figures at the .25 mg/ml concentration were 22 and 57 days, respectively. The lesser effectiveness in immature rats is demonstrated also by the reappearance of hair growth in the treated area in 19 of 23 rats of the former group after an average of 44.6 days of treatment. In contrast, regrowth did not occur during treatment of any adult rats. Similarly, prolonged inhibition did not begin in the immature rats until after an average of 62.9 days of treatment as compared with 16.3 days for the adults at the higher concentration of extract (Table I).

Discussion. These experiments show that age modifies tissue response to the local action of adrenocortical hormones. When growth is retarded by the presence of excessive amounts of these substances in the tissue fluids, it appears that the action of pituitary growth hormone is antagonized. Thus, growth hormone and corticotrophin exert antagonistic effects on the growth of bone in hypophysectomized rats (4). It is possible that young rats produce relatively more endogenous growth hormone than adults, or that their peripheral tissues are more responsive to it, which would explain why the inhibitory action of adrenocortical steroids is less pronounced in immature animals.

Since adrenocortical hormones may suppress growth of hair and certain aspects of the inflammatory response by a similar mechanism, one might expect that age would modify dosage requirements in the treatment of inflammatory diseases in children.

Summary. Adrenocortical extract inhibits hair growth less effectively in immature than in adult rats.

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Effects of Intravenously Administered Poly-D L-lysine in Rats. (20078)

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The recently synthesized water-soluble basic poly-α-amino acid poly-lysine(1) has been shown to have a number of actions *in vitro*. It inhibits the formation of thrombin in shed human blood(2), it inhibits fibrinolysis in a streptokinase-profibrinolysin-fibrin system(3), it is bacteriostatic(4,5), it reduces virus infectivity(6), it agglutinates chicken red cells (7) and human red cells(8) and is a hemolytic agent(9). The natural acidic polymers, heparin and poly-D-glutamic acid, and the synthetic water-soluble acidic poly-α-amino acid, poly-L-aspartic acid, have been shown to antagonize the clot delaying(2), the antifibrinolytic(3) the agglutinating(8), and the hemolytic action(9) of poly-lysine *in vitro*.

The present study reports the effects in rats of poly-D L-lysine on blood coagulation and red cells, the clinical and anatomical picture caused by toxic doses of this substance, and the antagonistic action of acid polymers.

Materials. Poly-D L-lysine hydrochloride with an average degree of polymerization-30 was prepared from D L-lysine according to Katchalski *et al.*(1). Poly-L-aspartic acid with an average degree of polymerization-150 was prepared according to Berger and Katchalski(10). Heparin, a Lederle solution containing 10 mg sodium salt of heparin per ml having anticoagulant potency of 1100 Toronto units per ml, was used as described below. Thromboplastin was prepared from human brain (11).

Methods. Solutions of poly-D L-lysine

TABLE I. Effect of Intravenous Poly-D L-Lysine on Blood Clotting Time and Prothrombin Consumption in Rats.

Poly-D L-lysine HCl, mg/100 g body wt	Clotting time, min.	Serum prothrombin, %	No. of rats
—	1.5–3.5	0	5
8	33	30	1
2	2.0–2.5	130–190	3
1.5	1.5–3.5	100–180	3
1	1.0–4.0	85–100	2
.5	3.0–3.5	50– 52	2
.2	3	45–110	2
.05	2	0– 57	2
.025 and lower	1.5–2.5	0	3

Rats receiving 8 and 2 mg and 2 rats receiving 1.5 mg polylysine hydrochloride/100 g body wt died within 10 min. after inj. Heart puncture immediately after death. Other rats sacrificed 10 min. after inj. Heart blood obtained immediately thereafter. Plasma prothrombin varied from 130–190% (expressed in % of human plasma prothrombin).

hydrochloride of various concentrations in buffered isotonic saline were injected slowly into rat tail veins in a total volume not exceeding 0.4 ml. Blood for coagulation studies was obtained by puncture of the heart exposed under ether anesthesia, avoiding admixture with tissue juice. Prothrombin of the serum, obtained one hour after clotting, was estimated by the method of Rosenfield and Tuft, using $BaSO_4$-treated oxalated human plasma as diluent(12). Rat serum prothrombin values are expressed in percentage of normal human plasma prothrombin content, measured by the same method. Blood clotting times were measured by the method of Pohle and Taylor at 37°C(13). Red cell counts were done on blood, obtained from the tail vein, in a Spencer counting chamber averaging the count of 2 chambers. Reticulocyte counts were determined from blood smears stained with brilliant cresyl blue and expressed as % of the number of red cells. Tissues were fixed in 10% formalin, sectioned at 8 μ and stained with hematoxylin eosin. Blocks were taken from the lungs, heart, spleen, liver, kidneys, lymph-nodes, muscle, skin and brain.

Results. 1. *Effect of intravenous administration of poly-D L-lysine on blood coagulation.* The results are given in Table I. The lethal dose of 8 mg per 100 g bodyweight prolonged the clotting time markedly. Lethal doses of 1.5-2 mg and sublethal doses did not prolong the clotting time, but a definite disturbance in thrombin formation, as indicated by elevated serum prothrombin values, was observed even with doses as low as 0.05 mg per 100 g bodyweight. The rather low prothrombin value of the serum of the rat given 8 mg polylysine may have been due to the inhibitory action of polylysine on the formation of thrombin in the procedure of prothrombin determination(2). It was found that the anticoagulant effect of intravenously administered polylysine in sublethal doses of 1.5 mg per 100 g bodyweight remained detectable up to 2-3 hours after injection and disappeared within 6 hours.

2. *Red cell agglutination.* The blood of rats injected intravenously with doses up to 2.5 mg of poly-D L-lysine hydrochloride per 100 g bodyweight did not show red cell agglutination. Red cell agglutination was observed only in the heart blood of the rat dying after injection of 8 mg per 100 g bodyweight.

3. *Red blood cell and reticulocyte count.* The results are given in Table II. Single or repeated intravenous injections of 1-2 mg of poly-D L-lysine hydrochloride per 100 g bodyweight (sublethal) caused a drop in the red cell count of 14 to 45% and a reticulocytosis ranging up to 16%. Single or repeated injections of 0.75 mg or less per 100 g bodyweight caused a variable drop in the red cell count. In 2 rats reticulocytosis without a fall in red cell level was observed. Peripheral smears showed no abnormality of the red cells. Bone marrow examinations in 2 rats revealed marked erythroid hyperplasia.

4. *Clinical and anatomical picture caused by toxic doses.* Rats injected intravenously with doses of 1.5 mg or more of poly-D L-lysine hydrochloride per 100 g body weight died within approximately 12 minutes. The larger the dose the earlier death occurred. In order of appearance the signs were: sneezing, irregular rapid respiration, cyanosis, increased movements, convulsions, frothy pink fluid issuing from the nose and death in flaccid paralysis. Doses of 1.2-1.5 mg of poly lysine hydrochloride per 100 g of body weight caused temporary dyspnea and slight cyanosis which disappeared within approximately three-quarters of an hour. The pertinent patho-

TABLE II. Fall in Red Cell Count and Reticulocytosis in Rats Receiving Poly-D L-Lysine Intravenously.

Poly-D L-lysine HCl, mg/100 g body wt, i.v.	Max fall in R.B.C.* % of initial value	Max fall in R.B.C.* On day	Max reticulocytosis %	Max reticulocytosis On day
1	24	3	9	5
1	17	3	7.4	6
1	14	6	5	6
1	20	3	7.4	2
.75†	0		0	
.5	0		0	
.25	0		0	
3 × 1.5 > 6 hr	40	3	12.8	5
2 × 1 ½ "	45	3	13	3
3 × 1 6 days	16	6	8	6
3 × 1 + 2 × 2 + 1.5 >8 days‡	31	9	16	11
3 × .75 > 4 hr	35	3	11	4
3 × .75 8 "	0		0	
2 × .75 24 "	0		0	
3 × .6 2 "	12	2	12.8	3
3 × .6 2 "	7	2	5.7	3
3 × .6 2 "	0		8	3
3 × .6 2 "	0		6.4	3

* Preinjection red cell counts done at least 3 times, within 3 to 6 days prior to inj., including day of inj. Only those rats used whose preinjection counts did not differ more than 600000 per cmm, *i.e.* approximately ± 3% from avg count.

† Rats not showing any change were counted at least 6 days.

‡ This rat did not die when receiving 2 mg/100 g body wt and apparently had become resistant.

logical findings were limited to the lungs and heart. The lungs of rats injected with lethal doses of poly-lysine were 2 to 3 times the normal size and weight, markedly distended, bright pink, and oozed a great deal of frothy pink fluid from the bronchial tree and cut surfaces. Microscopically the majority of the alveoli contained protein rich material, red cells or a mixture of these. Perivascular and peribronchial edema was marked. The heart was dilated predominantly on the right side and histologically there was evidence of cardiac edema in both left and right ventricles. No gross or microscopic findings in the other organs could be attributed to the polylysine. Rats were injected with sublethal doses of poly-lysine and were sacrificed 1½ to 3 hours after injection. Grossly the lungs of these animals were normal. Histologically a small number of alveoli contained hyalin eosinophilic membranes flattened against the alveolar septa. Slight to moderate perivascular and peribronchial edema was visible in the larger connective tissue septa. Nothing else of note was found in the remaining organs.

5. *Antagonistic action of heparin and poly-L-aspartic acid to poly-D L-lysine in vivo.* Heparin as well as synthetic poly-L-aspartic acid protected rats from the fatal effects of intravenous poly-D L-lysine in a weight ratio of 1:1 to 1.5:1,* either when mixed with the polylysine before injection or by intravenous administration not more than 3 minutes after injection of the poly-lysine. Histological examination of the organs of these rats showed no pathological changes. In only a few of several experiments however did the acidic polymers completely neutralize the disturbance in thrombin formation caused by polylysine. The *clot-delaying effect* of heparin *in vivo* could be neutralized by polylysine, either by mixing polylysine with it before injection or by intravenous administration of polylysine after injection of heparin. Effective acidic polymer to basic polymer ratio in all these experiments were 1:1 to 1.5:1. The drop in red cell count caused by polylysine could in some cases be prevented by mixing it with 1.5-2 times the amount of heparin before injection. However a slight reticulocytosis was observed sometimes even though

* Poly-L-aspartic acid injected in these quantities was not toxic, did not affect thrombin formation, nor did it cause red cell agglutination.

the red cell count did not fall.

Discussion. Some of the known *in vitro* actions of poly-D L-lysine, *i.e.* inhibition of thrombin formation(2), red cell agglutination (7,8) and red cell destruction(9), were also shown to occur in rats after intravenous administration. The *in vivo* action of polylysine on the blood coagulation mechanism manifested itself in disturbed prothrombin conversion while clotting times remained normal. The disturbance in thrombin formation might be attributed to antithromboplastic activity (2). Red cell agglutination did not occur following lethal and sublethal doses even when thrombin formation was disturbed. Only when a very high lethal dose of polylysine was given, the clotting time became prolonged and red cells became agglutinated. Red cell agglutination occurred apparently only after much of the polylysine was bound to the plasma proteins(8). Red cell destruction was probably due to direct damage to the red cell (9). The respiratory signs appearing after toxic doses of polylysine were probably due to pulmonary edema and hemorrhage, cardiac dilatation and edema. The mechanism of this has not been elucidated. No anatomical lesions were found in the central nervous systems to explain the neurological disturbances which might have been the result of sudden anoxia consequent to pulmonary and cardiac lesions.

The acidic polymers, heparin and poly-L-aspartic acid, were antagonistic to the lethal, hemolytic and coagulation disturbing effects of polylysine. Similar antagonistic actions were found *in vitro*(2,8,9).

Summary. Poly-D L-lysine was administered intravenously to rats. Lethal doses of 1.5-2 mg per 100 g body weight caused a disturbance in thrombin formation, pulmonary and cardiac edema and death within 12 minutes. The high dose of 8 mg polylysine per 100 g body weight caused red cell agglutination and prolonged clotting time. Sublethal doses also caused a disturbance in thrombin formation, slight hemolysis and reticulocytosis, and transitory symptoms of respiratory distress. Heparin and synthetic poly-L-aspartic acid were antagonistic to polylysine and prevented death of polylysine-treated rats, hemolysis and coagulation disturbance. These effects *in vivo* corroborated the observations on the biological action of polylysine *in vitro*.

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