of spread. However, it appears that adrenal extract so modifies the connective tissue ground substance as to accentuate the spreading. From the shape of Curve A, which demonstrates a definite alteration in the initial slow phase of diffusion, one may infer that the substrate (hyaluronic acid) has been so reduced in amount and/or modified in character that the substrate is no longer as sharply a limiting factor in the enzymatic activity as on the untreated side.

It has been shown previously(6) that irrespective of the enzyme concentration and/or the volume injected, all "normal" spreading activity is characterized by an initial slow phase followed by a more rapid phase of spreading. The observation in this study that indicator alone (Curve E) does not spread as rapidly in the ACE treated area as does the enzyme plus indicator emphasizes the fact that the result is not due entirely to the extreme thinning of the dermis(5), but that there has been some structural alteration in the ground substance which renders it more susceptible to hydrolysis by the enzyme.

The significant alteration in the slow phase of spreading in Curve B suggests that there is a concurrent systemic effect on the ground substance even though the treatment always had been localized. This alteration lends further support to the contention that the action of ACE is primarily on the slow phase since no significant difference from the alcohol controls (Curves D and C) is apparent in the rapid phase.

From the foregoing conclusions, one may assume that the difference in the slow phase of spreading in the ACE-treated skin may be characteristic of the alteration in the ground substance; the exact nature of this change is as yet unknown. These observations raise the possibility that prolonged action of adrenal cortical compounds on connective tissue ground substance might be a factor in the etiology of the collagen diseases.

Summary. An accentuation of hyaluronidase activity was observed in skin which had been treated locally wtih adrenal cortical extract for 4 months. This effect was the result of a probable alteration in the connective tissue ground substance.

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Effect of Antibiotics on Mortality from Internal Radiation.* (18199)

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Rats dead of P^{32} poisoning commonly show bacterial lesions at autopsy. Because of the possibility that bacterial toxemia might play a rôle in radiation death, an investigation was made of the protective effect of antibiotics.

Methods. A total of 248 white male rats of the C. F. Wistar strain weighing about 175 g each were evenly divided into 124 treated rats and 124 untreated controls. The animals were kept in individual cages in an air-conditioned unit permitting constant temperature and humidity. Radioactive phosphorus was injected intraperitoneally in amounts ranging from 3 to 6 μc/g body weight. Streptomycin and penicillin were employed in combination, since the tissue lesions showed both gram negative bacilli and gram positive cocci. The doses were 12 mg of streptomycin twice daily and 20,000 units of penicillin every other day and these were given intramuscularly in the hind extremities. Of the 124 rats which received antibiotics, 49 were pretreated for periods ranging from 3 to 10 days, while in 75 treatment was begun immediately after injection of the P^{32}. In all instances the antibiotics were discontinued

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TABLE I. Mortality in Treated and Control Rats.

Exp.	No. of rats	Dose of P^{32} (μc/g)	LD	Type	% mortality 10 days	15 days	21 days	30 days
1	30	3.0	27	Treated	0	0	0	0
				Control	0	20	27	27
2	30	3.8	47	Treated	0	0	7	13
				Control	7	20	33	47
3	29	3.5	67	Pretreated 5 days	0	0	14	14
				Control	0	36	43	67
4	30	5.5	67	Treated	0	27	47	47
				Control	33	53	67	67
5	30	5.0	73	Treated	0	13	27	33
				Control	0	40	47	73
6	16	4.5	75	Pretreated 2 wk	0	0	50	50
				Control	0	38	38	75
7	30	6.0	80	Treated	0	53	53	60
				Control	7	53	80	80
8	30	4.5	100	Pretreated 5 days	20	73	73	100
				Control	66	100		
9	23	4.5	100	Pretreated 3 days	0	14	14	33
				Control	7	60	100	

3 weeks after the P^{32} was given, since by this time the hemopoietic tissues are in the process of recovery. Varying amounts of the P^{32} were used in order to compare the effects of antibiotics over a fairly wide range of the mortality curve. However, in keeping with past experience, a perfect correlation between mortality and dosage was not obtained. On two occasions for example, injection of 4.5 μc of P^{32} per gram body weight gave an LD_{100}, while in other experiments with 5.0, 5.5 and 6.0 μc per gram, the respective LD figures were 73, 67, and 80.

Results. 1. *Mortality.* Table I gives the dose of P^{32} and the per cent mortality at 10, 15, 21 and 30 days. In all but one experiment the mortality at 30 days was lower for the treated animals than for the controls. The difference was marked in 4 experimental groups, *i.e.*, 14 as against 67%, 33 and 73, 13 and 47, and 33 as against 100. In 4 additional groups a protective effect was evident but less marked, the per cent mortality for treated and untreated rats being respectively 47 and 67, 50 and 75, 60 and 80, and 0 and 27. In one experiment the mortality for both treated and control animals was 100%. The overall figures were 83 dead out of 124 untreated rats (67%) as compared to 45 dead out of 124 treated rats (36%). Fig. 1 shows the mortality for one experimental group and Fig. 2 gives the total mortality at intervals up to 30 days.

There was no significant difference in per cent mortality for animals pretreated with antibiotics and those treated immediately after injection of the radioactive material. The data indicate that prolonged use of the antibiotics would not have given a significantly better survival rate. All animals which recovered were observed for at least 2 months and usually for a period of several months. Eight rats, 4 treated and 4 controls, died after 30 days, *i.e.*, between 5 and 7 weeks.

2. *Survival.* The control rats began to die earlier than those treated with antibiotics. This is shown in Fig. 2 which indicates a lag in mortality for treated rats at periods of from 10 to 30 days. However, the difference in average survival time (30 days) for all untreated and treated animals dead is not impressive, *i.e.*, 15 as compared to 19 days. This is probably due in part to compression of the survival curves in the higher radiation dose groups.

3. *Morbidity.* In general loss of weight was less marked in treated than in control rats. The treated animals consumed more food and water, showed greater activity, and had distinctly less diarrhea and bleeding than the control rats.

Discussion. The mechanism of radiation death has not been established. In instances where fatal outcome occurs within a few weeks rather than days, bacterial toxemia may well be a significant factor. Chrom(2) and Lawrence and Tennant(3) emphasized the frequency in experimental radiation death of infection by organisms of the colon-aerogenes group. Bacteremia was practically constant. Miller *et al.*(4) found a high incidence of bacteremia among mice receiving total

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2. Chrom, S. A., *Acta radiol.*, 1935, v16, 641.

3. Lawrence, J. H., and Tennant, R., ***J. Exp. Med.***, 1937, v66, 667.

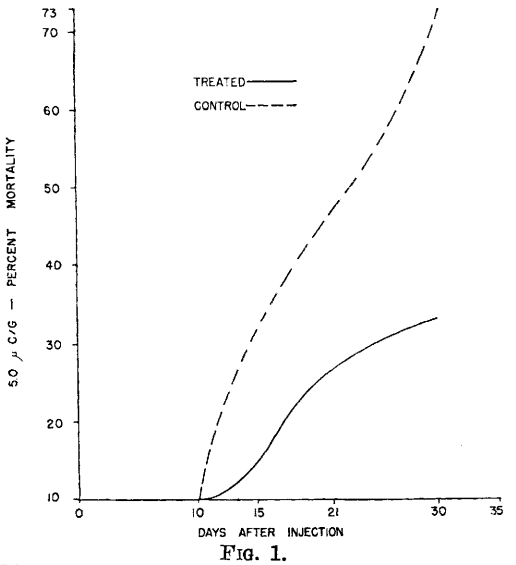

FIG. 1.
Mortality in rats receiving 5.0 μc P32/g body wt.

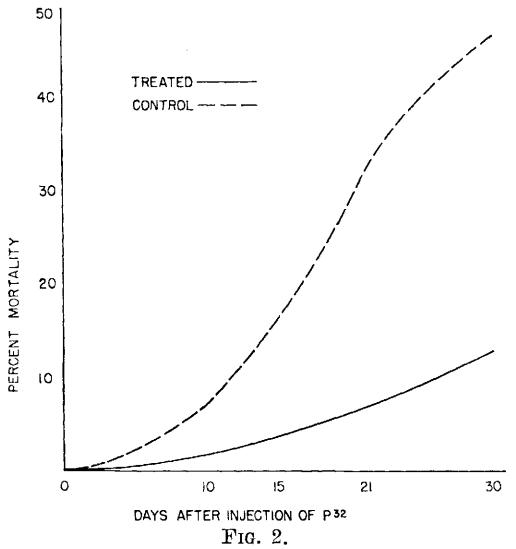

FIG. 2.
Total mortality in rats receiving 3 to 6 μc P32/g body wt.

body x-irradiation of 600 or 450 r. The frequency was greatest during the period of highest mortality.

Some experimental data are available on the therapeutic effect of antibiotics in radiation injury. Penicillin was used in goats exposed to radiation during the air atomic bomb explosion at Bikini. Since no controls were available, definite conclusions could not be drawn but the data suggested that penicillin might be of value(5). Cronkite(6) treated 16 dogs with aureomycin 250 mg qid and procaine penicillin 300,000 units intramuscularly daily, beginning immediately after irradiation with 600 r. The survival time was somewhat longer but the mortality was 100% in the control and treated groups. Howland and associates(7) found that aureomycin caused almost complete alleviation of the diarrheal state in rats and dogs given lethal doses (LD_{90} to LD_{100}) of x-irradiation. Survival of life was prolonged an additional 5 to 7 days. Furth and Coulter(8) gave aureomycin to 5 dogs which received an LD_{90} (450 r) of whole body radiation. The mortality 4 months postradiation was 40% as compared to 80% for 5 control animals. Miller *et al.* (9) reported that treatment with streptomycin alone or combined with penicillin caused a significant reduction in the mortality (30 days) of mice following total body x-irradiation with 450 r.

Our data indicate that antibiotics are beneficial in the treatment of rats injured by internal radiation. The results even suggest that such therapy may be of value with radiation doses in the high lethal range (LD_{70}-LD_{100}). Further experimental investigation appears warranted. Several items need to be clarified, *i.e.*, the relative merits of various preparations, especially in combination, the most advantageous portal of entry, the effect of altering the schedule of administration, the emergence of antibiotic resistant organisms.

It should be noted that antibiotics do not alter the course of radiation injury. Damage to sensitive tissues and organs and subsequent repair are similar in treated and untreated

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6. Cronkite, E. P., Personal communication.

7. Howland, J. W., Furth, F., Bennett, L. R., Coulter, M., McDonnel, G. M, 1949, UR-94, The University of Rochester.

8. Furth, F., and Coulter, M., 1950, UR-116, University of Rochester.

9. Miller, C. P., Hammond, C. W., and Tomplins, M., *Science*, 1950, v111, 719.

rats. Moreover, treated animals which recover are equally prone to develop long term radiation effects such as the development of osteogenic sarcoma(10).

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Summary. Streptomycin and penicillin in combination proved effective in reducing the mortality of rats given lethal doses of radioactive phosphorus. Morbidity was also reduced and survival time prolonged.

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Treatment of Pernicious Anemia with Crystalline Vitamin B_{12}.* (18200)

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Since the initial report by West(1) it has been suggested that one microgram of vit. B_{12} is approximately the equivalent of one U.S.P. unit of liver extract when administered parenterally to patients with pernicious anemia in relapse(2,3). Jones, Darby and Totter found that the daily intramuscular administration of 1 μg of vit. B_{12} for 22 days was followed by an excellent reticulocyte response and a satisfactory rise in hemoglobin and erythrocytes during the period of treatment (4). More recently, treatment of 30 patients with pernicious anemia in remission with vit. B_{12} concentrate in doses of one microgram daily by intramuscular injection every 3 to 4 weeks, failed to maintain optimal hematologic levels in 20 of the cases(5).

In the present study 8 persons with typical Addisonian pernicious anemia were divided into 2 groups. Four patients were treated with 1 μg of crystalline vit. B_{12} intramuscularly, daily, for 10 days, and thereafter with 14 μg every 14 days. The second group received 2 μg of vit. B_{12} intramuscularly, daily, for 10 days followed by 14 μg every week. All patients showed hyperchromic macrocytic anemia, histamine fast achlorhydria and megaloblastic bone marrow. X-ray examinations of the gastrointestinal tract, urinalyses, and blood chemistry determinations were all normal.

Results. A summary of the data is presented in Table I. The patients in the group receiving 1 μg of crystalline vit. B_{12} intramuscularly, daily, were observed for periods ranging from 53 to 108 days. The reticulocyte response in all instances was suboptimal. In only one case did the hemoglobin and erythrocytes reach normal levels, and this occurred after 108 days. The remaining 3 persons maintained stationary submaximal levels for 23 to 59 days. Patients G. and P. reached normal levels when treated with doses of liver extract which were greater than the equivalent of one U.S.P. unit a day (30 units a week for 2 months). The fourth patient, V. H., failed to report for liver extract therapy. All of the patients showed satisfactory clinical improvement as evidenced by increase in appetite, sense of well-being and gain in weight. Only one patient, V. H., showed signs of neurologic disease at the onset of treatment, and no change was observed after 93 days. The 4 patients receiving 2 μg of crystalline vit. B_{12}, intramuscularly, daily, were observed for periods varying from 48 to 250 days. The reticulo-

* Expenses for these studies were defrayed by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

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