Kärber, Reed and Muench, and moving average methods. 2. The standard deviation of the titrations was found to be greater than the error of individual assays, suggesting that titrations carried out on different occasions vary more than those performed at the same time. 3. None of the methods mentioned gave widely different estimates of the ED_{50}. In particular, the ED_{50}'s calculated by the Kärber method compared very favourably with those obtained by the probit method. 4. The Kärber method is proposed as a suitable alternative to the Reed and Muench procedure for the calculation of ED_{50} in routine titrations of virus and antiserum.

1. Morgan, I. M., *Am. J. Hyg.*, 1947, v45, 372.
2. Bell, E. J., *Am. J. Hyg.*, 1948, v48, 381.
3. Schwerdt, C. E., and Merrell, M., *Am. J. Hyg.*, 1952, v55, 268.
4. Finney, D. J., *Sankhya*, 1950, v10, 341.
5. Pizzi, M., *Human Biol.*, 1950, v22, 151.
6. Rhodes, A. J., Clark, E. M., and Shimada, F. T., *Canad. J. Publ. Health*, 1951, v42, 23.
7. Rhodes, A. J., and Clark, E. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 379.
8. Rhodes, A. J., Clark, E. M., Shimada, F. T., Donohue, W. L., and Ritchie, R. C., *Canad. J. Med. Sci.*, 1952, v30, 54.
9. Finney, D. J., *Probit Analysis*, Cambridge University Press, 1947.
10. Irwin, J. O., and Cheeseman, E. A., *J. Hyg.*, 1939, v39, 574.
11. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
12. Thompson, W. R., *Bact. Rev.*, 1947, v11, 115.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

Effect of X-Ray on Radioactive Phosphorus Turnover and Oxygen Consumption of Brain.*† (19797)

W. FLORSHEIM, C. DOERNBACH, AND M. E. MORTON.

From the Radioisotope Unit, Veterans Administration Hospital, Long Beach and the School of Medicine, University of California, Los Angeles.

Studies of the pathological effects of radiation on the central nervous system of adult mammals have shown no clearcut findings. Warren(1) presents evidence which indicates that at least "therapeutic radiation" has little effect on adult nerve tissue. Findings which demonstrate radiation damage to nervous tissue appear to result from effects on vascular components rather than direct damage to neural elements. The earliest experimental observations(2) of the effects of radiation on brain were in experiments where the whole body was irradiated; congestion and hemorrhage were the most consistent pathological findings.

The purpose of the present investigation was to demonstrate the presence or absence of alterations in the phosphorus metabolism of brain after whole-body irradiation with roentgen rays. In order to eliminate at least one variable, vascular circulation effects, studies of oxygen consumption and phosphorylation were done *in vitro* after *in vivo* irradiation of the animals.

Experimental. Six- to eight-week-old male albino mice were irradiated with 500 to 800 r and, in another experiment, with 19,000 r in air. A 250 kv General Electric Unit was used with 1 mm Al and 0.5 mm Cu filters at 15 ma output. The smaller dosages were delivered at 1 meter distance and the greater at 7 cm. The mice were killed by severing the spinal cord at various times after irradiation. The brain was excised as rapidly as possible and split along the longitudinal fissure into equal halves. The halves were weighed, minced, and then added to 3.0 cc of McIlwain's buffer(3) containing P^{32} as orthophosphate.‡ The tissues were incubated at 37.5°C for 3 hours

* Aided in part by a grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

† Grateful acknowledgement is made to Dr. F. Isaac for making available instruments for irradiation of the animals, and to Drs. H. W. Magoun and J. D. French for discussions.

TABLE I. Qo_2 and Phosphorus Uptake of Brain as Affected by X-irradiation.

Exp.	Dosage (r)	Hr post irrad.	No. samples	Qo_2 (μl/h) †	Uptake as % of incubation medium— Acid sol.	Residual	Lipid
1	500	19	7	452 ± 30*	.80 ± .08	.060 ± .008	.036 ± .008
	0	19	7	448 ± 46*	1.16 ± .16	.074 ± .008	.042 ± .008
2	500	0	6	378 ± 30	1.26 ± .22	.088 ± .006	.054 ± .006
	500	190	6	396 ± 30	1.76 ± .12	.104 ± .014	.062 ± .010
	0	0	6	386 ± 36	1.74 ± .18	.098 ± .002	.056 ± .008
3	800	18	6		1.1 ± .2	.15 ± .01	.12 ± .02
	0	18	6		1.2 ± .1	.12 ± .02	.09 ± .02
4	800	0	6		1.3 ± .2	.14 ± .02	.11 ± .01
	0	0	6		.95 ± .08	.12 ± .03	.12 ± .02
5	800	0	4		1.2 ± .3	.10 ± .005	.12 ± .01
	0	0	4		1 ± .05	.10 ± .008	.12 ± .01
6	19000	18	8		1.5 ± .2	.13 ± .01	.060 ± .008
	0	18	4		1.3 ± .3	.12 ± .01	.055 ± .007

* Extrapolated from 15 min. value.

† Results expressed as per 200 mg fresh brain tissue.

There was no significant difference in phosphorus turnover and oxygen consumption between the right and left hemispheres of the same animal. P from Fisher's(6) table of t was $<.1$ for protein and $<.8$ for the lipid fractions where $n = 19$. For oxygen consumption $p < .4$ where $n = 9$.

in either a Warburg apparatus under an atmosphere of oxygen (Exp. 1-2), or 3½ hours on a Dubnoff metabolic shaker under Carbogen (Exp. 3-6). An equal number of unirradiated brain halves were used for controls in each experiment.

Metabolic processes were stopped at the end of the incubation period by the addition to the media of an equal volume of 10% trichloracetic acid. The resulting suspension was fractionated by the procedure described by Rafelson(4) into acid-soluble, lipid and residual "protein" fractions.

Results and discussion. Data presented in Table I indicate that, at the radiation levels employed, the capacity of adult mouse brain tissue to utilize oxygen and to metabolize exogenous phosphate is not significantly impaired.

Quastler *et al.*(5) have reported that whole body irradiation at levels ranging from the minimal lethal dose to 10,000 r resulted in death within 3.5 days from effects on the gastrointestinal system, mainly hemorrhage. Doses of X-ray exceeding 16,000 r, however, produced death within an even shorter period after exposure from what appeared to be central nervous system involvement. Irradiation of the head alone was sufficient to produce the "hyperacute" radiation death. Yet, the present study, in which doses of radiation as high as 19,000 r were used, demonstrated no alteration of phosphorus metabolism.

It is possible that the lethal effects of such doses are associated with phases of cellular metabolism unrelated to phosphorylation mechanisms. However, if phosphorus turnover were a valid index of the metabolic state of a tissue, it would appear that there is no acute gross derangement of brain metabolism even after such large amounts of irradiation.

Conclusion. *In vivo* irradiation with 500 to 800 r of X-rays did not impair the ability of adult mouse brain tissue to metabolize exogenous radiophosphate and did not alter the Qo_2 of the minced tissue. Doses of 19,000 r produced no observable acute effect on the phosphorylation.

‡ The radiophosphorus used in this investigation was supplied by Oak Ridge National Laboratory on authorization from the Isotopes Division, U. S. Atomic Energy Commission.

1. Warren, S., *Arch. Path.*, 1943, v35, 127.

2. Heineke, H., *München. med. Wchnschr.*, 1903, v50, 2090.

3. McIlwain, H., Buchel, L., and Cheshire, J. D., *Biochem. J.*, 1951, v48, 12.

4. Rafelson, M. E., Winzler, R. J., and Pearson, H. E., *J. Biol. Chem.*, 1949, v181, 583

5. Quastler, H., Lanzl, E. F., Keller, M. E., and Osborne, J. W., *Am. J. Physiol.*, 1951, v164, 546.

6. Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936.

Received July 14, 1952. P.S.E.B.M., 1952, v81.