protein, and in two instances with histone. Isolated calf thymus, salmon sperm, human leukocyte and pneumococcal DNA also fixed complement with many of these sera. Similar reactions were not encountered in a limited control series including normal individuals and other pathological states. 2. Most active L.E. sera fixed complement with both nuclei and DNA in roughly parallel titer. However, exceptions were encountered and one serum reacted strongly with nuclei but failed to react with DNA. Cross-absorption experiments with nuclei and DNA suggested the presence of 2 distinct serum factors. 3. The L.E. factor appeared to be related to the factor responsible for complement fixation with nuclei but distinct from that responsible for DNA fixation. 4. The significance of these findings with respect to antibodies against nuclear constituents is discussed.

Addendum: Recent reports by Miescher (*Vox Sanguinis,* 1957, v2, 283) and Seligmann (*Comptes Rendus,* 1957, v245, 1472) also have presented evidence for the presence of antibodies to DNA in lupus erythematosus serum.

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Beneficial Effect of Quinoxaline 1,4-di-N-Oxide in Radiation Injury in Mice.* (23546)

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One of the most prominent features of radiation syndrome in mice is a pronounced bacteremia which appears during the first post-irradiation week and materially contributes to the overall mortality. The type of organisms involved seems to vary with the particular colony of mice used but *Pseudomonas pyocyaneus, Proteus vulgaris, Salmonellae* and various coliform organisms are usually involved. Coulthard and Hale(1) recently showed that quinoxaline-1,4-di-N-oxide was effective in counteracting infection by these organisms in mice. Upon this basis the present investigation was undertaken and the results obtained indicate that, although the drug is not 100% effective, it does materially decrease irradiation bacteremia and total mortality in mice.

Methods. Four hundred male, CF 1 mice, weighing an average of 25 g, were arranged in groups of 20 animals each according to the design given in Table I. The quinoxaline-1, 4-di-N-oxide was administered intramuscularly or orally as an acacia suspension in normal saline. Control mice received similar doses of acacia in saline. Four additional groups of 32 animals each were used to determine the effects of the drug on the course of radiation bacteremia and mortality. Eight animals each from medicated and non-medicated groups were sacrificed on post-irradiation days 5, 8, 11 and 14 and the bacterial content of the liver was determined using direct dilutions on Tryptose (Difco) agar. Pure colonies were isolated and identified. The entire small intestine was similarly treated using

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TABLE I. Effect of Oral Quinoxaline-1,4-di-N-Oxide on Radiation Mortality in Mice.

Medication	ST_{50} and range, days	Slope and range	Final mortality %	Final mortality Days
Saline control; day pre-R 3,2,1 *Radiated*	6.4 (5.81– 7.08)	1.25 (1.17–1.34)	100	9
Q 125 mg/kg/day; day pre-R 3,2,1	8.4 (7.5 – 9.41)	1.27 (1.18–1.37)	100	17
Q 250 " ; *idem*			50	68
Saline control; day post-R 1,2,3	7.5 (6.72– 8.37)	1.29 (1.19–1.40)	100	18
Q 125 mg/kg/day; day post-R 1,2,3	9.0 (7.81–10.38)	1.39 (1.26–1.54)	100	16
Q 250 " ; *idem*	7.2 (5.98– 8.68)	1.54 (1.35–1.76)	100	17
Saline control; day post-R 6,7,8	8.5 (7.66– 9.44)	1.27 (1.18–1.37)	100	15
Q 125 mg/kg/day; day post-R 6,7,8	7.9 (7.34– 8.51)	1.19 (1.13–1.26)	100	12
Q 250 " ; *idem*	9 (8.11– 9.99)	1.27 (1.18–1.37)	100	13
Q control; 250 mg/kg/day for 3 days *Non-radiated*	—	—	—	—

ST_{50} = Day on which 50% of animals alive. All values at P, 0.05; 20 animals/group.
Q = Quinoxaline-1,4-di-N-Oxide. Pre-R = Pre-irradiation. Post-R = Post-irradiation.

Liver-Veal agar and Nutrient agar. Total leukocyte and differential counts were performed on 190 additional mice following the drug and/or irradiation. Except during irradiation the animals were maintained in an air-conditioned room at 72 ± 5°F and were fed a diet of Rockland pellets supplemented weekly with additional vit. A and D. The 550 r radiation dose was administered from above and below the mice, with two 250 KVP Picker Industrial Units operating simultaneously. The technical factors were: 250 KVP; 15 ma; FOD 100 cm; filters 0.21 mm Cu inherent, 0.5 mm Cu parabolic and 1 mm AL; HVL 2.02; size of field—total body; r per min. measured in air 17.3 to 18.05. Both units were calibrated before and after each experiment with a Victoreen thimble r-meter. Animals were restrained in a plastic cage similar to the one described for guinea pigs(2). Results of mortality studies were analyzed statistically by the Litchfield method(3).

Results. Table I gives the results of the oral experiment. These data indicate a significant increase in the ST_{50} day in those groups premedicated with either 125 or 250 mg/kg of the drug. With the 250 mg/kg dose there was a marked reduction in total mortality. Post-irradiation medication does not appear to be effective. Similar results were obtained in the first intramuscular experiment in which the total survival for both doses was 35 and 30%/30 days, respectively. Beneficial results were obtained with pre-medication, 250 mg/kg in the second intramuscular experiment using 2 groups of 32 animals each. The ST_{50} day and its range was, controls 10.3 (9.5-11.2) and medicated 20 (16.4-24.4). Total mortality was, controls 100%/14 days and medicated 58%/118 days. The beneficial effects of quinoxaline-1,4-di-N-oxide were reflected in the overall appearance of mice because they showed none of the external signs of radiation injury—rough coat, pale skin and tail, diarrhea, watery eyes and lethargy—exhibited by the controls.

Table II gives the results obtained after culturing liver homogenates of irradiated control and medicated mice. The bacterial counts of livers admittedly measured a greater concentration of bacteria than would have been obtained from heart's blood, and in the later stages of the syndrome probably included organisms from infectional foci. However, all of these sources may be considered

TABLE II. Effect of Quinoxaline-1,4-di-N-Oxide upon Bacteremia in Irradiated Mice.

Post-irrad. sampling day	Bacterial count × 10^3 Control	Bacterial count × 10^3 Medicated
5	25 (2 bfL)	.07 (3 bfL)
8	29	1.5 (1 bfL)
11	441	1.2
14	1584*	.12† (2 bfL)

* Mean of 6 surviving mice.
† Without elimination of 2 of 8 mice having high bacteria counts, the mean would be 100 × 10^3.
bfL—No. of bacteria-free livers.

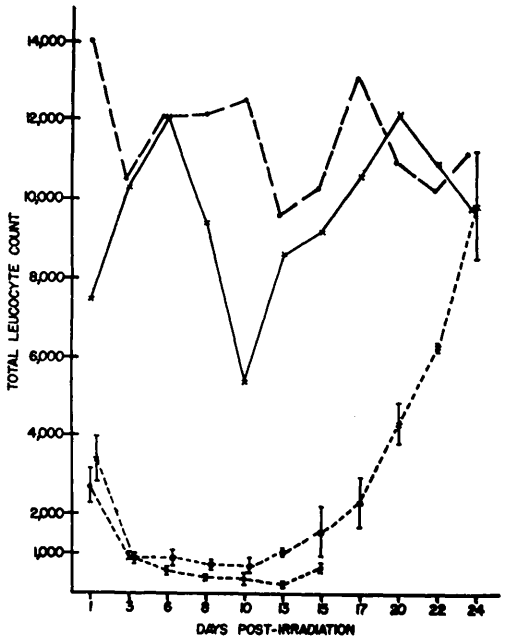

FIG. 1. Effect of irradiation and/or medication on total leukocyte count. Control non-irradiated ----; quinoxaline non-irradiated X——X; control irradiated —•—•—; quinoxaline irradiated X----X. Stand. deviations were from 400 to 2100 cells for non-medicated controls, and from 200 to 2000 cells for medicated controls.

to reflect state of infection in the irradiated mouse. The principal invaders in the mouse bacteremia were found to be lactobacilli, coliforms, pseudomonads and streptococci. A saccharomyces-like yeast was discovered as a surprising component of several bacteremic samples.

Because post-irradiation bacteremia has been demonstrated to be largely of enteric origin(4-6), the effect of the drug on small intestine flora was determined using individual plate counts of homogenized entire small intestine from normal animals. The mean bacterial counts from 2 groups of 8 mice each were: control 260.5×10^6 and medicated 15.5×10^6. The order of prevalence of intestinal organisms was as follows: lactobacilli, yeasts of dietary origin, coliforms, streptococci and pseudomonads. Their predominance in mouse bacteremia furnished further evidence of relationship of intestinal flora to irradiation bacteremia and their repression in the small intestine may have furnished part of the chemotherapeutic mechanism accounting for increased survival of irradiated medicated mice. The antibacterial effect of quinoxaline-1-4-di-N-oxide does not appear to be the entire mechanism involved in increasing survival of irradiated mice following 3 days pre-irradiation medication. Comparison of counts of high and low dilutions of liver and intestine homogenates indicated that there were no residues of the drug in the agar plates to influence the growth of bacterial colonies. Thus it appears unlikely that enough of the drug remained in the liver during sampling days to account for the increased survival. However, suppression of enteric organisms would materially decrease the extent of post-irradiation bacteremia thus increasing chances of survival.

The irradiation suppression of the bone marrow of both medicated and non-medicated mice (Fig. 1), indicates that the drug did not influence this phase of the radiation syndrome. However, even during the period of greatest bone marrow depression, the medicated animals always had twice as many circulating leukocytes as the controls. The return to normal leukocyte values in the medicated group follows the pattern described by Lawrence, Dowdy and Valentine(4). The differential counts in all cases reflected the usual picture of radiation injury followed by repair. From the control data in Fig. 1 it appears that quinoxaline-1,4-di-N-oxide caused a preliminary stimulation of the bone marrow followed by a depression then a second stimulation. However, daily leukocyte counts in 10 medicated animals indicated that such fluctuations were artifacts because there were no significant differences in leukocyte values over the control values during the medication days and 2 days post-medication. Moreover, the variation in total leukocytes in the control and medicated non-irradiated mice were within the limits reported by Gardner(5).

Discussion. Quinoxaline-1,4-di-N-oxide is one of the few drugs other than certain antibiotics or sulfhydryl compounds which has a beneficial effect on radiation injury in mice. The data reported indicate that the main beneficial action of the drug is related to its ability to decrease the number of bacteria in the intestinal tract. In this aspect the drug

differs from streptomycin in that it is effective when given prior to and not after irradiation when infection begins. Perhaps the drug also exerts a non-specific beneficial effect such as Treadwell *et al.*(8) demonstrated for estradiol benzoate.

Intestinal lactobacilli formed a formidable component of mouse bacteremia and appeared to have pathological significance in the radiation syndrome in this species. This mouse infection differs from that seen in the rat by Vincent *et al.*(9) where bacteremia lactobacilli counts were relatively low and did not correlate with severe irradiation effects. It is also true that lactobacilli were insignificant or missing from surveys of mouse irradiation bacteremias made by Miller *et al.*(7) and Gonshery *et al.*(10). Differences in lactobacilli concentrations between the present work and that of others(7,9,10) may have been due, in part, to differences in sampling and cultural procedures. Our methods, including homogenization of the liver and culture of the sample in poured, deep-agar plates, favored growth of microaerophilic lactobacilli. Differences in laboratory diet could also have been involved.

Summary. It has been shown that premedication with Quinoxaline-1,4-di-N-oxide is beneficial to irradiated mice, significantly increasing both the ST_{50} day and total number of survivors. The effect has been related to a significant reduction in number of enteric organisms causing the usual irradiation bacteremia. It has been demonstrated that lactobacilli were the predominating invaders under conditions of our experiments. The drug does not appear to have an effect on bone marrow.

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Cardiovascular Responses of Unanesthetized Rats During Traumatic and Endotoxin Shock.* (23547)

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The available information on the direct measurement of arterial pressures in the unanesthetized rat is scant(1,2), and no data have been found where such studies were made during shock from trauma. Chambers *et al.*(3) determined the apparent systolic pressures of unanesthetized rats before and after physical trauma by an indirect method, but they made no systematic presentation of the data.

Thomas(4) recently pointed out the marked similarities of symptoms and pathological changes reported to occur after endotoxin intoxication and traumatic shock, and Schweinburg *et al.*(5) have suggested endotoxins as a factor in shock. It is the purpose of the present investigation to obtain com-

* Dr. David Tennent prepared the sample of endotoxin used.