Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 112 FEBRUARY 1963 No. 2

SECTION MEETINGS

MARYLAND
University of Maryland Medical School — January 17, 1963

NORTHWEST
University of British Columbia — November 17, 1962

SOUTHWESTERN
University of Arkansas Medical Center — November 9-10, 1962

Recovery of Central Nervous System Functions Impaired by Lethal Head X-Irradiation.* (28014)

V. NAIR, H. SUGANO† AND L. J. ROTH
Department of Pharmacology, University of Chicago, Chicago, Ill.

In connection with our interest in blood brain barrier (BBB) permeability changes, we have been studying the irradiated rat brain (10,000 r to head) utilizing such specific indicators as I^{131}-labeled serum albumin (RISA, Abbott) and S^{35}-sodium sulfate. We have been unable to demonstrate any marked alteration in BBB permeability in the head irradiated rat employing RISA and S^{35}-sulfate and the technics of radioassay and autoradiography(1,2). On the other hand, utilizing the same methodology, we were able to demonstrate a marked increase in penetration of S^{35}-sulfate as well as RISA into the brain of rats subjected to metrazol convulsions(2). That alterations in brain permeability exist in metrazol convulsions has been shown by others using RISA and dye indicators(3,4). The head irradiated rats under our conditions showed gross pathological changes, progressive weight loss and died within 8-10 days. These results posed the question of the possible existence of derangements in central nervous system (CNS) activity or function in these head irradiated rats even though there was no marked BBB permeability changes. Neuronal damage in the absence of demonstrable BBB permeability changes is not unknown(5).

The present study was undertaken to explore this possibility in the head irradiated rat under the same irradiation conditions as

* This work was supported in part by a research contract from Atomic Energy Commission.

† Rockefeller Scholar from Dept. of Pharmacology, Kyushu Univ., Fukuoka, Japan.

previously employed by us for permeability studies(2). As indices for measuring irradiation induced changes in CNS activity or function, we have employed 1) electroencephalographic changes and 2) response to maximal electroshock.

Materials and methods. Male Sprague-Dawley rats weighing about 200-220 g were used in these studies. Food and water were given *ad libitum.*

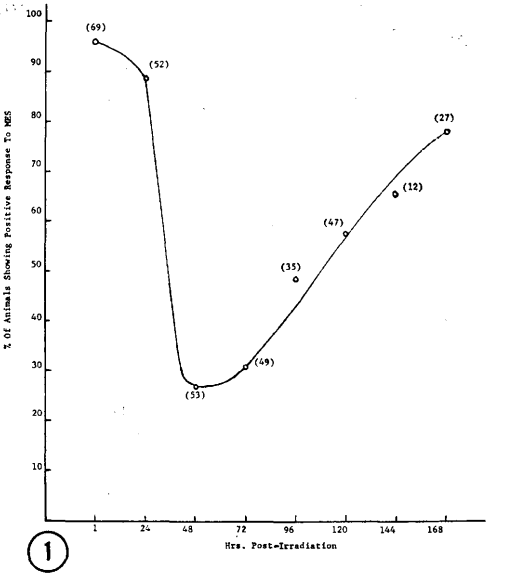
X-Irradiation. The head alone of the rats was irradiated from a 250 KV 30 mA General Electric Maxitron X-ray machine. The filters employed were 0.5 mm Cu and 1 mm Al. The half value layer of the beam was 1.45 mm Cu. While being irradiated the animals were in plastic holders with lead shield (3.5 mm thick) over the entire body except the region of the head and they were placed on a platform which was rotated continuously to ensure equal exposure throughout the brain. The dose given was 10,000 r at 295 r per minute at a focal distance of 57.7 cm. After irradiation, the animals were transferred to air-conditioned quarters.

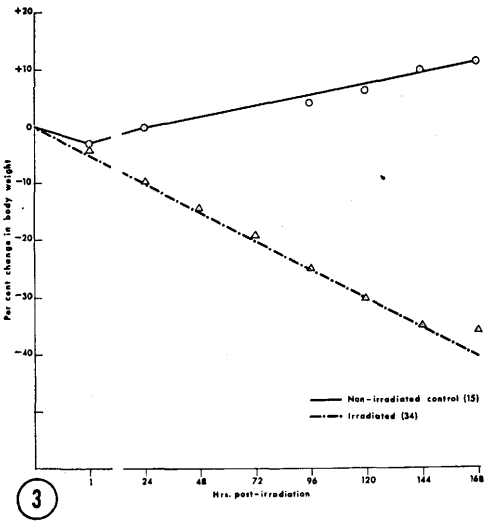
Electroshock. The apparatus employed to produce the electroshock seizures is a constant current stimulator (Wahlquist, Salt Lake City, Utah) of the type described by Woodbury and Davenport(6). The maximal electroshock seizure pattern (MES) test was performed according to the method of Toman *et al.*(7). Maximal seizure was induced by 150 mA delivered through corneal electrodes for 0.2 second. Prior to irradiation rats were subjected to this test and only those showing the typical maximal seizure pattern (tonic-clonic) consistently for 3-4 days when shocked at 24 hour intervals were used for irradiation and subsequent MES studies. The response of the head irradiated rats to MES test was measured at various intervals following irradiation (at 1, 2 or 3, 24, 48, 72, 96, 120, 144 and 168 hours post-irradiation). Non-irradiated control rats were also subjected to MES test in the same manner and at the same shock intervals as the irradiated rats.

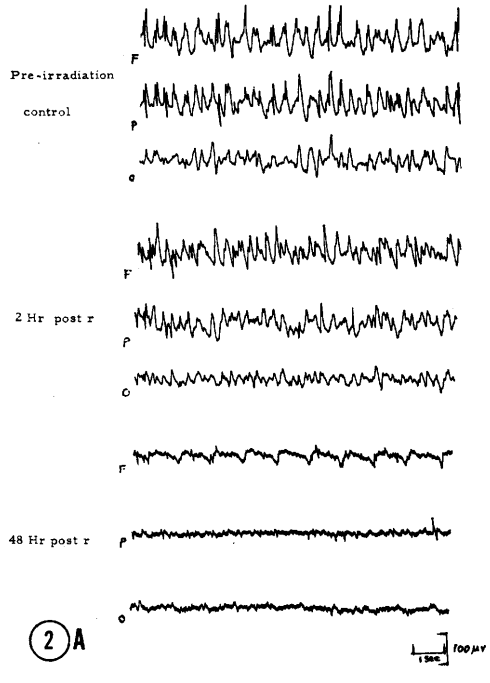
EEG. EEG was recorded using stainless steel wires as electrodes. Rats were anesthetized with nembutal (50 mg/kg) and the electrodes implanted on the frontal, parietal and occipital surface of the rat skull. The 3 monopolar leads were connected to an Offner Type R Dynograph and EEG tracings obtained from the anesthetized animals. Since variations in degree of anesthesia can influence the EEG and lead to erroneous results, this factor was controlled by taking the EEG record in each case at 15-20 min after the loss of righting reflex. Consistency was thus maintained. The EEG tracings were repeated daily for 2-3 days prior to irradiation and only those showing reproducible records were selected for irradiation. This record was then selected as the pre-irradiation control EEG and is used for comparison with the tracings from the irradiated animals. At specified intervals after irradiation the rats were anesthetized with nembutal and EEG tracings again obtained at 20 min following the loss of righting reflex. Thus electroencephalograms of the irradiated rats were obtained as controls and at 1, 2 or 3, 24, 48, 72, 96, 120, 144 and 168 hours post-irradiation.

Results and discussion. The MES test is a measure of the spread of excitability. Under the supramaximal stimulus employed in this test, normal adult rats show all the components of typical maximal seizure pattern (tonic-clonic). Toman *et al.*, (*loc. cit.*) found that clinically recognized anticonvulsant drugs abolish the hind limb tonic extensor component of maximal seizures even when these drugs fail to raise appreciably the threshold for electroshock and they used this test to detect and evaluate anticonvulsant agents.

The most striking effect induced by radiation on the response to MES test was the progressive decrease in percent of animals showing hind limb tonic extension, *i.e.*, the progressive increase in protection against electroshock (Fig. 1). This apparent anticonvulsant action reached a maximum at about 48 hours post-irradiation and was subsequently lost. The response of the non-irradiated controls to electroshock was not altered when subjected to MES test in the same manner and at the same shock intervals as the irradiated rats. At 1 hour post-irradiation, out of 69 animals shocked, 63







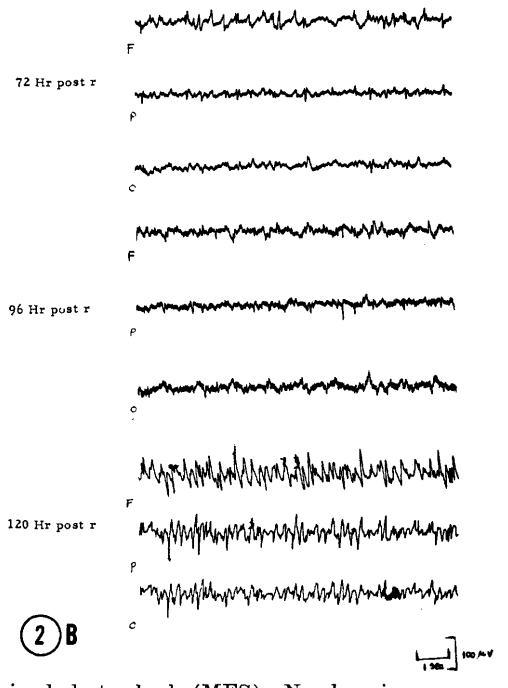


FIG. 1. Response of head irradiated rats to maximal electroshock (MES). Numbers in parentheses refer to No. of animals.

FIG. 2. EEG changes in a rat following head irradiation. F, P, and O refer to frontal, parietal and occipital leads.

FIG. 3. Effect of head irradiation and electroshock on body wt of rats. Each point represents mean wt of No. of animals indicated in parentheses. Controls were subjected to all the treatments except irradiation that were given to irradiated animals (such as restraining in plastic holders and electroshock). In the case of shocked animals, weight was taken before submitting them to MES test. Electroshock alone did not significantly alter body wt of controls or irradiated rats. Initial wt loss indicated at 1 hr in control animals is a result of restraining in plastic holders for 35-40 min.

showed positive response (tonic extension of hind limbs), 5 partial extension and one no extension. In expressing the data graphically, 2 partial extensions have been counted as one positive extension. Thus at 1 hour post-irradiation, 95% of the animals shocked showed a positive response. The response at ½, 2 or 3 hours post-irradiation was essentially the same as that shown at 1 hour. At 24 hours post-irradiation, 88% of the animals tested showed extension signifying a decrease of 7% from the 1 hour period. At 48 hours post-irradiation, only 11 out of 53 or 27% of the irradiated animals showed the positive response to electroshock. Thus the greatest decrease in percent of animals (61%) responding positively to MES between any 2 successive days falls between the 2nd and 3rd day following irradiation. The irradiated rats after 48 hours showed progressive recovery in their ability to respond to maximal electroshock (Fig. 1). There was an increase of 4-5% in the number of animals showing extension at 72 hours post-irradiation. After this period the recovery trend continues until 168 hours or death, whichever was earlier. Between 144-168 hours post-irradiation, many of the animals died, although some of them survived up to 9 days. At 120 hours and thereafter, the animals appeared very sick with eyes closed and dried exudate often covering the margin of the eyelids. Those animals that were repeatedly shocked daily until 120 hours post-irradiation appeared very sensitive to handling and this posed considerable difficulties in subjecting them to further electroshock. Extra care had to be taken to ensure proper electrical contact over the edematous and exudate filled eyes. At 168 hours post-irradiation, most of the animals were terminal and in respiratory distress. Few survived this period. It is of interest that in spite of these factors, over 70% of the animals showed a positive response to electroshock at this late period. In other words, at least as regards the ability to respond positively to MES test, a large proportion of the irradiated animals had recovered this function by 168 hours and were comparable in this respect to those at the 30 hours post-irrad ation period.

The EEG of the non-irradiated rat is cha acterized by regular waves of 6-12 c/s (basic waves) and superimposed waves 16-20 or more c/sec (small fast waves X-irradiation at the dosage employed these studies (10,000 r) produced no signi cant changes in the EEG of rat at 1, 2 or hours post-irradiation. However, significa alterations both in the amplitude and fr quency of EEG appeared about 24-48 hou post-irradiation (Fig. 2). There were on moderate changes in the EEG prior to t 48 hour period, while by 48 hours th reached a maximum and about 50% of t animals at this period showed blacko (electrical silence) or near blackout in th EEG. After 48-72 hours post-irradiation, t rat began to show recovery in that the EE began to indicate progressive improveme and by 120 hours 45% of the anim showed an EEG similar to their pre-irra ation record. Furthermore, at this late po irradiation period, in spite of their debilitat condition, there was no blackout in the EE of any of the animals examined. A typi pattern of recovery in a rat is illustrated Fig. 2. The pattern is reminiscent of the sponse of irradiated rats to electroshock that a depression followed by recovery again seen.

There was a progressive decrease in bo weight following irradiation but it was linear decrease and does not parallel the sponse to MES test (Fig. 3). The irradiat rats lost about 30-35% of their pre-irra ation weight by the 7th or 8th post-irra ation day. That the results observed in t irradiated rats are not related to the loss body weight is further supported by the a sence of a similar response as seen in t irradiated animals in the fasted (24- hours) non-irradiated controls. The fast controls responded with tonic extension hind limbs. In this connection the findi of Rosenthal and Timiras(8) are of intere They noted that there was no direct relati between alterations in rate of body grov and changes in threshold after irradiati Previous work in other laboratories l

shown that CNS response to electrical stimulation can be modified by X-irradiation(8,9). In most of these cases, however, doses lower than that used in the present studies were employed for irradiation and a reduction in threshold for electroshock seizures was noted. Rosenthal and Timiras(8,10) found that whole body X-irradiation at 250 and 500 r increased brain excitability in rats and that the irradiation induced changes in convulsive reactivity persisted for at least 180 days following a single exposure to 500 r X-irradiation. Whether these contrasting differences in the response of the CNS to X-irradiation are a function of the dose of irradiation remain to be explored.

We have considered the possibility that the observed effects may be secondary to peripheral effects. Accordingly, we have examined the response of head shielded rats to MES test following 1000 r to the rest of the body. Preliminary results from 11 rats indicate no similarity to the results obtained in the head irradiated series. Fifty percent of the head shielded rats died by 96 hours post-irradiation. The head shielded rats showed no significant protection against electroshock at any of the time intervals examined, consequently the question of recovery does not arise here. This also excludes the possibility that the results obtained in the head irradiated rats may arise from scattered radiation to parts other than head. It would appear then that the anticonvulsant effect and recovery phenomenon noted in the head irradiated rats is a CNS effect.

The mechanisms underlying this phenomenon are as yet obscure. However, the following points are clear from these studies: 1) X-irradiation under the conditions of our studies induces a block in the transmission or conduction processes in the CNS necessary for exhibition of hind limb tonic extension following maximal electroshock. 2) This effect is not permanent and the animal recovers its ability to respond positively to maximal electroshock thus suggesting recovery of central nervous system functions impaired by a lethal dose of X-irradiation.

Summary. The effects of X-irradiation on the response to maximal electroshock and on EEG have been studied in head irradiated rats at various intervals following irradiation. A single application of 10,000 r to head of rats was found to induce a progressive increase in protection against maximal electroshock. This apparent anti-convulsant effect reached a maximum by about 48 hours post-irradiation and was then rapidly lost. Most of the animals died by the 7th or 8th day following irradiation. It is of interest that the irradiated animals progressively recover their ability to respond to maximal electroshock after the 48 hour period in spite of their otherwise increasing moribund state. EEG records following irradiation also suggest a similar recovery effect. These findings are interpreted as indicating that there is a progressive recovery of certain central nervous system functions impaired by a lethal dose of X-irradiation.

1. Nair, V., Palm, D., Roth, L. J., *Nature,* 1960, v188, 497.

2. Nair, V., Roth, L. J., *Fed. Proc.,* 1962, v21, 422.

3. Lending, M., Slobody, L. B., Mestern, J., *Am. J. Physiol.,* 1959, v197, 405.

4. Bjerner, B., Broman, T., Swensson, A., *Acta Psychiat. et Neurol.,* 1944, v19, 431.

5. Clemente, C. D., Holst, E. A., *A.M.A. Arch. Neurol. Psychiat.,* 1954, v71, 66.

6. Woodbury, L. A., Davenport, V. D., *Arch. Int. Pharmacodyn.,* 1952, v92, 97.

7. Toman, J. E. P., Swinyard, E. A., Goodman, L. S., *J. Neurophysiol.,* 1946, v9, 231.

8. Rosenthal, F., Timiras, P. S., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 267.

9. Gangloff, H., Haley, T. J., *Radiation Research,* 1960, v12, 694.

10. Rosenthal, F., Timiras, P. S., *ibid.,* 1961, v15, 648.

Received November 5, 1962. P.S.E.B.M., 1963, v112.