per 10^{10} trypanosomes. Even higher degrees of binding have been reported[6,12] which are not commensurate with the quantity of sulfhydryl found by us. This might be construed as evidence for binding as a monothioarsenite or by some other means not involving the sulfhydryl group. However, it is more likely that the discrepancy may be attributed to conversion of disulfide to sulfhydryl during exposure or to a sulfhydryl level in the living trypanosome higher than that found in this investigation. The presence of substrate is important to the degree of binding,[7] and it is not unlikely that during centrifugation the closely packed trypanosomes suffer a relative glucose deficiency, which would yield low values of reduced sulfhydryl. That oxidation during the preparation of the sample was not grossly responsible for low values was tested by comparison with samples of wet protein whose dry weight equivalent was reasonably established by count.

Summary. Normal and arsenic-resistant strains of *T. equiperdum* do not differ significantly with respect to their sulfhydryl content, but the former shows a considerable surplus of disulfide groups. The sulfhydryl content of normal *T. hippicum* is not appreciably different from that of an arsenic-resistant strain, but the disulfide content of the arsenic-resistant strain is higher than that of the normal strain. It is concluded that these differences are not the cause of the resistance but may be rather the result of some cytological reconstitution which probably also gives rise to resistance. The sulfhydryl content is found to be of the same order as the degree of arsenic binding.

11 Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. intern. pharmacodynamie*, 1932, **43**, 186.

12 Eagle, H., *J. Pharm. and Exp. Therap.*, 1945, **85**, 265.

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Agents Influencing Experimental Radiation Injury. Effects of Folic Acid and Pyridoxine.

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In a previous communication by one of us,[1] it was noted that a high mortality rate occurred among tumor bearing mice which were treated with X-rays for therapeutic purposes. The survival time even of successfully treated animals was short. It has been assumed that the animals might have succumbed due to either the absorption of the disintegrated tumor, or to same toxic substances produced by secondary, or scattered, radiation. The latter assumption was based on observations made from earlier experiments using the tissue culture technique.[2] Thus it was noticed that tissue fragments remaining in the medium in which they were irradiated needed less dosage for preventing their growth *in vitro*, than those which were washed in Tyrode solution and transferred to a fresh non-irradiated medium. It was concluded that some toxic substances might have been produced in the culture medium by radiation. Similar observations were made by other investigators.[3,4] The so-called "radiation sickness" occurring among human patients treated with X-rays may also be the result

1 Goldfeder, Anna, *Radiology*, 1945, **44**, 283.

2 Goldfeder, Anna, *Radiology*, 1938, **31**, 73; 1940, **35**, 210.

3 Luria, S. E., and Exner, F. M., *Proc. Nat. Acad. Sci.*, 1941, **27**, 370.

4 Evans, T. C., Slaughter, J. C., Little, E. P., and Failla, G., *Radiology*, 1942, **39**, 663.

of the action of similar toxic substances produced by ionizing radiation.

Attempts are being made to remove or counteract the undesirable effects produced by radiation. This report deals with such an attempt.

Materials and Methods. White mice, Swiss strain inbred, weighing between 20 and 22 g were used. These were obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine. They were kept in metal cages and fed with Purina dog chow and water *ad libitum* for about 2 weeks prior to radiation, and their appearance, weight, and blood levels were noted.

It was necessary, first of all, to devise a procedure whereby the experimental object would receive a most uniform dose of radiation, and which would permit *reproducible* results within the least limits of error. It is known that biologic effects of radiation do not depend upon the physical calculated dose delivered, but upon the amount of radiation absorbed by the exposed object. Where the biologic object is very small, such as a drosophila egg or a tissue fragment, the amount of radiation delivered is equal to the amount absorbed. When, however, it concerns a larger object, the amount of radiation absorbed depends upon the size and shape of the object and upon the secondary radiation produced by back-scatter. To simplify matters a device was used in which the X-ray beam hits a single mouse placed in a separate compartment, thus permitting uniform radiation over the entire body surface. This procedure differs from those previously used by other investigators for similar experiments, in which batches of mice were exposed.[5,6,7] The procedure consists of the following:

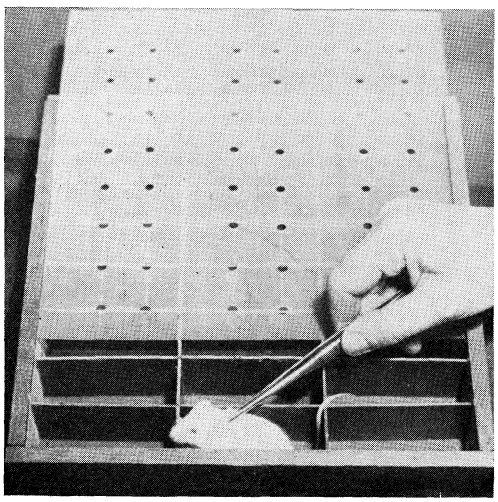

FIG. 1.
Wooden box with 24 compartments and perforated plastic cover in which mice were irradiated.

A square wooden box of white pine, 24x24 cm in size and 4 cm in depth, was divided by plastic partitions into 24 compartments of identical size and shape (7.5 cm in length, 2.7 cm in width, and 4 cm in depth). The box can be closed by a cellulose acetate cover in which small air holes of about 5 mm in diameter were made. One mouse was placed in each compartment and the cover was gradually slipped over the mice in the box (Fig. 1).

This box was placed directly in the useful beam of the X-ray tube at a distance of 80 cm. The distance from the target of the X-ray tube to the center of the body of the middle mouse was 82 cm, which was considered the target-tissue distance for the purpose of this experiment. A mouse at the corner of the box is necessarily at a slightly greater distance from the target, approximately 84 cm, but it receives only about 4% less radiation than the central animal. This degree of uniformity is satisfactory for the present purpose. The cellulose cover absorbs 2½% of the incident radiation, and this was taken into account in computing dosage.

The physical factors used were: 200 KV peak pulsating potential generator; 20 ma.; ½ mm Cu plus 1 mm Al filtration; half value layer 0.9 mm Cu; field 25x25 cm uniform at 82 cm target-skin distance. A dosage rate of 14 r/min. measured in air was obtained under the above conditions. The physical measurements were made by placing the ionization chamber through a hole in the center of one of the side walls of the box,

[5] Henshaw, Paul S., *J. Nat. Cancer Inst.*, 1944, **4**, 477.

[6] Ellinger, F., *Radiology*, 1945, **44**, 125.

[7] Quastler, H., *The Am. J. Roent. and Radium Ther.*, 1945, **51**, 449.

Fig. 1. The ionization chamber was introduced parallel to the bottom of the box, perpendicular to the side, and was located in the center of the box. The measurements were taken with and without mice in the box. The difference between these measurements was approximately 2% more with the mice in the box. This figure is so small that it precludes any significant effect from scatter.

Doses were applied in single exposures, and based on measurements made in air.

Blood samples were obtained by the following technical procedure, which permitted the use of minimum amounts of blood without loss of accuracy. Short lengths of capillary tubing were calibrated with mercury to contain 5 cu mm. For taking blood samples the mouse was placed in a special device (Fig. 2). The tail of the mouse was first cleansed with alcohol and then wiped dry with cotton. The tip of the tail was snipped off with a pair of sharp scissors and the initial drop of blood wiped away. The tail was then gently massaged until a good sized drop accumulated and the capillary was placed in contact with the drop of blood. When full, the capillary tube was dropped into a small test tube containing 0.25 cc of a 1% acetic acid solution. This was gently shaken until a homogeneous suspension of the blood cells and the diluting fluid was obtained. A smear for a differential count was taken and the tail wiped dry. Lastly, a drop of collodion was applied to the tip of the tail to stop any further bleeding.

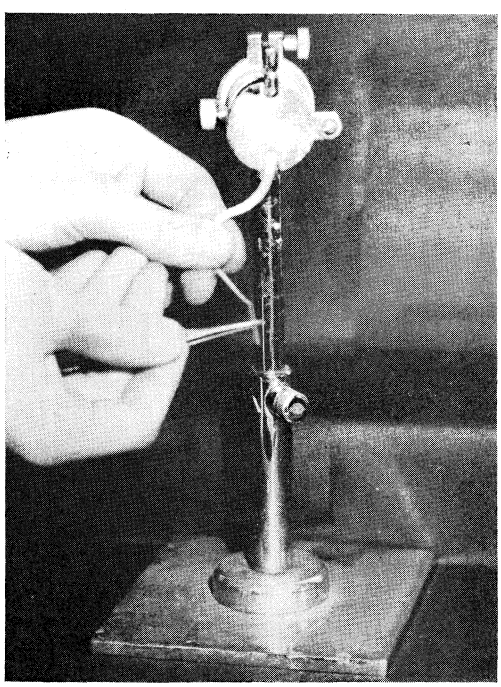

FIG. 2.
Mouse holder, and 5 cu mm capillary tube for blood samples.

The blood counts were done in the regular manner with the Bright-Line Improved Neubauer blood counting chamber, using the dilution factor of 500 to give the number of white blood cells in 1 cu mm of blood.

Pyridoxine and folic acid were chosen as the first test agents for the following reasons: The former has been widely used for some time clinically and shown to have a beneficial effect in preventing nausea of X-ray treated cases;[8,9,10] (the mechanism of its action is still obscure). The folic acid (*L. casei* factor) proved to have a beneficial effect in cases of sprue,[11,12] and in some degree on leucopenia. Since diarrhea and leucopenia are also symptoms of radiation sickness, it was of interest to test the efficacy of folic acid in these respects.

In evaluating the effects of irradiation produced by a given dosage, the following criteria have been used: (1) weight of the animals and their general appearance, (2) peripheral blood levels, (3) survival time in days following irradiation, and (4) morphological appearance of liver and spleen.

The observations made on W.B.C. counts and survival time of the irradiated, medicated, and non-medicated mice will be reported here.

Results and Discussion. The average white blood count of the control, non-ir-

8 Scott, L. O., and Tarleton, G. J., *Radiology*, 1946, **47**, 386.

9 Maxfield, J. R., McIlevain, A. J., and Robertson, J. W., *Radiology*, 1943, **41**, 383.

10 Shervon, L. M., *Brit. J. Radiology*, 1946, **19**, 369.

11 Watson, C. J., Serbell, W. H., Kelevey, J. L., and Daft, F. S., *Am. J. Med. Sci.*, 1945, **210**, 463.

12 Hernadez, R. L., and Spies, T. D., *Am. J. Radiology and Radium Therapy*, 1946, **56**, 337.

TABLE I.
Effect of 200 r and 500 r on Survival Time and W.B.C. Count of White Mice.

	200 r	500 r
Lowest avg W.B.C. count	8000	1000
Duration of falling W.B.C.	2 days	10 days
Period of recovery	5 "	38 "
Deaths in leucopenic phase	0%	45%
Occurrence of first death	28 days	8 days
Period of 50% mortality	39 "	21 "

TABLE II.
Effect of Folic Acid on the Survival Time and W.B.C. Count of Mice Irradiated with 350 r.

	Medicated mice		
	Oral folic acid	Intramuscular folic acid	Control mice
Lowest avg W.B.C.	3,000	2,000	2,000
Duration of falling W.B.C. count	4 days	3 days	9 days
Period of recovery (W.B.C. normal avg 15,000)	22 "	25 "	23 "
Deaths in leucopenic phase	0%	2%	26%
Occurrence of first death	30 days	24 days	13 days
Period of 50% mortality	53 "	100 "	43 "

radiated mice used in these experiments was found to lie within 15,000-19,000. In order to ascertain the amount of radiation giving the most clear-cut results, preliminary experiments were carried out. With less than 200 r no consistent changes in blood count or any definite diminution in survival time within 4-6 weeks following radiation could be noted.

A group of 20 mice giving an average W.B.C. count of 17,000 was exposed to 200 r; the W.B.C. count fell to 8,000 on the second day following irradiation, and returned to normal levels (taking the average of 15,000 W.B.C. for the mouse) on the 5th day. None of the animals died in the leucopenic phase; the first deaths occurring on the 28th day after radiation; 50% had died on the 39th day. Table I summarizes the results.

A dose of 500 r applied to a group of 20 mice resulted in severe roentgen injury. The W.B.C. counts fell to very low levels. The leucopenia persisted longer than after a dose of 200 r, and resulted in a considerable proportion of deaths during this phase. The mice commenced to die at the end of the first week, and the 50% mortality fell on the 21st day. Table I summarizes the results.

It was considered that the acute trauma of this large dose resulted in dissolution of the experimental animals too soon for any possible benefit from medications to become apparent; for this reason an intermediate dose of 350 r was accepted as a standard for this investigation.

*Experiment with Folic Acid.** Four groups, 12 mice in each, were exposed to 350 r. As it was noted from preliminary experiments, 350 r is apparently sufficient to produce a sharp reaction with marked leucopenia during which a few animals die; most of the irradiated animals, however, survive long enough to show the more chronic radiation injury, and the effect of drugs on their survival.

One group was given 20 γ of folic acid orally daily following irradiation; another was given 15 γ of folic acid by intramuscular injection; and along with each of these treated groups a control group of animals was irradiated but no medication given.

In Table II are recorded the average results obtained from the experiments with folic acid.

The daily injections of 15 γ of folic acid for a period of 22 days (7 days prior to radiation and 15 days after radiation), each mouse

* Dr. Stanton M. Hardy, Medical Director of Lederle Laboratories, supplied the synthetic folic acid, under the commercial name, "Folvite." This is equivalent to 15 mg/cc of folic acid. This was diluted with saline solution to make 15 gammas in 0.1 cc for intramuscular injections.

TABLE III.
Effect of Pyridoxine on the Survival Time and W.B.C. Count of Mice Irradiated with 350 r.

	Medicated mice		
	Pyridoxine pre-radiation	Pyridoxine post-rad.	Control mice
Lowest avg. W.B.C. count	1800	2000	2500
Duration of falling W.B.C. count	6 days	4 days	10 days
Period of recovery	14 "	25 "	22 "
Deaths in leucopenic phase	17%	60%	85%
Occurrence of first death	9 days	2 days	3 days
Period of 50% mortality	100 "	16 "	14 "

thus receiving a total of 330 γ, greatly prolonged the average survival time of irradiated mice. This prolongation was evidenced by the fact that no animals died after the 25th post-radiation day. This drug had no obvious effect on the hematologic response, although it decreased the mortality rate in the leucopenic phase and afforded significant protection from delayed X-ray death after recovery of the leucopoietic system. This effect, however, was not manifest when folic acid was administered orally.

Experiment with Pyridoxine Hydrochloride. Pyridoxine hydrochloride (Squibb, for parenteral use), was used as a protective agent in 3 groups of 12 mice each, irradiated with 350 r. In one group, 50 γ of the drug were administered by intramuscular injection for 7 days prior to irradiation, and 13 days after irradiation, each mouse thus receiving a total of 1,000 γ; in a second group administration was begun after irradiation, and the third group was used as irradiated unmedicated control. The average results obtained from this experiment are summarized in Table III.

Analysis of the data recorded in Table III reveals that pyridoxine hydrochloride greatly prolongs the average survival time of irradiated mice. The protective effect is not produced unless the drug is administered before irradiation. This is evidenced in the 50% mortality rate occurring within 100 days, as compared to 16 days for the post-radiation treated mice, and that of 14 days for control irradiated, unmedicated mice. Pyridoxine does not appear to influence the onset, severity, or duration of the leucopenia, but gives a definite protection against the delayed roentgen injury, as no animals died after recovery of the leucopoietic system. In this respect, pyridoxine hydrochloride resembles folic acid.

An average loss of weight of 1 to 2 g per irradiated mouse, experimental and control, was noticed during the leucopenic phase. This loss of weight, however, was regained within 3-4 days by the surviving mice.

Both pyridoxine and folic acid seemed to have diminished and in some cases prevented diarrhea of the irradiated mice. Only about 10% showed this symptom within 3 days following radiation; while all of the irradiated unmedicated mice had diarrhea. The usual ragged appearance of the hair of irradiated animals was not noticed on those medicated either with pyridoxine or with folic acid. In particular, the hair of the mice treated with pyridoxine appeared more sleek and luxurious than those treated with folic acid.

Since the results obtained from the preliminary experiments recorded above seemed to indicate that folic acid and pyridoxine hydrochloride prolonged the survival time of irradiated mice, both these agents were tested on an additional 124 mice divided into three groups, using the same technique as previously described.

The results are summarized in the accompanying curves, Fig. 3 and Fig. 4, in which the average daily W.B.C. count and the proportion of animals surviving were plotted against time in days for a period of 100 days.

As can be noted in the graph, Fig. 3, of 32 mice treated with folic acid, 20 (62%) are still alive; and of 24 mice treated with pyridoxine hydrochloride 14 are living (58%), as compared to 68 controls, irradiated non-medicated mice, of which 13 (19%) are alive.

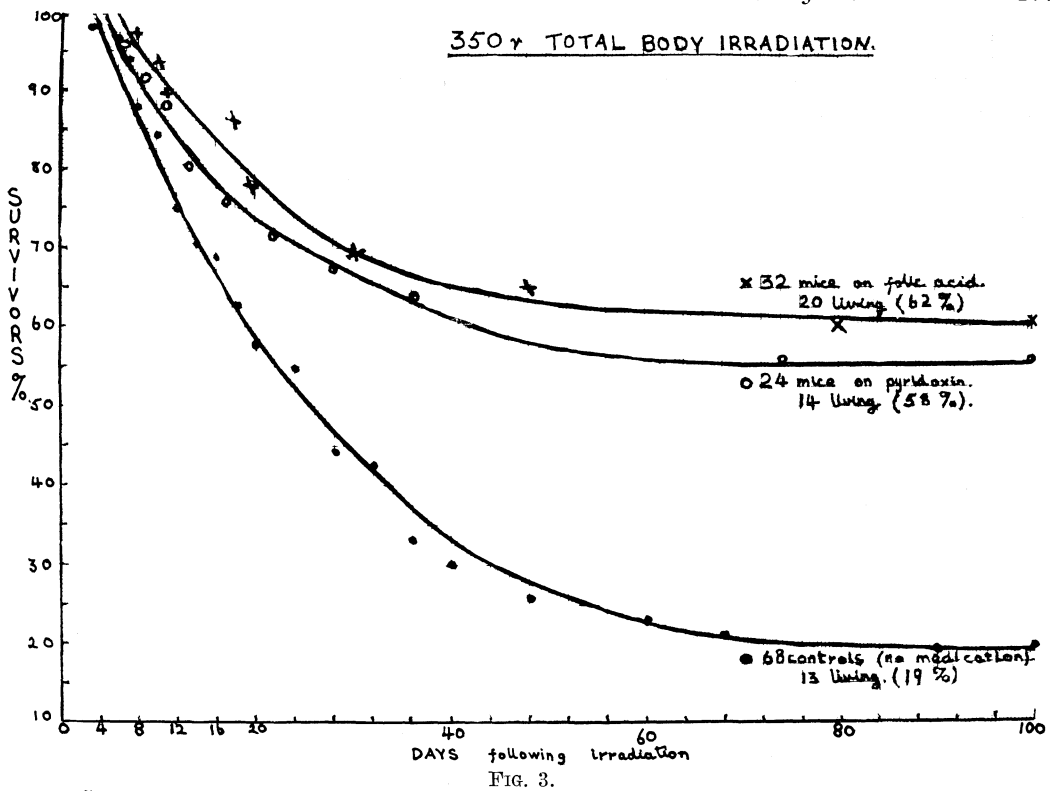

FIG. 3.
Curves showing % of survivors plotted against days following irradiation with 350 r of control mice, and mice medicated with folic acid, and pyridoxine.

From the experimental data reported here it might be concluded that folic acid and pyridoxine hydrochloride extend the survival time of mice exposed to 350 r.

Regarding the observations made in this study, reference is being made to 2 most recent publications along this line. In one, protective action of desoxycorticosterone acetate against X-ray-induced liver changes in mice was reported by Ellinger.[13] In the other, a reduction in the mortality rate of irradiated dogs treated with a flavonone, rutine, was reported by Rekers and Field.[14] To quote from these authors, "following irradiation of dogs with 350 r, 16 of 25 (64%) of the irradiated dogs succumbed within 13 to 30 days, whereas only 3 of 25 (10%) rutine-treated dogs died 16, 28, and 31 days post-radiation." These investigators attributed the beneficial effect in preventing capillary fragility, usually caused by radiation, to rutine. This effect was evidenced by the lack of pulmonary and intestinal hemorrhages in the medicated dogs, in contrast to the hemorrhagic condition noticed in irradiated, unmedicated dogs.

Possibly the beneficial effect of folic acid and pyridoxine hydrochloride reported in this paper, bears some relationship to the mechanisms of action of the preparations used by the other two investigators. This may be ascertained in our future histological studies of the internal organs of the animals used in our experiment, a great number of which are still alive.

Summary and Conclusion. White mice, exposed to X-radiation, given a medium dose of 350 r in a single exposure was found satisfactory under the experimental conditions described in the text. Folic acid and pyridoxine hydrochloride were used as possible protective agents. No appreciable effect of these drugs on the hemopoietic system was noted. On the other hand, it was found that

[13] Ellinger, F., *Science*, 1946, **104**, 502; PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 31.

[14] Rekers, P., and Field, J. P., *Science*, 1948, **107**, 16.

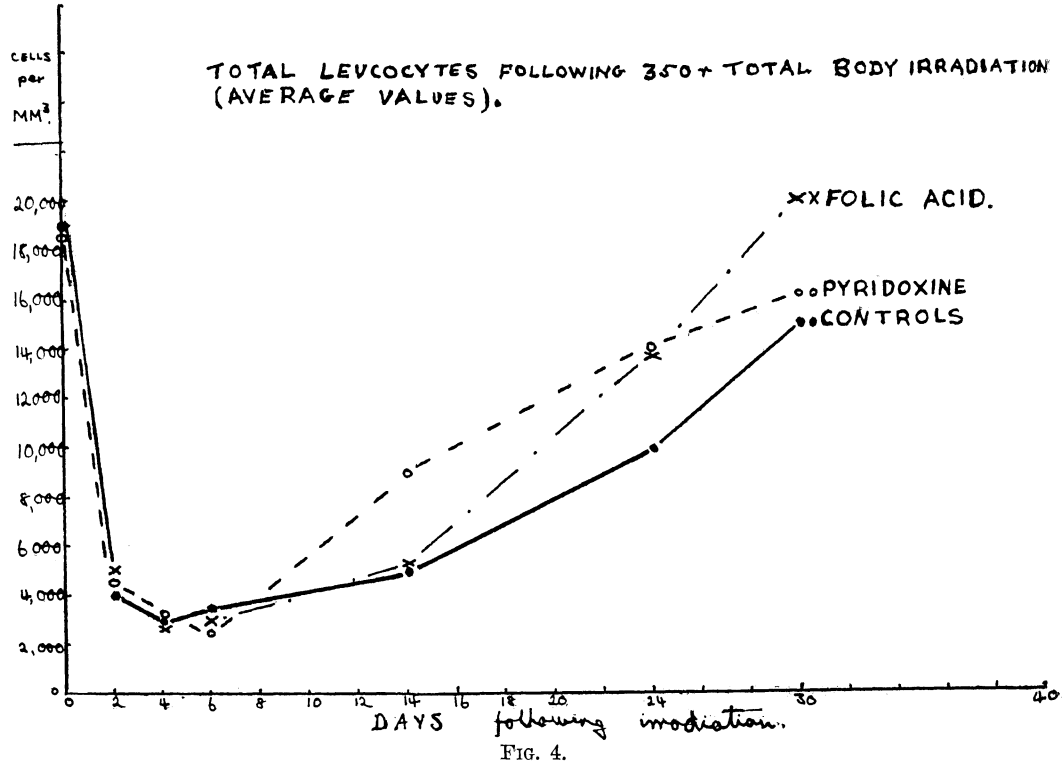

FIG. 4.

Curves showing average total W.B.C. counts plotted against days following irradiation with 350 r of control mice, and mice medicated with folic acid, and with pyridoxine.

15 γ of folic acid injected daily during a period of 7 days prior to radiation and 15 days after radiation prolonged significantly the survival time of the irradiated mice. Similar observations were made with pyridoxine hydrochloride. Thus, irradiated mice injected with 50 γ of pyridoxine hydrochloride intramuscularly daily 7 days prior to radiation and 13 days post-radiation, extended significantly the life span of the irradiated mice, as compared with the irradiated, unmedicated mice.

One of the authors (A.G.) wishes to express sincere appreciation to the members of the Physical Laboratory of the Department of Hospitals, and to the members of the Department of Radiation-Therapy, Bellevue Hospital, for their helpful co-operation.

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Comparative Effectiveness of Two Penicillin Treatment Schedules in Pneumococcal Infections of Mice.

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The methods currently employed for penicillin therapy are based largely on the principle that adequate blood levels of the antibiotic must be constantly maintained throughout the period of treatment. Consequently, since penicillin is rapidly excreted after its injection in aqueous solution, repeated doses at 3-hourly intervals are usually given to re-