Evaluation of Multichain Polyglutamic Acid as a Possible Plasma Expander.* (27027)

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The search for plasma substitutes is prompted by growing interest in their use in cases of simultaneous mass injuries. Many substances have been developed, but none fully conforms to the requirements of an ideal plasma volume expander(1,2). New substances are therefore being investigated continuously. The use of the natural α-glutamyl polypeptide isolated from *B. subtilis* as a possible plasma volume expander was examined by Bovarnick and his collaborators(3,4,5,6). When the molecular weight and diameter of the macromolecule were increased by coupling chemically several natural polyglutamic acid chains to yield a branched polymer, rate of renal excretion decreased considerably. Evaluation of sodium poly-α, L-glutamate as plasma expander has been reported by Kenny(7). At a concentration of 3%, sodium poly-α, L-glutamate was found to be similar to 6% dextran in its effect as a plasma expander, but the linear acidic polyamino acid at these high concentrations was too toxic for clinical trial.

The work reported here summarizes observations on the behavior *in vivo* of multichain polyglutamic acids(8), and their evaluation as plasma expanders. Multichain polyamino acids are synthetic branched polymers whose molecules are composed of linear polymeric chains attached to a polyfunctional core(9,10). The different polymer batches had polylysine cores of average degrees of polymerization of 70-140, and side chains varying in average length from 13 to 18 (Table I). The polymers are accordingly chemically described as multi-(copoly-L-glutamyl-L-aspartyl) poly-L-lysine.

Materials and Methods. The polymers were prepared by the method of Yaron and Berger (8). Three batches of different compositions and molecular weights were used (Table 1). Ultracentrifugation revealed that each batch contained about 15-20% of a low-molecular weight fraction.

The soluble sodium salts of the polymers were used throughout as 2-3% solutions in 0.9% sodium chloride solution. The solutions were adjusted to pH 7.0.

Seven mongrel dogs weighing 3.2 to 7.7 kg were fasted 12 to 20 hours before experiment, with free access to water. Six rabbits weighing 1.7 to 2.4 kg were used without preparation. The animals were anesthetized with intraperitoneal and intravenous sodium thiopentone. Hypovolemia was produced in some animals by arterial bleeding. Twenty to 30 ml/kg were removed in 10 to 20 minutes immediately prior to the polymer infusion. Other animals were given the polymer solution without prior bleeding. Twenty-five to 40 ml/kg of polymer solution was infused intravenously in 10 to 20 minutes. In 2 normovolemic rabbits the polymer dose was divided into 10

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TABLE I. Amino Acid Contents, Molecular Weight and Sedimentation Constant of Polymers used in Infusion Experiments.

Polymer batch No.	No. of amino acid residues— in backbone Lysine	per one side chain Aspartic acid	Glutamic acid	Molecular Wt	Intrinsic sedimentation constant* (Svedberg units)
125/10	140	2.2	11.4	260,000	11.0
25/11	70	3.4	13.2	150,000	5.7
60/11	70	2.7	15	160,000	6.4

* Intrinsic sedimentation constant was determined on benzyl derivatives in dimethylformamide, 20°C.

daily injections, no anesthetic being used. In one normovolemic dog the infusion lasted 1 hour. No anesthetic was added after starting the infusions.

Arterial blood pressure was measured in some dogs from the femoral artery with a mercury manometer and recorded on a kymograph. No anticoagulant was used. Blood hematocrit and erythrocyte sedimentation rate were measured on oxalated blood samples taken at intervals during experiments. Polymer concentrations in plasma and in urine were determined colorimetrically by the Safranin-O method of Bovarnick(4) with some modifications. Gross pathological examinations were carried out on animals that died.

Results. Plasma expanding properties. In all the animals that were bled prior to infusion, blood pressure dropped to shock levels after the bleeding. During infusion of the polymer solution blood pressure returned to almost normal levels. In all animals the infusion caused a drop of the hematocrit. The magnitude of this drop was that to be expected on the assumption that the polymer was initially distributed only in the plasma. No significant differences were found in these plasma expanding properties among the 3 batches of polymer, nor between polymer solutions of 2 and 3%.

Plasma and urine levels. During the first 2 to 3 hours after infusion, 10 to 20% of the injected polymer was recovered from the urine. Plasma polymer concentration dropped correspondingly by about 20% during the same period. No further polymer excretion in the urine was detected. The polymer recovered from the urine was shown by ultracentrifugation to represent the low-molecular weight fraction of the polymers. Subsequently plasma polymer concentrations dropped exponentially, reaching a level of 50% after approximately 48 hours (Fig. 1). In 2 rabbits the polymer dose was injected over a period of 10 days. Six days later the calculated total polymer in the plasma was 2.6 and 4.4% of the initial dose respectively. There was no significant difference in rates of disappearance from the blood among the 3 polymer batches.

Toxicity. The 2 rabbits which received the polymer dose in 10 daily injections showed no toxic reactions to the material. The other 4 rabbits showed increasing weakness and died

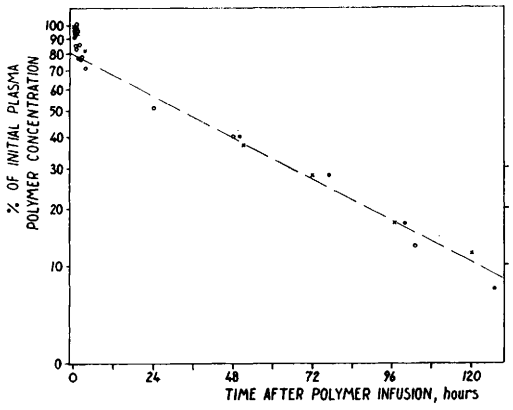

FIG. 1. Rate of disappearance of polymer from plasma in 3 dogs after intravenous infusion.

within 21 hours. One rabbit, not bled previously, died with signs of pulmonary edema after 11 hours. All 7 dogs died within 6 days after infusion. During this period they suffered from diarrhea, loss of appetite, occasional vomiting and progressive weakness.

Apart from the restorative effect of the polymer solutions on blood pressure of the shocked animals, a transient fall of blood pressure occurred at the beginning of infusions in normovolemic as well as hypovolemic animals. A capillary bleeding tendency was observed at site of incisions. Blood clotting time was not prolonged. After death small quantities of bloody fluid were found in the serous cavities of some animals. Subserosal and submucosal hemorrhages were found in a few intestinal segments in most dogs and in one rabbit. Clumping of red blood cells and an increased sedimentation rate were caused by the polymer both *in vivo* and *in vitro*. Apart from these minor bleeding manifestations no macroscopic changes were observed after death.

Discussion. The polymer samples investigated were shown to be adequately retained in the circulation and were efficient in abolishing the features of hemorrhagic shock in rabbits and dogs, but they proved to be toxic. Severe gastrointestinal disturbances occurred, and mild hemorrhagic and hypotensive symptoms were found. Minor falls in blood pressure could be produced by rapidly injecting a few ml of polymer solution during infusion.

These toxic manifestations can be compared to the findings of Kenny(7), that linear synthetic polypeptides of glutamic acid caused a

drop in arterial blood pressure in intact dogs, and that the dogs died within 5 days after constantly losing weight.

There is as yet no explanation as to the cause of toxicity of these polypeptides. The bleeding tendency did not cause major hemorrhages. The increased red blood cell aggregation and sedimentation rate are non-specific phenomena which are caused by all macromolecular substances which were investigated (11). The postmortem gross findings in our animals were not sufficient to explain their deaths.

The polypeptides investigated by Kenny, as well as our polymers, have multiple free negative charges whereby their oncotic efficiency is considerably enhanced due to the Donnan effect, as shown by Rosenthal *et al*(6). On the other hand, these negative charges may possibly interfere with the normal function of the cell membrane, and cause serious metabolic disturbances. It seems, therefore, desirable to extend this investigation to other, non-charged or isoelectric, water-soluble linear and multichain polyamino acids.

Summary. Some biological properties of 3 batches of a multichain polyamino acid, multi-(copoly-L-glutamyl-L-aspartyl) poly-L-lysine, were examined *in vivo* in dogs and in rabbits. The polymer was administered intravenously as 2 to 3% solutions of its sodium salt by one or by repeated injections. In some animals hypovolemia was produced by arterial bleeding prior to infusion. A low-molecular weight fraction of the polymer, amounting to about 10 to 20%, was excreted in the urine within a few hours. The rest of the injected polymer was not excreted in the urine, and disappeared gradually from the blood, with a constant half-concentration time of about 48 hours. As shown by its influence on blood pressure and on hematocrit, the polymer was able to combat the manifestations of hypovolemic shock. However, severe toxic manifestations appeared in many animals, consisting of diarrhea, vomiting, loss of appetite, a transient drop in blood pressure and a tendency to capillary bleeding. Progressive weakness of the animals was observed, leading to their death. The cause of toxicity of these polypeptides has not been found. Possibly their multiple negative charges may be responsible. It appears worthwhile to examine non-charged or isoelectric polyamino acids with regard to their plasma expanding properties and their toxicity.

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Preservation of Rabbit Spermatozoa: Fertility Results from Frozen Semen.* (27028)

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The influence of cold on animal spermatozoa has been studied for a considerable time. De-

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