TABLE I. Effects of Hormone Treatment on Amount of Spreading Factor.

Controls and treatments	No. of animals	§	Assay of spreading factors‡ Avg final bleb, mm	Area of spread, mm², mean ± S.D.
Aqueous buffer solution, pH 6	8		16 × 17	59.7 ± 13
Castrate ♀	5†		16 × 16	47.1 ± 12.6
10 day pregnant ♀*	"		21 × 21	192.5 ± 16.5
1 μg estradiol benzoate*	"		16 × 18	72.3 ± 13.3
5 " " *	"		20 × 21	176 ± 16.4
5 mg progesterone*	"		18 × 19	114.7 ± 14.5
1 μg E. B. + 5 mg progesterone*	"		21 × 22	209 ± 16.9
1 " + 25 GPU relaxin	"		17 × 18	86.4 ± 14.1
5 " + " " "	"		20 × 22	191.7 ± 16.5
5 mg progesterone + 25 GPU relaxin	"		18 × 19	114.7 ± 14.5
1 μg E. B. + 5 mg progesterone + 25 GPU relaxin	"		21 × 22	209 ± 16.9

* Inserted from previous exp. for comparison(5,6).
† Castrate female rats.
‡ .2 cc extract + .1 cc Evans blue dye.
§ Avg initial bleb 14 × 14 mm.

Groups of 5 rats were treated as follows: a) 1 μg estradiol benzoate and 25 guinea pig units of relaxin; b) 5 μg estradiol benzoate and 25 guinea pig units of relaxin; c) 5 mg progesterone and 25 guinea pig units of relaxin; and d) 1 μg estradiol benzoate, 5 mg progesterone and 25 guinea pig units of relaxin.

Results. The extracts from both the 1 μg and 5 μg levels of estradiol benzoate plus relaxin showed only slightly more mammary spreading factor than the same levels of estradiol benzoate alone. The addition of relaxin to the 5 mg of progesterone failed to increase the amount of spreading factor in the mammary gland extracts. Similarly the injection of 1 μg estradiol benzoate, 5 mg progesterone and relaxin produced no increase in the amount of spreading factor in comparison with a group administered estradiol benzoate and progesterone alone (Table I).

Summary. The injection of 25 guinea pig units of relaxin daily for 10 days to castrate female albino rats as an addition to estradiol benzoate and to progesterone failed to increase, significantly, the elaboration or activation of the mammary gland spreading factor in comparison with the steroid hormone action.

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Effect of Age on Local Action of Adrenocortical Hormones.* (20077)

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The direct application to rat skin of adrenocortical extract or alcoholic solutions of several

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TABLE I. Duration of Local Treatment Required to Inhibit Growth of Hair in Immature and Adult Rats.

	No. of rats	Mean duration of treatment (days) — Initial inhibition	Regrowth	Prolonged inhibition*
(a) One mg/ml conc.				
Immature rats				
Extract-treated	23	38 ±10.7†	44.6±10.0‡	62.9±15.5
Ethanol-treated	14	0	0	0
Adult rats				
Extract-treated	17	16.3± 4.8	0	16.3±19.7
Ethanol-treated	14	0	0	0
(b) .25 mg/ml conc.				
Immature rats				
Extract-treated	5	57 ± 3.0	82§	69 ±19.8
Ethanol-treated	4	0	0	0
Adult rats				
Extract-treated	3	22 ±11.2	0	29 ± 7.0
Ethanol-treated	2	0	0	0

* By "prolonged inhibition" is meant a state of retarded hair growth which lasted for a minimum of 6 consecutive weeks.

† Stand. dev. ‡ Nineteen rats showed regrowth. § One rat showed regrowth.

adrenal steroids induces inhibition of hair growth(1,2) and marked connective tissue changes(3) which are limited to the area of treatment. This report demonstrates that a longer period of treatment is required to inhibit hair growth in immature than in adult rats.

Procedure. Daily percutaneous application of hog adrenocortical extract† to immature rats was begun 3 days after birth and to adult rats at approximately 70 days of age. Control animals in both groups received comparable volumes of the solvent, 25% ethanol. Because of the difference in surface area between newborn and adult rats, an attempt was made to administer approximately the same amount of hormone per unit area of skin rather than a constant quantity to a constant area in both age groups. The volume administered was the amount required to moisten the skin of the right dorsal surface of the neck within an area bounded by the right ear, center of the scapula, dorsal mid-line and lateral mid-line. The volume applied daily to newborn rats was .02 ml. This amount was increased gradually to 0.1 ml at about 40 days of age, 0.1 ml being the volume given to adult rats from the beginning of the experiment. Since two concentrations of extract were employed, *i.e.*, the equivalent of 1 mg and .25 mg of cortisone per 1 ml as determined by the liver glycogen test, the immature rats received at the beginning of the experiment 20 or 5 μg per day which was increased to the adult dosage of 100 or 25 μg at 40 days of age. In each experiment, littermate male and female newborn rats were divided into extract- and ethanol-treated groups with adult rats being run in parallel. Adult rats and the immature rats after weaning were kept in individual cages. The patterns of hair growth on the dorsum of the neck were drawn weekly. Since the normal pattern of growth is bilaterally symmetrical, inhibition was revealed by the absence of pigment (black-hooded Long-Evans rats were used) or hair on the right side at a time when these appeared on the left. The hair was clipped weekly in adults and in the young after 35 days of age. Because of the extremely dense and rapid growth of hair on young rats, they were clipped twice weekly prior to 35 days of age in order to insure adequate moistening of the skin with the applied fluids.

Results. The greater resistance of immature rats to the local growth-inhibiting action of adrenocortical extract is shown in several ways. The mean number of days of treatment required to elicit the response in adults is less than half that required for young rats, at the 1 mg/ml concentration 16 and 38 days being required, respectively (Table I). Comparable

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