of the "supernate" is 7 times that of the "granule fraction". In contrast the tumor influencing activity in the "granule fraction" is slightly higher than that of the "supernate fraction". (Table III). These findings indicate that the "lymphosarcoma factor" is not identical with the spreading factor (hyaluronidase).

Summary. A factor has been demonstrated in testis extract which inhibits the growth of a transplanted lymphosarcoma in AKm mice.

This factor is of unknown nature, but appears to be different from the Duran-Reynals spreading factor.

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17158. Effects of Urethane on Living Tissue.*

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Ethyl Carbamate (urethane) has been shown to be a useful agent in the treatment of wound infections.[1,2] When extensive wounds or large areas of intact skin are exposed to the prolonged application of 10% urethane solution[2] about 20 to 30% of the patients treated develop nausea and vomiting. This suggests that the drug is absorbed through open wounds and normal skin surfaces. The nausea and vomiting reported by Howe and Weinstein,[2] and Howe[1] in urethane treated wound infections and by Paterson *et al.* in leukemia patients receiving the drug by mouth[3] appeared to be benign in nature. However, because the treatment of human wound infections requires prolonged exposure to large amounts of this drug, experiments were conducted to investigate its effect on the organs of animals treated with similar doses.

A group of 10 guinea pigs was given lethal amounts of urethane administered intraperitoneally in rapidly ascending doses over a short period of time (Table I, Pigs 1-10). The gross and histological tissue changes were compared with those of a second group of 10 animals receiving daily subcutaneous injections of urethane in dosages simulating those used in the topical treatment of infected wounds in man (Table II, Pigs 14-23). Hypnotic effects ranging from drowsiness and ataxia to terminal anesthesia were noted. Some animals recovered and ate heartily after 8 hours of complete anesthesia. In the treatment of a large infected wound a person is exposed to a maximum of 60 g of urethane per day, although only a fraction of this is absorbed due to loss in dressings. The animals in Group II received 0.1 g per 100 grams of body weight per day for 28 consecutive days which is about twice as long as the average duration of therapy in 39 urethane treated patients.[2] This is comparable to the maximal dosage used in man, and it was given subcutaneously to insure complete absorption. Dosages were corrected weekly for fluctuations in weight. Control animals (Table I, Pigs 11-13; Table II, Pigs 24-26) received corresponding amounts by volume of normal saline. Diet consisted of Purina chow, lettuce and cabbage. All animals in Group II gained weight and at the end of the experiment were healthy and had lustrous silky coats. They were killed on the 29th day.

Examination of the organs was made immediately after death. Sections were fixed in 5% formaldehyde solution, imbedded in paraffin and stained with hematoxylin eosin. Brain, striated muscle, myocardium, lungs,

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1 Howe, C. W., *Surg., Gynec. and Obst.*, 1948, **87**, 425.

2 Howe, C. W., and Weinstein, L., *Surg., Gynec. and Obst.*, 1947, **84**, 913.

3 Paterson, E., Thomas, I. A., Haddow, A., and Watkinson, J. M., *Lancet*, 1946, **1**, 677.

TABLE I.
10% Urethane Intraperitoneally in Lethal Doses.

Pig No.	Wt in g	Days treated	No. inj.	Total dose, g	Fate
1	248	9	5	0.99	Died
2	250	13	6	1.32	"
3	316	13	6	1.47	"
4	267	9	5	1.02	Killed
5	269	6	4	0.83	"
6	842	3	3	7.57	Died
7	821	3	3	7.81	"
8	906	3	3	8.19	"
9	757	3	3	6.80	"
10	842	3	3	7.58	"
11*	757	3	3	0.58†	Killed
12*	250	10	6	0.11†	"
13*	270	9	5	0.09†	"

* Saline treated control.
† Grams of NaCl.

TABLE II.
10% Urethane Subcutaneously.

Pig No.	Initial wt, g	Total No. daily inj.	Total dose, g	Final wt, g
14	442.0	28	13.81	581.6
15	353.2	20*	8.19	468.4
16	396.7	20*	8.46	393.4
17	344.5	28	10.47	432.1
18	412.9	28	13.23	528.3
19	399.0	28	12.44	492.4
20	355.5	28	10.96	412.2
21	391.2	28	12.19	503.7
22	441.5	28	13.82	556.6
23	274.1	28	9.67	440.5
24†	434.4	28	1.18‡	563.7
25†	392.4	28	1.10‡	558.1
26†	390.4	28	1.18‡	514.1

* Injections omitted after 20th day due to development of pneumonia. Animal recovered.
† Saline treated control.
‡ Grams of NaCl.

liver, spleen, pancreas, kidneys, adrenals and gonads were examined.

Findings. Only the significant changes are described. In the group of animals submitted to intraperitoneal injections of lethal doses of urethane (Group II) there was congestion in the lungs, liver and spleen and occasionally in the kidneys. Vascular changes characterized by endothelial swelling and desquamation, mural edema and extravasation of plasma and formed blood elements into the surrounding tissues were occasionally present. Cytoplasmic vacuolization was observed in the hepatic cells and glandular epithelium of the renal tubules. A striking hyperplasia of mesenchymal cells was noted in the lungs and spleen and to a lesser degree in the myocardium and liver. Frequent hepatic cell changes consisting of an increase in fat droplets, cytoplasmic swelling and rarefaction were present. The central lobular cells in 3 animals[3,8,10] displayed loss of cellular outlines. The kidneys revealed no glomerular damage. There were hemorrhages in the cortex and medulla in animals 2 and 9.

In the animals subjected to sublethal injections of urethane over long periods of time (Group II), the striking feature was a mesenchymal cell reaction in the lungs and spleen. Cytoplasmic vacuolization of the hepatic cells was frequently seen. Vascular engorgement in the lungs, liver and spleen often accompanied by mural edema and by escape of plasma and formed blood elements were occasionally encountered.

The kidneys showed no significant findings.

The tissues at the site of injection revealed blood pigment and recently extravasted blood cells in the subcutaneous tissues. Independently from these hemorrhagic changes, a mesenchymal reaction consisting of histiocytes and lymphocytoid cells was found in a number of guinea pigs.

Microscopic sections of the lungs in two control animals showed engorgement of blood vessels, mural edema, minimal endothelial damage and red cell extravasations. In the same two animals there were dilated hepatic sinusoids with red cell extravasations and increased cellularity in the pulp substance of the spleen.

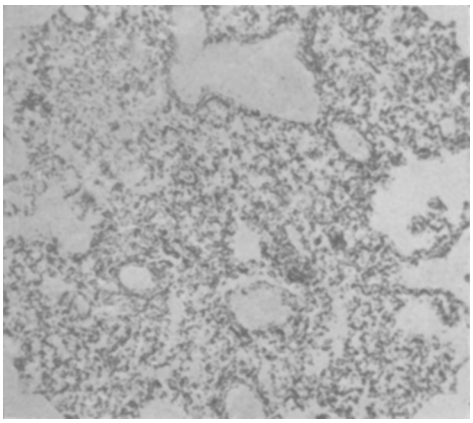

FIG. 1.
Diffuse septal mesenchymal cell infiltration in lung of a guinea pig; × 25.

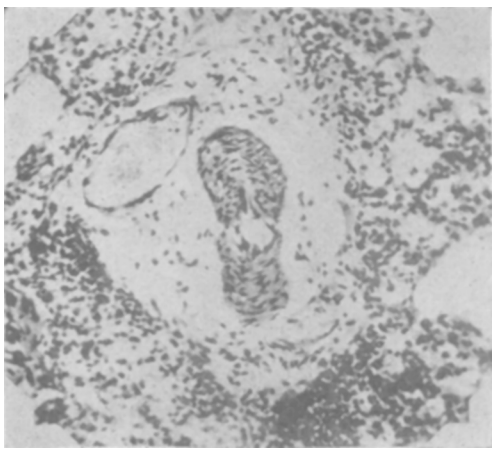

Fig. 2.
Pulmonary artery and vein displaying perivascular edema and mesenchymal reaction; × 125.

Discussion. The changes in Group I animals which died from lethal doses of urethane were not significantly greater than those in Group II which were killed in apparent health, to explain the death of the former on a morphologic basis. It is our opinion that death occurred through disturance of the respiratory mechanism as a result of the central hypnotic action of the drug and that the tissue changes in Group II were compatible with healthy life.

Vascular dilation and engorgement in the lungs, liver and spleen at times accompanied by mural changes were consistently seen. The frequent finding of areas of perivascular edema or hemorrhage without evidence of damage to vessel walls might indicate a reversibility of these mural changes. Vascular injury comparable to the mildest forms seen in the urethane treated animals were also observed in two of the saline treated control animals. Although urethane induced renal damage has been reported in specific strains of mice,[4,5] the glomerular apparatus in our guinea pigs remained essentially intact.

The hepatic changes compare with those previously reported by Doljanski and Rosin[6] in a study of urethane treated rats. The cellular damage consisted mainly of cytoplasmic vacuolization, a condition met with under a variety of pathological states. This condition has been produced by perfusion with 0.6 NaCl Solution[7] and Ringer's Solution[8] and vacuolization has been noted as early as 10 minutes after perfusion started. Raum[7] considered it a reversible phenomenon.

A striking reaction of cellular mesenchyma was consistently found in both groups of animals, most apparent in the lungs and spleen. A similar proliferation of mesenchy-

[4] Dunn, T. B., and Larsen, C. D., *Fed. Proc.*, Part II, 1946, **5**, 220.

[5] Kirschbaum, A., and Bell, E. T., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 71.

[6] Doljanski, L., and Rosin, A., *Am. J. Path.*, 1944, **20**, 945.

[7] Raum, J., *Arch. f. Exp. Path. u. Pharmakol.*, 1892, **29**, 353.

[8] von Skromlik, E., and Hunnerman, T., *Z. f. d. ges. exp. Med.*, 1920, **11**, 349.

mal cells is mentioned by Nettleship *et al.*[9] in urethane treated mice. Paterson *et al.*[3] reported fibrosis in the bone marrow, liver and spleen of urethane treated leukemia patients and the authors have noted excess fibroplasia in excised sections of urethane treated wounds.

It is conceivable that the induction of a degree of vascular dilation and excess fibroblastic proliferation would be beneficial in the treatment of healing wounds.

Although the evidence is insufficient to exclude a direct action of urethane on liver cells, it was productive of comparatively slight hepatic and renal damage even when given in lethal doses. These changes are apparently causally dependent upon vascular injury which is reversible.

Conclusions. (1) Doses of urethane simulating those used in the topical treatment of infected wounds in man are not productive of toxic parenchymal changes in the organs of guinea pigs subjected to prolonged daily subcutaneous injections. The animals remained healthy and gained weight.

(2) A marked mesenchymal cell reaction was consistently seen in the lungs and to a lesser extent in the liver and myocardium of urethane treated animals.

[9] Nettleship, A., Henshaw, P. S., and Meyer, H. L., *J. Nat. Cancer Inst.*, 1943, **4**, 309.

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17159. Anticonvulsant Action of 2-Substituted-1,3-Propanediols.

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3-(o-Toloxy)-1,2-propanediol, called myanesin, possesses two important pharmacological properties: it causes transient paralysis of skeletal muscles and has an anticonvulsant action.[1] The pharmacological examination of numerous related ethers of glycerol has shown that there was no simple relationship between their paralysing and anticonvulsant activities. The observation that nuclear substitution of 3-phenoxy-1,2-propanediol markedly affected paralysing activity but did not have any influence on anticonvulsant action[2] suggested the examination of the anticonvulsant properties of 2-substituted-1,3-propanediols.†

The anticonvulsant action of the compounds was determined in male, white mice, weighing 18 to 22 g. Metrazol 100 mg per kg, which caused convulsions and deaths in 99% of the animals, was injected intraperitoneally together with graded doses of the compound under test. Ten mice were used at each dose level. The animals were observed for 90 minutes following the injection and the incidence of convulsions and deaths noted. The relative efficacy of the substances was evaluated by finding graphically the dose protecting one half of the animals from convulsions and deaths. This mean protective dose of 13 experimental compounds and of phenobarbital and myanesin is given in Table I. Several of the investigated substances had anticonvulsant properties of a similar order as myanesin (No. 3, 6, 7, 9). Compounds containing butyl radicals (No. 4 and 8) were inactive. When a phenyl or phenoxy group was a substituent, activity was decreased or lost (No. 11, 12, 13). 2,2-Diethyl-1,3-propanediol, called DEP, possessed outstanding anticonvulsant properties. A short description of its pharmacological properties appears to be of interest.

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[1] Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, 1946, **1**, 265.

[2] Berger, F. M., *J. Pharm. and Exp. Therap.*, 1948, **93**, 470.

† I am obliged to Mr. W. A. Lott of the Squibb Institute for Medical Research for supplying me with some of these compounds.