TABLE II. Intracerebral Cross-Immunity Tests in Mice.

Exp.	Tested with*	Previously immunized with†	Results in tested mice‡
1	SLE	EK	S,S,S,S,S,S,S
		Controls	3,3,3,4,4,4,4,5,5,5
2	EK	SLE	7,10,11,S,S,S
		Controls	2,3,4,4,4,4,4,4,5,5
3	Jap B	EK & SLE	4,4,7
		Controls	3,4,4,4,5,5,5,5
4	West Nile	EK & SLE	5,5,6,7
		Controls	4,4,4,5,5,5,6

* Test dose consisted of a 10^{-2} dilution prepared from an infected mouse brain suspension stored in solid carbon dioxide.

† Immunized mice had survived a previous intracer. challenge with a 1% suspension of the homologous strain of virus.

‡ Figure indicates day of death; S indicates survival.

virus. Conversely of 6 mice that were resistant to SLE, 3 were now resistant to the EK virus while 3 succumbed after prolonged incubation periods. Seven of the above survivors now immune to both EK and SLE were divided into 2 groups Three mice were examined for cerebral immunity to Jap B virus and 4 were tested against W. Nile virus. They all succumbed to a 10^{-2} dilution of virus inoculated intracerebrally.

Discussion. It was previously shown that by serological methods the viruses of epidemic keratoconjunctivitis and St. Louis encephalitis could not be distinguished(1,2). The present immunity results confirm the close relationship that exists between the 2 viruses and seemingly it is only by means of intracerebral inoculation in rabbits and guinea pigs that the viruses can be told apart. It is not apparent at the present time whether the results of the immunity tests in mice are of significance or whether they merely represent differences that might be found to occur among different strains of the same virus. The current results do not add anything new to previous reports but do confirm the remarkable similarities that exist between the viruses of epidemic keratoconjunctivitis and St. Louis encephalitis.

Summary. Intracerebral inoculation of epidemic keratoconjunctivitis virus caused a fatal encephalitis in rabbits and guinea pigs, whereas inoculation of St. Louis encephalitis virus caused a non-fatal pyrexia in guinea pigs and elicited no response in rabbits. Mice were equally susceptible to both viruses while rats were refractory. Guinea pigs recovered from EK infection were also immune to SLE and those animals recovered from SLE were immune to EK. Rabbits convalescent from SLE were immune to EK, but the reverse experiment could not be done. Although EK immune mice resisted SLE, the reverse was not completely true.

Received September 13, 1951. P.S.E.B.M., 1951, v78.

Modification of Sensitivity to X Radiation by Morphine Sulfate.* (19113)

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Lowered oxygen tension has been shown to prevent damage from X radiation in a variety of organisms(1-4). Anoxia has been produced

* Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

† We are indebted for statistical analyses to Dr. A. W. Kimball, Jr., of the Biometrics Section, ORNL Mathematics Panel.

in mammals by various methods, and has been shown to protect the animals from radiation lethality to some extent. Bacq and Herve (5) produced histotoxic anoxia with cyanide before irradiation, and demonstrated increased survival in mice so treated. Storer and Coon (6) produced a high degree of methemoglobinemia in mice by injection of p-aminopropriophenone (PAPP), and demonstrated that protection against X rays resulted. This finding has been confirmed independently in this laboratory. Another method of reducing oxygenation of tissues is by the production of anoxic hypoxia, by reducing the amount of oxygen in the atmosphere as in the work of Dowdy *et al.*(3), or by depressing the respiratory center. Morphine is outstanding in its ability to depress the respiratory center. Therefore, a series of experiments was performed to determine whether the administration of morphine would protect animals against the lethal effects of X radiation.

Materials and methods. Animals used were ZBC strain mice,‡ 10 to 12 weeks old at the time of injection, and weighing between 25 and 30 g. Morphine sulfate was dissolved in sterile physiological saline in a concentration of 15 mg/ml; 0.1 ml of this was injected per 25 g mouse. Injections were made intramuscularly with sterile precautions 30 minutes before exposure to the radiation. At the time of exposure to the X rays all mice showed positive Straub tests, and analgesia was seen in decreased pain responses to pinching of the tail and feet. Factors of irradiation were: 250 kvp, 30 ma, TSD 93.7 cm, filtration 3 mm Al, inherent filtration 1 mm Al, HVL 0.4 mm Cu. The dose rate, measured with a 100 r Victoreen r-meter, was 56.5 r/min. Mice were exposed in groups of 20, individually compartmented in a Lucite box. The maximum variability in dose delivered to the various compartments was 2%. Controls were uninjected irradiated mice, since a preliminary experiment indicated that the injection of 0.1 ml of physiological saline had no effect on X-ray mortality. Figures reported are for 30-day mortality, as no deaths occurred after this period.

Results. A preliminary experiment was performed using morphine dosages of 24, 60, and 90 mg/kg, and X-ray doses of 800 and 900 r. The results indicated that enough protection was afforded by morphine to merit further investigation. Of the 3 doses tried, 60 mg/kg showed maximum protection with no mortality from the drug; this dose was used throughout the remainder of the work. The observed pattern of response to the injection of morphine was a preliminary excitation, lasting for 15 to 30 minutes, followed by a more prolonged depression, as described by Krueger, Eddy, and Sumwalt(7). Thus, the X-ray exposures were begun at the beginning of the depression period. The weight loss of the irradiated animals, though not accurately quantitated, was less in the morphine-treated groups than in the controls.

The results of the experiment designed to test the effect of morphine sulfate on the LD_{50} of whole-body X radiation are given in Fig. 1. Each point represents 40 mice except the 880 r control group and the 630 r and 670 r morphine-treated groups, which represent 20 mice. Results were calculated by the probit method. The figures indicate that if more experiments were carried out under conditions similar to those outlined above, in 95% of such experiments the LD_{50} of the untreated groups would fall in the interval between 595 r and 620 r. The interval for the morphine-treated groups is 819 r to 842 r. Since the confidence intervals for the 2 groups are far

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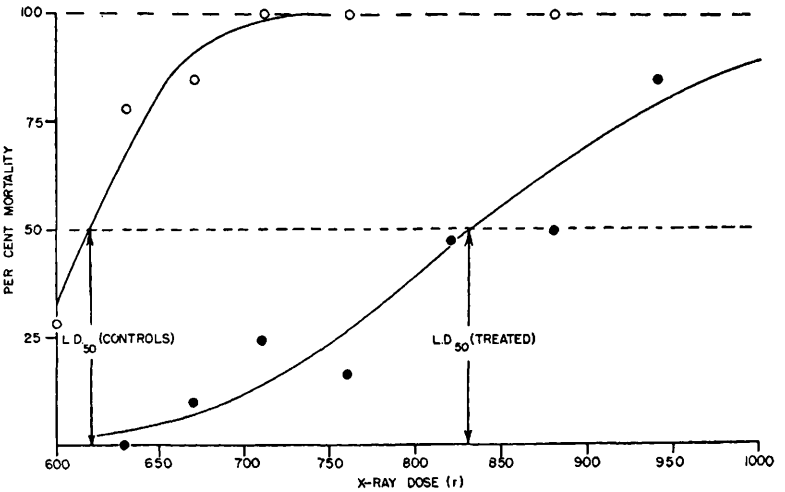

FIG. 1. Effect of morphine sulfate on LD_{50} of x radiation. ○ = control; ● = morphine-treated (60 mg/kg). Each point represents 20 to 40 mice.

from overlapping, it is clear without formal statistical treatment that the LD_{50}'s of the 2 groups differ significantly, at the level of 0.1% or less. Fig. 1 represents the probit fits to the observed mortalities, and indicates an increase in LD_{50} from 609 r in the controls to 830 r in the treated group.

Discussion. Morphine has long been known to affect many systems, such as the autonomic nervous system, central nervous system, and endocrines. However, only 2 of the actions of morphine seem to offer possible explanations of its effect in increasing the radioresistance of mice. The first of these is the hyperglycemic effect which morphine has been shown to have on various species(8), probably through an adrenal mechanism. Loiseleur and Velley(9) have shown that rats can be protected from the lethality of X rays by intraperitoneal injections of very large amounts of glucose 45 minutes before irradiation. Baclesse and Loiseleur(10) have demonstrated a reduction of cutaneous sensitivity of rabbits to X rays by intramuscular glucose injections. Both of these studies utilized exceedingly large doses of glucose near the lethal level; on the other hand, the rise of blood glucose after an injection of morphine is quite variable, and rarely exceeds 60 mg %. Hence, it seems unlikely that the hyperglycemic action of morphine can account for its protective ability.

The other effect of morphine which might serve to explain its protective action is its pronounced depression of the respiratory center. This depression of respiration results in a decrease of oxygen uptake by the blood, resulting in turn in decreased tissue oxygenation. This end effect is similar to that produced by placing animals in 5% oxygen(3), by producing methemoglobinemia(6), or by histotoxic anoxia(5). It is interesting that the curves of Fig. 1 have the same shape and the same difference in slope between graphs of control and treated mice as do the curves of Hollaender *et al.*(4) for the oxygen effect in *E. coli*. It seems likely that the mechanism of action of morphine in protecting mice against the lethal effect of radiation is a decrease of oxygen tension in some radiosensitive tissue or tissues. This parallels the conditions in *E. coli*(4) and other organisms(1,2).

The possibility remains that the protection afforded by morphine is on a purely physical basis, *e.g.*, through formation by morphine of a substrate for the action of activated products of water irradiation. This can be tested by injecting into mice an alkaloid structurally similar to morphine, but with different effects

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on the respiratory center. It is planned to test the effects of thebaine and of n-allylnormorphine in this manner, which should furnish further evidence for the role of the respiratory center in protection against radiation toxicity.

Summary. Morphine sulfate, injected intramuscularly into ZBC male mice 30 minutes before exposure to total-body X-radiation, raises the LD_{50} of the radiation from 609 r to 830 r. Possible mechanisms of this effect are discussed.

Received September 14, 1951. P.S.E.B.M., 1951, v78.

Effect of Oral Terramycin Prior to Whole Body X-Radiation.* (19114)

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Severe radiation injury is often complicated by bacteremia(1-3). The intestinal tract probably serves as the main portal of entry for organisms. This study was made to determine whether reduction or alteration in the intestinal flora by antibiotic therapy prior to radiation would be effective in lowering mortality.

Method. Two hundred white male rats of the C. F. Wistar strain having a mean weight of 195 g were separated randomly into 2 groups of 100 each. Animals were kept in cages having dimensions of 22 by 20 by 15 inches in groups not exceeding 10 rats per cage. Terramycin hydrochloride (10% aqueous solution) was administered orally, using a French No. 8 soft rubber catheter, by a modification of the technic described by Levin(4). The antibiotic was given in 5 doses of 100 mg each, at intervals of 10 to 14 hours, beginning 48 hours prior to radiation and ending 1 to 4 hours prior to radiation. No antibiotic was used after radiation. The rats were weighed 9, 3, and 1 day prior to x-radiation, daily for the first 6 days following radiation, and thereafter at 2 or 3 day intervals. The experiment was discontinued at the end of 30 days. X-radiation was carried out with a 250 KV, 15 MA unit at a distance of 44.3 cm using ½ mm copper and 1 mm aluminum filters. The dosage rate was approximately 60 r per minute. The total dose was 660 r.

Results. (1) Mortality. The 30 day mortality for the control group was 72% and for the treated group 48%. Analysis of the data by the method of Chi square gives a value of 4.08. This is statistically significant. (2) Survival. The mean survival time of the rats in the control group was 10.6 days as compared to 15 days for the treated group. The mean difference is 4.4 days and the mean standard error is 1.3 days. Statistically this difference is highly significant. Fig. 1 shows a plot of per cent mortality against days after x-radiation. The control rats began to die earlier than the treated rats and had a higher death rate up to the 12th day. From the 4th to the 12th day the mean death rate for the control group was 6.2% per day and for the treated group 2.6% per day. From the 12th to the 30th day the mean death rate for the control group was 0.9% per day and for the treated group 1.4% per day. However, when the death rate is calculated on the basis of the number of rats alive on the 12th day, the death rate for the control group is 2.3% per day and for the treated group 1.7% per day. (3) Morbidity. In general this paralleled the death rate. Morbidity was greater in the

* This work performed under contract between the U. S. Atomic Energy Commission and Western Reserve University, Contract No. W31-109-eng-78. Terramycin furnished by courtesy of Chas. Pfizer and Co., Brooklyn, N. Y.

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