5 are higher than in Exp. 3 even though the contaminant was almost completely removed in the purification of the arterenol.‖

These experiments represent the first direct evidence that arterenol is converted to epinephrine and completes the reaction sequence postulated to lead from phenylalanine to epinephrine.

Summary. C^{14}-epinephrine has been found in the adrenal glands of rats administered α-C^{14}-arterenol intraperitoneally. Its presence has been demonstrated by paper chromatography and by isotope dilution using a new method, involving reaction with pyridoxal-5-phosphate, for separating these catecholamines.

‖ We wish to thank Dr. William Drell who developed this method and demonstrated that it completely separated arterenol from epinephrine and 3,4-dihydroxyphenethylamine. Furthermore, a paper chromatogram of carrier 3,4-dihydroxyphenethylamine hydrochloride which had been recrystallized from a solution containing the purified C^{14}-arterenol hydrochloride showed no radioactivity at the R_F of the carrier.

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Investigations on Polyvinylpyrrolidone in Mice Given Brucella Endotoxin. (22646)

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Studies in these laboratories with endotoxins prepared from various species of Gram-negative bacteria, including brucellae, have confirmed the observations that these endotoxins cause profound vascular disturbances in animals, terminating in collapse and death (1,2). More precise measurements with non-lethal doses of brucella endotoxin have shown that the daily temperature rhythm of mice is affected by small amounts of the endotoxin (3). The lethal effects of endotoxin in mice can be prevented by administration of adrenal corticosteroids or chlorpromazine, and tolerance to lethal doses of endotoxin can be produced by administration of sublethal amounts of heterologous endotoxins(1,4,5,6). The hypothermia of mice given brucella endotoxin also can be counteracted with added corticosteroid(7).

The present studies were undertaken to determine if the polymer, polyvinylpyrrolidone (PVP), would protect mice against brucella endotoxin. Originally introduced as a plasma expander by Hecht and Weese(8), interest turned to using PVP as a "detoxifying" agent. Toxic substances not usually excreted in the urine were reported to be "adsorbed" to PVP and excreted as such(9,10,11). PVP not only protected animals, but it was described as also effective in the treatment of human cases of tetanus and of diphtheria(12,13,14). According to these investigators, the most effective form of PVP was incorporated in the preparation known as Periston N (PVPN), having a smaller molecular weight than the original PVP (molecular weights: PVP~100,000, PVPN~12,600). More recently in a therapeutic trial on 10 patients with severe tetanus, no benefit attributable to PVPN was noted(15). Likewise, in mice PVPN given 3 hours after tetanus toxin was ineffective, but it protected when given within one hour after

TABLE I. Statistical Analysis of Data Presented in Fig. 1.

Comparison No.	Mortality rates compared: Stock	Time, hr	PVPN*	X^2	P
1	ABC	24	0	13.73	<.01
	ABC	24	+		
2	ABC	48	0	27.53	<.01
	ABC	48	+		
3	DBC	24	0	.06	>.32
	DBC	24	+		
4	DBC	48	0	.42	>.32
	DBC	48	+		
5	ABC	24	0	3.09	≃.08
	DBC	24	0		
6	ABC	48	0	1.34	>.16
	DBC	48	0		
7	ABC	24	+	25.49	<.01
	DBC	24	+		
8	ABC	48	+	31.02	<.01
	DBC	48	+		

* 0 = No PVPN inj. + = PVPN inj.

toxin administration(14). In the present investigations PVPN* was tested for a possible protective effect against brucella endotoxin. the 2 agents being administered concomitantly.

Materials and methods. Mice, 5 to 6 weeks of age, were used for testing. The mice had Purina Fox Chow and tap water *ad libitum* and were kept in an airconditioned room, maintained at about 78°F, illuminated by day and darkened by night. A first series of experiments was carried out on hybrids of the A and C stocks (ABC mice). Because of availability, further experiments were carried out on DBC mice, obtained by mating DIF_1 hybrids to the D (dilute brown) stock. As the results on DBC mice were found to be at variance with those previously obtained on ABC mice, a third series of experiments was undertaken. The effects of endotoxin in each of these additional experiments with and without added PVPN were compared alternately in ABC and DBC mice. A Boivin-type of endotoxin made from a smooth culture of *Brucella melitensis* was employed, the details for its preparation having been presented elsewhere(3). Different lots of endotoxin were employed, the doses averaging 1.5 mg per 20 g of body weight. But in any given experiment the same lot of endotoxin was used. A 6% solution of PVPN was used in a dose of 0.5 ml per mouse. All injections were made into the tail vein. Except for one experiment on ABC mice in which it was given 5 minutes after the endotoxin, the PVPN was introduced simultaneously through the same syringe containing the endotoxin. Mortality rates are reported for 24 and 48 hours post-injection. The rates at these times were representative of the PVPN effect recorded in some experiments at shorter and longer intervals as well.

Results. The results of all the experiments are summarized in Fig. 1, and a statistical analysis of these data is shown in Table I. As noted in Fig. 1, a total of 453 ABC mice and 350 DBC mice were used in these studies. The outstanding and consistent result was that ABC mice given endotoxin and PVPN survived in significantly larger numbers than DBC animals treated in the same manner. While PVPN protected ABC mice against the lethal action of the endotoxin, this protection

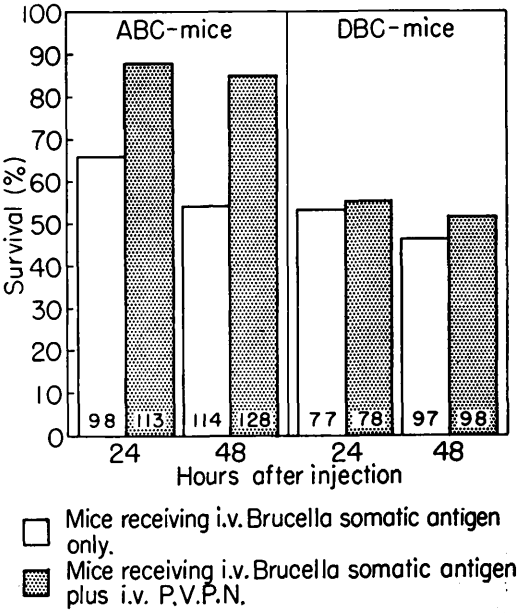

FIG. 1. Comparative survival rates in populations of ABC mice and DBC mice given brucella endotoxin alone, and brucella endotoxin plus Periston N (PVPN). No. of mice constituting each treatment group is shown at bottom of corresponding column.

* Obtained from Die Farbenwerke Bayer, Leverkusen, Germany.

was lacking in the DBC mice, (Fig. 1) from the rates recorded at 24 as well as at 48 hours after injection of endotoxin. When the number of mice comprising these comparative studies is taken into consideration, the differences in per cent of survivals are quite significant. That PVPN protected ABC mice against endotoxin is supported by the results of the statistical analyses given in comparisons 1 and 2 of Table I.

The data in Fig. 1 also suggest that ABC mice may be less susceptible to the endotoxin than the DBC mice, and this might account for the protection offered to ABC mice by PVPN. The differences in survival rates between ABC and DBC mice at 24 and 48 hours after injection are however slight, and they are not significant at the 5% level, as seen in comparisons 5 and 6 in Table I. This slight difference in the susceptibility to the endotoxin does not appear to be sufficient to explain the large difference between ABC and DBC in their response to PVPN. The latter difference is analyzed in comparisons 7 and 8 in Table I, based on survival rates in those groups of ABC and DBC given PVPN as well as endotoxin. Whether data for survival rates at 24 or 48 hours are analyzed, the inter-population differences in survival rates are significant below 1% ($P<0.0005$).

Discussion. These experiments demonstrate that PVPN provides protection for mice against Brucella endotoxin. PVPN significantly increased survival rates of ABC mice when given 5 minutes after endotoxin injection, as well as when given in the same syringe with the endotoxin. But this protection is limited, with populations of ABC mice protected, but not DBC mice. Only a single injection of PVPN was given in evaluating the effect on survival rates. It would be of interest to see whether repeated injections of PVPN may have more general protective effects. In evaluating the effects of polymers in animals given endotoxin, two major considerations must be kept in mind. There exist not only intra-species differences in the response to the polymer, as the present experiments emphasize, but varying results must be anticipated when interpreting the differences between species of animals. When PVP alone is administered to dogs, shock is produced(16). Further differences within the same species may be forthcoming if there is a variation in the molecular weight of the polymer. Moreover, under certain experimental circumstances, related compounds of larger molecular weight have been found to be harmful in rabbits(17).

Summary and conclusions. Brucella endotoxin was administered intravenously to groups of mice and survival rates were recorded at 24 and 48 hours post-injection. The effect of a synthetic polymer on such survival rates was evaluated in 2 genetically different populations of mice—ABC mice (hybrids of the A and C stocks) and DBC mice (obtained by mating DIF_1 hybrids to the D stock). The polymer, polyvinylpyrrolidone (PVP) was used, in the form of Periston N (PVPN), having a molecular weight about 12,600. The mortality rates induced by endotoxin were modified favorably by PVPN in ABC mice, but not in DBC mice. It may be concluded that PVPN beneficially influences survival from Brucella endotoxin in at least one population of hybrid mice. The observation that another population of mice did not gain appreciable benefit from the same dose of PVPN may deserve further studies aimed at elucidating the mechanisms of host response to this agent.

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Fibrinolytic and Coagulant Activities of Certain Snake Venoms and Proteases.* (22647)

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In a search for an ideal agent for the intravascular dissolution of thrombi and emboli in man, investigators have studied heparin(1,2, 3), coumarins(4), trypsin(5), chymotrypsin (3), streptokinase(6), staphylokinase(7), and plasmin(8). Certain snake venoms have long been known to be fibrinolytic *in vitro*. In addition, many of these are also known to contain other proteins with coagulant, hemolytic, neurotoxic or other properties which would contraindicate their clinical use. A survey of available snake venoms was undertaken to compare their *in vitro* lytic actions against fibrinogen, fibrin, formed fibrin and formed whole blood clots with various other proteolytic agents. Coagulant and hemolytic properties were also studied.

Materials. Bovine fibrinogen was a 1% solution of Armour's Fraction I. Human fibrinogen was a crude fraction prepared by 25% saturation of human plasma with ammonium sulfate, collection of the precipitate, dialysis, and adjustment of the clottable protein concentration to 0.5%. Plasmin was a spontaneously activated dog serum unglobulin fraction(9). Plasminogen was prepared from human barium sulfate adsorbed plasma by 20-fold dilution, adjustment of pH to 5.3, collection of precipitate and dissolution in a volume of 0.85% NaCl equal to 1/10 the original plasma volume. The fibrinogen was removed as fibrin after addition of a trace of thrombin (1 unit/10 ml). Streptokinase (Varidase, supplied through the courtesy of Lederle Laboratories) was dissolved in 0.85% NaCl to contain 200 units per ml. Uroprotease was prepared from human urine as described by Celander and Guest(10). Trypsin and chymotrypsin (crystalline, courtesy Armour Laboratories) were 0.1% solutions in 0.85% NaCl. Ficin (Delta), bromelain (courtesy Pineapple Research Institute of Hawaii), papain (Worthington), and pepsin (Worthington) were prepared as 1% solutions in 0.85% NaCl. Snake venoms (supplied through the courtesy of Wyeth and Co.; Ross Allen's Reptile Institute; Burroughs, Wellcome; and Hynson, Wescott and Dunning, Inc.) were dissolved as 1% solutions in 0.85% NaCl. *Stypven* (Burroughs, Wellcome) was used as a 0.2% solution. Thrombin (Thrombin, Topical, Parke Davis) was prepared to contain 100 units per ml.

Methods. Fibrinolysis was estimated by observing lysis time in a clot containing 0.2 ml material to be tested, 0.2 ml fibrinogen, 0.2 ml borate buffer (pH 7.6) and 0.05 ml thrombin. Intimate mixing of the lytic agent and fibrin was obtained by a 2-tube mixing method(11), in which the fibrinogen and buffer were poured into the lytic agent and thrombin, at zero time, and subsequently poured back and forth three times. *Formed clot lysis. Fibrin*: Human fibrinogen clots

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