Evaluation of Sodium Poly-α, L-glutamate as a Plasma Expander.*† (24776)

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In recent years many high molecular weight materials both of natural and synthetic origin have been tested as plasma expanders. A preparation with entirely satisfactory properties is still lacking; hence new expanders are still being investigated. Bovarnick *et al.*(1,2) and Rosenthal *et al.*(3) reported on the preparation and pharmacological properties of a γ, D-glutamyl polypeptide of bacterial origin. The work here described concerns evaluation as plasma expander of an α, L-glutamyl polypeptide of synthetic origin.

Materials and methods. Sodium poly-α, L-glutamate (SPG)‡ was prepared by the method described by Blout and Idelson(4). Two batches (#S-2035-186C and #R-4273-112) were used. Weight average molecular weights of polymers as determined by viscosimetric and light scattering technics were approximately 80,000 and 51,000 respectively (equivalent to degrees of polymerization of 620 and 395). The SPG was dissolved in 0.9% sodium chloride solution and autoclaved at 15 lbs/sq. in. for 20 minutes, after which pH of solution was adjusted to 7.4. In plasma expanding experiments male dogs were used after sedation with morphine sulfate (4 mg/kg, subcutaneously). The expander solution was infused into femoral vein at 40 ml/kg for 10 minutes. Plasma volumes were measured with radioactive iodinated serum albumin (RISA, Abbott) 1, 6, and 12 hours after administration of the expander. In experiments with hypovolemic dogs blood was first removed from femoral artery *i.e.*, 40 ml/kg for 7 to 20 minutes. The acute effects of SPG on right atrial and arterial pressures were studied in intact dogs which had been anesthetized with pentobarbital sodium (35 mg/kg, intraperitoneally). Dog heart-lung preparations were made according to Starling, for details see Krayer and Mendez(5). SPG was determined by modification of safranin O method described by Bovarnick *et al.*(2); the standard curve was linear over the range 0 to 500 μg of SPG.

Results. Plasma expanding experiments. The expanding properties of 3% SPG (batch #S-2035-186C) were compared with those of 6% dextran (Abbott) in normovolemic dogs and were similar (Table I). Analysis of variance revealed that the apparent difference between effects of the 2 expanders is not significant ($p>0.05$). The results obtained with hypovolemic dogs (Table I), show that 3% SPG (batch #S-2035-186C) and 6% dextran have similar expanding properties. The greater effect of dextran at 1 hour is consistent with the known hypertonicity of a 6% solution(6).

Toxicity studies in dogs. Acute effects. Rapid injection of 400 ml of 3% SPG solution (40 ml/min) in an intact anesthetized dog caused a rise in right atrial pressure and a fall in arterial blood pressure. Injection of a 6% solution of dextran at the same rate also resulted in increase of pressure in the right atrium, but at the same time arterial pressure rose. Respiration was unchanged during both injections. The drop in arterial blood pressure after SPG could be due either to impairment of cardiac output in spite of increased filling pressure, or to a dilating effect on peripheral vascular system. The effect of SPG upon cardiac output was investigated in the heart-lung preparation. Addition of as little as 40 ml of 3% SPG resulted in immediate

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TABLE I. Expanding Properties of 3% SPG and 6% Dextran in Normovolemic and Hypovolemic Dogs.

Expander	3% SPG	6% dextran	3% SPG	6% dextran
Dog status	Normovolemic		Hypovolemic	
No. of dogs	4	3	4	4
Dog wt, kg	9.7	10.0	9.8	10.0
Vol blood withdrawn, ml			388 *	400 *
" expander infused, "	389	401	393	400
Post-infusion time, hr	Plasma vol, ml			
0	540	611	580†	564†
1	894	939	789	838
6	768	780	697	674
12	720	736	687	664

* Hematocrits: SPG dogs 47%; dextran dogs 48%.
† These values are pre-bleeding levels.

rise in right atrial and pulmonary pressures and a striking decrease in cardiac output. Addition of 200 ml dextran was without any effect. The nature of the deleterious effect of SPG upon cardiac output was not further analyzed.

Chronic toxicity. A dose of 6% SPG (batch #R-4273-112) was injected intravenously at 40 ml/kg into 3 male dogs. All dogs died within 22 hours Two male dogs died on sixth day after intravenous injection of 3% SPG at 40 ml/kg. Plasma level of SPG, which immediately after injection was 785 mg %, fell to a mean of 249 mg % within 5 hours and remained at this level for at least 3 days. During post-injection period the dogs steadily lost weight (Table II).

Effects of SPG on blood. During *in vivo* experiments it was noted that SPG had effects on the blood. It increased erythrocyte sedimentation rate (ESR) and caused agglutination of erythrocytes; these effects were maintained for at least 3 days following injection of SPG (Table II). *In vitro* studies were made by mixing 0.5 ml of solution to be tested with 2.5 ml of either citrated human blood, or heparinized dog blood, or heparinized rabbit blood. Three % SPG increased ESR (human 23-25 mm/hr; dog 33-35 mm/hr; rabbit 44 mm/hr) and caused agglutination of erythrocytes in all 3 blood samples, whereas saline and 6% dextran had no effect.

Blood and urine levels of SPG. Retention of SPG by blood (Table II) has already been noted. Analysis for SPG of urines, collected during experiments with normovolemic and hypovolemic dogs, revealed that only small amounts (from 6 to 15%) of the injected dose were excreted during the first 6 hours after administration. None of the animals excreted any appreciable amount of SPG after 6 hours.

Discussion. Although 3% solution of SPG was equivalent to a 6% solution of dextran in expanding the plasma volume in dogs, further studies proved the polypeptide to be too toxic for clinical trial. In the dog heart-lung preparation, the drug impaired cardiac output. Such a deleterious effect would explain the decrease in arterial pressure in the intact dog following SPG. The experiments did not allow conclusions as to the precise mechanism of the effects on the heart. The effects of

TABLE II. Single Dose Toxicity Studies with 3% SPG (40 ml/kg I.V.) in Dogs.

	Post-inj. time, hr							
	0	0.25	5	24	48	72	96	120
Blood SPG, mg %	0	785	249	245	240	228		
ESR, mm/hr	1	28	22	27	29	33		
Hematocrit, %	47	28	34	39	43	41		
Erythrocyte agglutination	0	+	+	+	+	+		
Dog wt, kg	10			9.3	9.5	9.0	8.7	8.1
Survival time	Both dogs died at 6 days. (With 6% SPG all 3 dogs died within 24 hr.)							

SPG on blood, particularly agglutination of erythrocytes, would certainly alter some of the physical properties of blood. It is interesting to note that Rosenthal *et al.*(3) mention no effect, other than an "expected increase" in sedimentation rate, on human erythrocytes with their bacterial γ, D-glutamyl polypeptide. That the increase in sedimentation rate observed with SPG is not just a non-specific effect of all macromolecules, can be concluded from the fact that commercial dextran had negligible effects on ESR of human and dog bloods in our studies. Synthetic α, L-copolymers of glutamic acid and lysine have also been investigated with respect to their effects on blood *in vitro*. It has been found (Klein, Fasman and Blout, personal communication), that those copolymers containing more than 65 mole % of glutamic acid behaved similarly to SPG in that they caused agglutination of erythrocytes and an increase in viscosity of plasma; those in the 50-60 mole % range exhibited no such toxic effects. Determination of blood levels of SPG revealed that after the first 5 hours further elimination of the drug did not occur. Such a finding was compatible with the observations of urinary excretion of SPG, where it was noted that no appreciable amount was excreted after the first 6 hours. In contrast to the toxic effects of SPG reported here, Maurer (7) found in immunological studies that no detectable antibodies could be produced against the material (batch #S-2035-186C) either in man, rabbit, or guinea pig.

Summary. (1) A synthetic polypeptide, sodium poly-α, L-glutamate (SPG), has been evaluated as a plasma expander by comparing it with dextran in both normovolemic and hypovolemic dogs. (2) SPG at a concentration of 3% was equivalent to 6% dextran as a plasma expander. (3) Further studies with SPG proved it to be too toxic for clinical trial. It was observed to impair cardiac output in the dog, and to cause agglutination and an increase in sedimentation rate of both human and dog erythrocytes. (4) A considerable part of the injected dose remained in the blood at 5 hours after injection and no evidence of further elimination over the next 3 days was observed.

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Effects of Cortisone and Herpes Simplex Virus on Metabolic Processes. I. Alterations in HeLa Cell Metabolism.* (24777)

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Recently various effects of cortisone and related steroids on cell growth and on carbohydrate and nitrogen metabolism have been reported(1-3). Gavosto *et al.*(2) suggested that cortisone increases gluconeogenesis in animals leaving a negative nitrogen balance due to enhanced transamination processes. Independently, Rosen and Roberts(3) substantiated these claims. As yet one can only speculate as to the effect of cortisone on metabolism of isolated tissue culture systems. Although cortisone treatment may be dramatically effective in limiting inflammatory re-

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