TABLE II. Analysis of Data Presented in Table I.

Source	Sum of squares	Degrees of freedom	Mean square
Patients	85	11	7.7
Phenaglycodol	18	1	18.0*
Phenobarbital,	50	2	25.0*
25 mg *vs* 100		(1)	19.4*
mg blank		(1)	30.6*
Days	63	3	21.0*
Remainder	1,176	270	4.35
Total	1,392	287	

* Significant at $p > 0.05$.

erated by humans without excessive central nervous system depression. Comparable doses can therefore be given.

Usually drugs effective in grand mal are expected to increase the severity of petit mal and vice versa. Since phenaglycodol is effective in petit mal and the generalized seizures occurring in patients with focal brain damage, it may prove advantageous in epilepsy of the mixed type.

Conclusions. Phenaglycodol (Ultran, Lilly) is an effective anticonvulsant in epilepsy associated with focal brain damage.

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Protection of Mice with Polyvinylpyrrolidone Against Lethal Doses of Tetanus Toxin. (22955)

HEINZ BICK, ROBERT S. ABERNATHY, AND WESLEY W. SPINK
Department of Medicine, University of Minnesota Medical School, Minneapolis, Minn.

The polymer polyvinylpyrrolidone (PVP) was introduced as a plasma expander by Hecht and Weese(1). Other German investigators(2,3) subsequently demonstrated that in experimental animals, injections of PVP caused urinary excretion of various dyes normally retained in the body. Stern(4) showed later that in mice PVP enhanced renal excretion of dyes, prevented storage of dyes in the reticuloendothelial system, and also displaced dyes already stored in the reticuloendothelial system. Prompted by these results, Schubert (5) and later Krech(6) described in both experimental animals and human cases a beneficial action of PVP against exotoxins of tetanus, diphtheria, and botulism. Further experience of the German workers(7-11) indicated that PVP with a molecular weight of 12,600 (Periston N or PVPN) was more effective, presumably because it was more rapidly and completely eliminated in urine than the original PVP having a molecular weight of 30,000.

Our previous study demonstrated that PVPN protected ABC mice against lethality of brucella endotoxin(12). In experiments summarized here, the first objective was to confirm the enhancing effect of PVPN upon urinary excretion of trypan blue in animals. Following this demonstration, the action of PVPN upon lethality of tetanus toxin was investigated and compared to its effect on trypan blue. As will be shown, the action of PVPN upon these two agents differed significantly.

Material and methods. In investigations relating to excretion of trypan blue, male albino rabbits were selected for use, since accurate quantitative collections of urine could easily be obtained by urethral catheterization. At various intervals following intravenous injection of trypan blue (13 mg/kg body weight), PVPN* in an amount approximating 20% of estimated blood volume was given intravenously. Urine was collected by urethral catheterization both prior to and following injection of PVPN. The amount of dye in the urine was determined colorimetrically, using as standards known amounts of trypan blue

* Obtained from Die Farbenwerke Bayer, Leverkusen, Germany.

TABLE I. Effect of PVPN on Urinary Excretion of Trypan Blue in Rabbits.

Treatment	Time of PVPN admin., hr	No. of animals	% total dose trypan blue excreted after Periston-N 2 hr	4 hr
Saline		7	0	0
PVPN	S*	10	22 (15-33)	24 (16-36)
"	24	3	9 (8-12)	12 (11-12)
"	48	1	12	13
"	72	1	4	4

* S = Simultaneous.

dissolved in normal rabbits' urine. The amount of trypan blue excreted in urine was then expressed as per cent of total dose injected. Male ABC mice, averaging 20 g in weight, were used in experiments with tetanus toxin. All injections were made either intravenously through tail vein or intraperitoneally. The tetanus toxin† was stored at 4°C until use. Challenge was made with 0.1 ml of various dilutions, which were always freshly prepared to avoid spontaneous conversion to toxoid. Further treatment consisted of 0.5 ml of PVPN (6% solution) or 0.5 ml of sterile saline. When the toxin and PVPN were given simultaneously, they were mixed and injected from the same syringe. All other injections were given from separate syringes.

Results. Effect of PVPN on Excretion of Trypan Blue. Injection of trypan blue gave immediate bluing of skin and sclerae in control rabbits. No appreciable excretion of the dye in urine was apparent over a 72 hour period. When PVPN was given simultaneously with trypan blue, the degree of tissue staining was often less than in controls, but this was not consistent. The most striking and consistent effect of PVPN was to cause excretion of trypan blue in urine (Table I). Maximal excretion of the dye occurred within 2 hours; a small additional amount was found at 4 hours but none thereafter. When PVPN was given simultaneously with the dye, all rabbits tested promptly excreted grossly blue urine containing a considerable portion of the dye. Animals receiving PVPN 24 or 48 hours after trypan blue also had blue urine, but excreted less of the dye. Even at 72 hours a slight but definite excretion of trypan blue occurred in one rabbit.

Effect of PVPN on Lethality of Tetanus Toxin. Two lots of tetanus toxin were used. Both produced typical tetanic muscular spasms and generalized convulsions when given either intravenously or intraperitoneally. Since the majority of studies were made with lot #29577, a detailed titration of its potency is presented in Table II.

The effects of injections of PVPN upon lethality of tetanus toxin are shown in Table III. When tetanus toxin and PVPN were given intravenously and simultaneously (Exp. 1), excellent protection resulted. However, if therapy with PVPN was delayed for 24 hours (Exp. 2), no protection occurred. When toxin and PVPN were given at the same time intraperitoneally (Exp. 3), the only significant protection was against the minimal lethal dilution of 1:65,000. In Exp. 4, the effects of varying the timing, the number, and route of injections of PVPN were evaluated against toxin administered intraperitoneally. Neither multiple intraperitoneal injections nor intravenous injections afforded any protection against this challenge.

To confirm these results, the efficacy of PVPN was tested against a second lot of tetanus toxin (#29495). This was found to be less potent than lot #29577, necessitating injections of more concentrated dilutions to obtain lethality. As shown in Exp. 5, Table III, protection with PVPN was found only when toxin and PVPN were given intravenously.

Discussion. Studies with trypan blue con-

TABLE II. Lethality of Tetanus Toxin (Lot #29577) in ABC Mice.

Dilution of toxin (× 1000)	Intrav. Deaths*	Intrav. Avg day of death	Intraper. Deaths*	Intraper. Avg day of death
1: 5	5/5	1	5/5	1
1: 20	5/5	2	5/5	2
1: 40	2/2	3	5/5	2
1: 50			5/5	2
1: 60	5/5	3	4/5	4
1: 70			0/5	—
1: 80	4/5	3	0/5	—
1:100	7/7	4		

* No. dying/No. challenged.

† Obtained from Merck-Sharp & Dohme, West Point, Pa.

TABLE III. Effect of PVPN on Lethality of Tetanus Toxin in Mice.

Exp.	Dilution of toxin (× 1000)	Route* of toxin	Route* of PVPN	Timing of PVPN (hr)	Results of treatment: PVPN Deaths†	PVPN Avg day of death	Saline Deaths†	Saline Avg day of death
1	1:60	I.V.	I.V.	S‡	0/20		15/15	5
	1:80	"	"	S	2/20	7	10/10	5
2	1:80	"	"	S	2/10	7	"	4
	"	"	"	24	10/10	4		
3	1:50	I.P.	I.P.	S	20/20	2	15/15	2
	1:55	"	"	S	7/10	7	10/10	3
	1:60	"	"	S	8/10	4	12/15	4
	1:65	"	"	S	2/10	7	10/10	3
4	1:50	"	"	S	9/10	4		
	"	"	"	24	10/10	2		
	"	"	"	24 + 48	9/10	3	15/15	2
	"	"	"	S + 24 + 48	10/10	3		
	"	"	I.V.	S	"	2		
	"	"	"	24	"	2		
5	1: 2	I.V.	"	S	0/35		27/35	6
	"	I.P.	I.P.	S	40/41	4	31/41	4
	1: 4	"	"	S	20/20	6	18/20	7

* I.V. = Intrav.; I.P. = Intraper. † No. dying/No. challenged. ‡ S = Simultaneous.

firm the observations by Schubert(2,5) and Stern(4) that polyvinylpyrrolidone has a "washing out" effect upon dye stored in tissues. In contrast, however, such an action upon tetanus toxin was not observed. Although distinct protection with PVPN was demonstrated, this occurred only under restricted experimental conditions in which both toxin and PVPN were given intravenously and simultaneously. Lack of protection when therapy with PVPN was delayed has also been noted by Krech(6). This observation suggests that PVPN has no effect on tetanus toxin which has already been fixed by the tissues. Such an explanation has also been proposed by Benchelt.‡ The ineffectiveness of PVPN against toxin given intraperitoneally may result in a similar fashion. The explanation of the discrepancy between actions of PVPN on stored trypan blue and on fixed tetanus toxin is not clear but may reflect either a greater affinity of tissues for tetanus toxin than for trypan blue or an alteration in the chemical or physical properties of the toxin molecule by tissues so that PVPN can no longer combine with it.

These experiments point out the limited magnitude of the protective action of PVPN against tetanus toxin. This would suggest that therapy of human tetanus with PVPN would be of limited value, since only rarely can patients be treated simultaneously with liberation of toxin in the body. In support of this is the observation that PVPN had no beneficial effect in 10 patients with severe tetanus(13).

Summary. Intravenous injection of polyvinylpyrrolidone (PVPN-molecular weight 12,600) caused urinary excretion of trypan blue in rabbits, even when given 48 hours following injection of the dye. PVPN also protected mice against tetanus toxin but only when both toxin and PVPN were given intravenously at the same time. Since no protection was observed when therapy with PVPN was delayed or when the toxin was given intraperitoneally, it was suggested that PVPN was ineffective against tetanus toxin fixed in the tissues.

‡ Personal communication.

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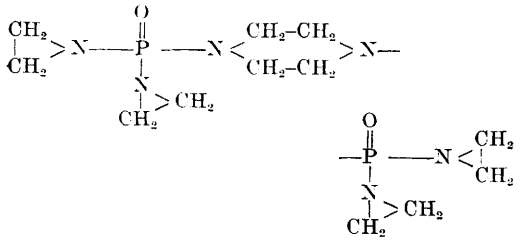
IV. Chemotherapy of Tumors in Rats with Piperazine-1,4-*bis* (N,N'-diethylenephosphondiamide).* (22956)

M. L. CROSSLEY, J. B. ALLISON, D. R. SEEGER AND E. KUH

Bureau of Biological Research, Rutgers University, New Brunswick, N. J., and Calco Chemical Division, Am. Cyanamid Co., Bound Brook, N. J.

This investigation is a continuation of the study of ethylenephosphoramides in the treatment of transplantable tumors in rats (1).

Materials and methods. Piperazine-1,4-*bis*(N,N' - diethylenephosphondiamide), (PDA) is a white crystalline compound; m.p. 185-186°C; very soluble in water and alcohol but almost insoluble in acetone and ether. It is slightly hygroscopic. Its structural formula is:



It has been reported to be active against a granulocytic chloroleukemia of the rat.† Adult, male Sprague-Dawley and Wistar rats, averaging about 150 g were used. In each experiment there were, as a rule, normal control (NC), tumor control (TC), drug control (DC), and tumor treated (TT) groups consisting of 8-10 animals each. The number of groups varied with dose levels of the drug chosen for comparison in any one experiment. An isotonic saline solution of the drug prepared weekly and kept at about 5°C, was given in one daily dose by intraperitoneal injection, the volume being that required to provide the desired dose in mg/kg of body weight of each rat at time of treatment. The Flexner-Jobling carcinoma and the Walker 256 carcinosarcoma were studied in Sprague-Dawley rats, the sarcoma Rl in Wistar rats. Methods of implantation and measurement of growth were essentially the same as those previously described(2). The animals were weighed periodically, length and width of the tumors measured in millimeters and size expressed as mm^2. Therapeutic effectiveness was determined by comparing average size of tumors of treated groups with that of untreated controls. When possible tumor weights were also compared. Regression was considered complete when the tumor was no longer palpable, no regrowth occurred in about 2 weeks, and gross examination at autopsy showed no evidence of tumors. Treatment of rats with Flexner-Jobling and Rl tumors began 5 days after implantation and with Walker Carcinosarcoma 256 3 days. The rats were group pair-fed, using 18%

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† Sparks, S. *et al.* abstract published.