for such substances the production of a localized inflammatory reaction in the peritoneal cavity may be a prerequisite of their effectiveness.

Recently, a high molecular weight proteinaceous material with potent stimulatory activity on the nonspecific host resistance to infections has been obtained from the protoplasm of gram-negative bacteria (2–4). This substance, named "protodyne", is of particular interest because it is devoid of many toxic effects that are characteristic of endotoxin (5) and, unlike endotoxin, is not inactivated by plasma (6).

The experiments described in this paper were carried out to determine whether the protective action of protodyne involves principally an increase in the local intraperitoneal defense mechanisms or whether protodyne produces a more generalized stimulation of the defense mechanisms of the host. This was investigated in several ways: (a) by comparing the protective action of protodyne with that of bovine serum albumin (BSA), which is known to increase host resistance to infections when given intraperitoneally (7, 8); (b) by studying the protective action of protodyne in bacterial infections after intravenous administration to eliminate or at least diminish the influence of intraperitoneal events; and (c) by evaluating the effect of protodyne in viral infections, which generally are not influenced by local inflammatory reactions.

Materials and Methods. Mice: Male Swiss-Webster strain mice, weighing approximately 20 g, were obtained from Charles River for the bacterial protection tests. For viral protection tests Charles River CD-1 mice, weighing 10 g, were employed.

Protodyne: Protodyne utilized for the experiments in this study was extracted from *Escherichia coli* as previously described (2, 3).

Bovine serum albumin: Five times recrystallized bovine serum albumin (BSA) was purchased from Nutritional Biochemical Company, Cleveland, Ohio.

Bacterial protection tests: Groups of 20 mice were treated by the intraperitoneal (ip) or intravenous (iv) route with tenfold serial dilutions of either protodyne or BSA. The mice were challenged by the ip or iv route 1 to 4 days later with an appropriate predetermined dose of the high virulent *Salmonella typhimurium* strain ST_1 (LD_{50} 180 organisms ip), the less virulent *S. typhimurium* strain 3S (LD_{50} 6.6×10^5 organisms ip), or *Streptococcus mastitidis* (SP_1) (LD_{50} 200 organisms ip). The challenge doses were selected to kill 80 to 100% of the untreated control mice within 48 to 96 hr. Both treatment and challenge doses were injected in 0.2 ml volume.

Protection was estimated by calculating the PD_{50}, the dose of the compound (mg/kg) which protects 50% of the animals from death at a time when 90% or more of

TABLE I. The Protective Action of Protodyne or BSA Given Intraperitoneally to Mice 24 hr Prior to Intraperitoneal Challenge with *S. typhimurium* (3S).[a]

Treatment		Cumulative no. surviving/ total at hr:		PD_{50}[b] 95% CL[c]
Sample	Dose (mg/kg)	24	48	(mg/kg)
Control	Untreated	6/30	2/30	—
Protodyne	10.0	19/20	19/20	0.17
	1.0	16/20	14/20	(0.057–0.51)
	0.1	10/20	9/20	
BSA	10.0	9/20	9/20	12.0[d]
	1.0	9/20	8/20	(0.59–246)
	0.1	8/20	5/20	

[a] Challenge dose, 3.6×10^8 *S. typhimurium* (3S) or 545 LD_{50} dose.
[b] PD_{50} calculated on basis of survivors at 48 hr.
[c] Confidence limits.
[d] Estimate; accurate determination not possible because of flat slope.

the control animals were dead. In making these determinations, the drug was usually given at three different dose levels. When the agents were given at only one or two dose levels, the time at which 50% of the animals were dead, the LTD_{50}, was calculated ac-

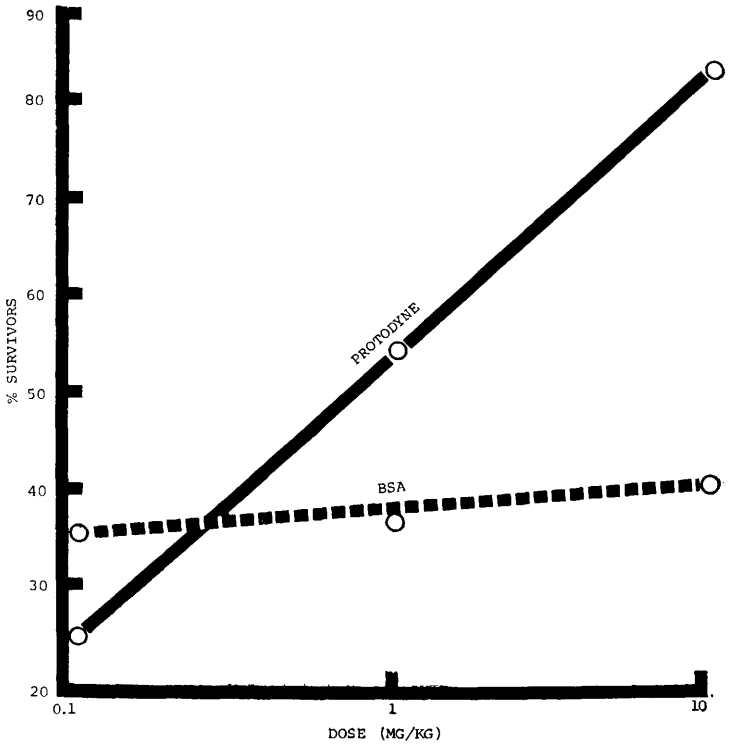

FIG. 1. Comparative protection of protodyne and BSA given intraperitoneally to mice prior to intraperitoneal challenge with *S. typhimurium* (3S). Composite of 15 separate experiments using over 200 mice at each dose level. Survival values 48 hr after challenge with 1.5 to 4.8×10^8 *S. typhimurium* (3S). Only 8.3% of the 350 untreated control mice survived this challenge.

TABLE II. The Protective Action of Protodyne or BSA Given Intraperitoneally to Mice 24 hr Prior to Intraperitoneal Infection with *S. mastitidis* (SP_1).[a]

Treatment		Cumulative no. surviving/ total at hr:		PD_{50}[b] 95% CL[c]
Sample	Dose (mg/kg)	24	48	(mg/kg)
Control	Untreated	2/30	1/30	—
	Saline	2/30	1/30	—
Protodyne	10.0	17/20	17/20	0.17
	1.0	17/20	13/20	(0.085–0.34)
	0.1	8/20	4/20	
BSA	10.0	2/20	1/20	
	1.0	2/20	1/20	>10
	0.1	1/20	1/20	

[a] Challenge dose 1.8×10^8 microorganisms.
[b] PD_{50} based on survivors at 24 hr.
[c] Confidence limits.

cording to the method of Litchfield and Wilcoxon (9).

Viral protection tests. Columbia SK virus was extracted from the brains of mice 72 hr after infection and stored frozen at —70°. Mice were challenged ip with 10 to 20 LD_{50} of virus 2 hr after injection of protodyne or BSA. Deaths were recorded daily for 12 days.

Results. The results of a typical experiment in which groups of mice were treated ip with graded doses of protodyne or BSA 24 hr prior to ip challenge with *S. typhimurium* are given in Table I. Both agents increased the number of animals that survived infection. The protection afforded by protodyne differed from that provided by BSA by being stronger and by showing a well marked dose–response relationship. The more intense protective action of protodyne as compared with that of BSA is reflected in the PD_{50} values. Thus, the PD_{50} value, *i.e.,* the amount of drug necessary to protect one-half of the animals from death, was 0.17 mg/kg of protodyne compared with 12.00 mg/kg of BSA.

The protective action of protodyne showed a dose–response relationship in the sense that larger doses of the substance produced greater protection than smaller doses. BSA,

TABLE III. The Protective Action of Protodyne or BSA Given Intravenously to Mice 48 hr Prior to Intraperitoneal Challenge with *S. typhimurium* (ST_1).[a]

Treatment		Cumulative no. surviving/ total at days:			PD_{50} 95% CL[b]
Sample	Dose (mg/kg)	1	3	5	(mg/kg)
Control	Untreated	19/20	13/20	4/20	—
	Saline	18/20	12/20	5/20	—
Protodyne	10.0	20/20	19/20	14/20	0.35
	1.0	20/20	20/20	13/20	(0.086–1.4)
	0.1	20/20	18/20	7/20	
BSA	10.0	19/20	18/20	8/20	32[c]
	1.0	18/20	11/20	2/20	(4.6–220)
	0.1	19/20	10/20	1/20	

[a] Challenge dose: 3.0×10^7 *S. typhimurium* (ST_1).
[b] Confidence limits.
[c] Extrapolated value.

TABLE IV. Survival Time of Mice Given Protodyne 10 mg/kg or BSA 10 mg/kg Intravenously and Challenged after 1 or 4 Days with *S. typhimurium* (ST_1).[a]

Time treatment to challenge (days)	LTD_{50} (95% CL) (hr)		
	Control (saline)	Protodyne (10 mg/kg)	BSA (10 mg/kg)
4	23.8 (18.3–31.0)	84.0 (70.6–100)	23.0 (16.8–31.5)
1	17.8 (13.7–23.2)	86.0 (76.8–96.0)	16.7 (11.9–23.4)

[a] Mice challenged by ip route with 4.8×10^7 *S. typhimurium* (ST_1) or 2.7×10^6 LD_{50}.

on the other hand, tended to give a protective response of the same magnitude irrespective of the dose administered within the 0.1 to 10 mg/kg range. The results are apparent from Fig. 1, which summarizes 15 separate experiments. Over 200 mice were used at each dose level. Only 29 out of 350 untreated control animals (8.3%) survived the infection. The steep slope of the dose–response curve of protodyne contrasts sharply with the flat slope of the BSA dose response.

In *Streptococcus mastitidis* infections protodyne provided good protection but BSA was ineffective (Table II). Infection with this strain produces death of the majority of the control animals within 18 to 24 hr. Thus, there may not have been enough time for the development of local inflammatory reactions in BSA-treated animals and this may be the reason for the complete lack of effectiveness of BSA in these rapidly lethal infections.

In additional experiments with *S. typhimurium* as the challenge organism, protodyne or BSA was administered intravenously to circumvent the possibility of establishing a localized inflammatory reaction in the peritoneal cavity. Results from a typical experiment are shown in Table III. Protodyne at the two higher dose levels produced significant protection. BSA had some protective action only at the highest dose level but this protection was weak.

To obtain additional data on the protective action of intravenously administered BSA, further experiments with BSA or protodyne employing a single dose of 10 mg/kg iv were conducted. The animals were challenged with *S. typhimurium* either 1 or 4 days after treatment. The results given in Table IV indicate that the mean survival time (LTD_{50}) was significantly prolonged only by protodyne but not by treatment with BSA. The results were similar whether the bacterial challenge was carried out 1 or 4 days after administration of the drugs.

In Columbia SK virus infection in mice (Table V), protodyne produced a dose-related decrease of mortality. It also increased the survival time of mice, an effect that was very marked at the higher dose of protodyne. BSA, on the other hand, produced only a slight increase of survival time that was not dose related and did not significantly affect the mortality of the animals.

Discussion. It has been known since the early observations of Metchnikoff (8) that heterologous serum given intraperitoneally may protect guinea pigs from *Vibrio cholerae* infections. Philipson (7) reviewed the protective action of serum and carried out additional experiments. Paratyphoid B vaccine given intraperitoneally to mice enhanced resistance to *Shigella* infections induced by the intraperitoneal route. Resistance to *Shigella* was minimal when the animals were infected by other routes. Immunization with paratyphoid vaccine did not increase *Shigella* agglutinins in the mouse serum. It was not possible to transfer the enhanced resistance to infection from vaccinated mice to normal mice by the injection of serum or whole blood. In rabbits, intraperitoneal administration of normal rabbit, mouse, or horse serum increased resistance to *Shigella* infections given by the intraperitoneal route. As a result of these studies, Philipson concluded that local phagocytic defense mechanisms that take place in the peritoneal cavity may be the

TABLE V. The Effect of Protodyne and BSA on Columbia SK Infections in Mice (intraperitoneal administration).

	(mg/kg)	Dead/total	Mean survival (days)
Protodyne	100	18/40	9.35
	10	32/40	6.43
BSA	100	37/40	5.65
	10	35/40	5.63
Control	—	58/60	4.47

main factor responsible for the increased resistance to infections observed after intraperitoneal administration of serum.

Our investigations show a striking difference in the capacity of BSA and protodyne to increase host defenses against bacterial and viral infections. Although increased resistance to *S. typhimurium* infection can be obtained by pretreating animals with BSA by the intraperitoneal route, such protection is weak and is not dose dependent. These findings suggest that the increased resistance induced by intraperitoneal administration of BSA is primarily due to irritation and inflammation of the peritoneal cavity. In contrast, the dose dependent and much more powerful protection produced by the administration of protodyne must involve a stimulation of defense mechanisms which remained unaffected by the administration of BSA.

These differences in effects produced by the intraperitoneal administration of protodyne and BSA are further supported by the findings obtained in mice infected with *S. mastitidis*. In these rapidly lethal infections, protodyne showed a protective action of a magnitude similar to that observed in mice infected with *S. typhimurium*. BSA, on the other hand, did not show any protective activity against the streptococcal infections.

Stimulation of local intraperitoneal defense mechanisms could not play an important part when resistance-increasing agents are administered by the intravenous route. Under these conditions BSA, even in large doses, produced at best an insignificant degree of protection and did not increase the survival time of the animals. In contrast, the marked increase of the survival time of animals treated intravenously with protodyne again suggests that this agent stimulates general defense mechanisms of the body in an entirely different manner, which is not dependent on local events taking place in the peritoneal cavity. This conclusion is also supported by the protective action of protodyne in animals infected with the Columbia SK virus and the lack of any effect of BSA in this viral infection.

Summary. Protodyne, a high molecular weight proteinaceous material derived from the protoplasm of gram-negative bacteria, increases resistance to infection in mice whether given by the intraperitoneal or intravenous routes. It thus differs from bovine serum albumin which increases nonspecific resistance to infections only if given intraperitoneally, presumably by setting up local inflammatory reactions in the peritoneal cavity. BSA, even if given intraperitoneally, is ineffective in rapidly lethal streptococcal infections in which protodyne has good protective action. Protodyne is also effective in Columbia SK infections while BSA is not.

1. Pfeiffer, R., and Issaeff, Z., Hyg. Infektionskr. **17**, 355 (1894).

2. Berger, F. M., Fukui, G. M., Ludwig, B. J., and Rosselet, J. P., Proc. Soc. Exp. Biol. Med. **127**, 556 (1968).

3. Berger, F. M., Fukui, G. M., Ludwig, B. J., and Rosselet, J. P., Colloq. Int. Cent. Nat. Rech. Sci. **174**, 285 (1969).

4. Berger, F. M., Fukui, G. M., Ludwig, B. J., and Rosselet, J. P., Symp. Ser. Immunobiol. Stand. **15**, 15 (1971).

5. Rosselet, J. P., Murphy. S. G., Adamski, R. J., Fletcher, M. J., and Ludwig, B. J., J. Bacteriol. **98**, 434 (1969).

6. Fukui, G. M., Goldenbaum, E., and Berger, F. M., Nature (London) **218**, 362 (1968).

7. Philipson, J., Acta Pathol. Microbiol. Scand. Suppl. **32**, 1 (1937).

8. Metchnikoff, E., Ann. Inst. Pasteur, Paris **7**, 403 (1893).

9. Litchfield, J. T., and Wilcoxon, F., J. Pharmacol. Exp. Ther. **96**, 99 (1949).

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