SOME EFFECTS OF X-IRRADIATION ON OXYGEN UPTAKE AND SEROTONIN LEVELS IN RAT BRAIN TISSUE SLICES

APPROVED:

James R Lott
Major Professor

S. J. Norton
Minor Professor

J. K. G. Silvey
Director of the Department of Biology

Robert B. Toulouse
Dean of the Graduate School

SOME EFFECTS OF X-IRRADIATION ON OXYGEN UPTAKE AND SEROTONIN LEVELS IN RAT BRAIN TISSUE SLICES

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF ARTS

By

John F. Hines, B. A.

Denton, Texas

January, 1966

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

PART I

OXYGEN UPTAKE IN RAT BRAIN TISSUES X-IRRADIATED *IN VITRO*

CHAPTER I

INTRODUCTION

The recent increased interest in biochemical and physicochemical processes in the central nervous system includes the effect of ionizing radiation. According to Livshits (9) and Van Cleave (15), the available evidence is often contradictory, but comparative studies of the effect of radiation on certain metabolic functions in the brain and other organs strongly suggests a CNS peculiarity. Whether this apparent difference is due to the blood-brain barrier or due to more direct localized effects is uncertain. Until recently, it was believed that the enzyme systems of the respiratory cycle and tissue respiration, in general, were relatively unaffected by ionizing radiation. A selection of tissue respiration studies since 1946 has been reviewed by Ord and Stocken (12), and by Snezhko (13). Unfortunately, in their reviews, these workers do not distinguish between the results derived from tissues irradiated *in vitro* and those irradiated *in vivo*. Moreover, the literature is relatively void in its reports on effects of X-irradiation on the respiration of nervous tissues. The oxidative processes in rabbit brains, however, have been intensively studied by Snezhko, using potentiometric methods. He found that whole body exposure to acute doses of X-irradiation brought about an increased oxygen tension in the brain tissues which was phasic in character. He concluded that this effect was a direct one on the respiratory apparatus in the tissues.

Recently, workers have found that the CNS of mammals and even ganglia of invertebrates are extremely sensitive to ionizing radiation. Haley (8) reported electroencephalographic (EEG) changes after 400 r, whereas, Garcia and Buchwald (7) reported EEG changes with dosages of less than one roentgen. Not only electrical alterations have been noted at low dosages, but also behavorial changes have been reported by Garcia and Kineldorf (6) and Miller (11). It is generally agreed that in recent years the bioelectrical techniques for measuring functional changes in the CNS produced by irradiation have outdistanced those for detecting biochemical and/or structural changes. To date, it has been shown that a considerable dosage of ionizing radiation is required (greater than 1000 r) before measurable biochemical changes are noted (1, 10, 13). Moreover, it is well established that certain tissues irradiated *in vitro* and *in vivo* are more radiosensitive than others (Errera and Forssberg (4). The data concerning changes in respiration of irradiated nervous tissues is conflicting. Florsheim (5) found no change in the oxygen uptake of minced mice brain tissues after whole body irradiation. Egana (2), on the other hand, reported enhancement in the respiration of specific areas in rat brain tissues following the injection of p^{32}, a beta emitter, prior to tissue removal.

It is unfortunate that most of the data concerning the effects of radiation on the respiration of nervous tissues, including the brain, have involved measurements made on tissue from previously irradiated animals. Under these circumstances, the problems of uniform dosages and indirect effects due to circulating humoral agents formed in the animal are formidable handicaps. To offset these obstacles it was decided that the present study would be carried on isolated tissue slices where uniform doses could be applied and circulating agents be avoided. Moreover, by removing the tissues to be studied from specific areas from the brain and irradiating each in vitro it was felt that a better understanding of the effects of X-irradiation on the biochemical integrity of these tissues could be made.

Summarily then, the aims of this study were as follows: (1) to determine the changes in respiration in tissues from specific brain areas X-irradiated in vitro using a direct monometric procedure; (2) to determine differences in radiosensitivity of specific areas in the brain as reflected by changes in oxygen uptake; (3) to shed some light on the nature of the biochemical lesions in nerve tissues brought about by ionizing radiation.

CHAPTER BIBLIOGRAPHY

1. Aurand, K. and H. Pauley, "Zui Beeinflussung der Gebeatmung durch Rontengstrahlen," Zeitschrift fur Naturforschung, IX, (1954), 506-507.

2. Egana, Enrique, "Some Effects of Ionizing Radiation on the Metabolism of the Central Nervous System," International Journal of Neurology, III, No. 3-4, (1962), 631-647.

3. ______________, and M. I. Velarde, "A Comparative Study of Normal and Beta Irradiated Rats, The Effects of Hydrogen Peroxide on the Respiration of the Central Nervous System," Comparative Neurochemistry, ed., D. Richter, New York, Pergamon Press, (1964), 275-278.

4. Errera, Maurice, and Arne Forssberg, "Mechanisms in Radiobiology," I. General Principles, Academic Press, New York, (1961), 261.

5. Florsheim, W., K. C. Dorenback and M. E. Morton, "Effects of X-Ray on Radioactive Phosphorus Turnover and Oxygen Consumption of Brain," Proceedings of Society of Experimental Biology and Medicine, LXXXI (1952), 121-122.

6. Garcia, J. and D. J. Kimeldorf, "Some Factors Which Influence Radiation Conditioned Behavior of Rats," Radiation Research, XII, (1960), 719-722.

7. Garcia, J., N. A. Buchwald, G. Bach y Rita, B. H. Feder, R. A. Koelling, "Electroencephalographis Responses to Ionizing Radiation," Science, CXL (1963), 289-291.

8. Haley, T. J., "Changes Induced in Brain Activity by Low Doses of X-Irradiation," International Atomic Energy Commission, Christoph Reisser's Söhne, Vienna, 1962.

9. Livshits, N. N., "Physiological Effects of Nuclear Radiation on the Central Nervous System," Advances in Biological and Medical Physics VII, Academic Press, New York, (1960), 173-248.

10. Mendelsohn, M. L., "Response of PAH Transport and Oxygen Consumption in Rabbit Kidney Slices to Graded Doses of X-Ray and Several Metabolic Inhibitors," American Journal of Physiology, CLXXX, (1955), 599-604.

11. Miller, Dorothea Starbuck, "Effects of Low Level Radiation on Audiogenic Convulsive Seizures in Mice," Response of the Nervous System to Ionizing Radiation, ed., T. J. Haley and R. S. Snider, Academic Press, New York, 1962.

12. Ord, M. G. and L. A. Stocken, "Biochemical Lesion in Vivo and in Vitro," Mechanisms in RadioBiology, ed., Maurice Errera and Arne Forssberg, Academic Press, New York, (1961), 259-334.

13. Pauly, H. and B. Rajewsky, "The Effects of X-Irradiation on Metabolism and on Enzymes," Progress in Radiobiology, ed., J. S. Mitchell, B. E. Homes and C. L. Smith, Pub. Charles C. Thomas, Springfield, (1955), 32-43.

14. Snezhko, A. D. "Change in Oxygen Uptake of Brain Tissue After X-Irradiation," Biophysics, II, (1957), 70-82.

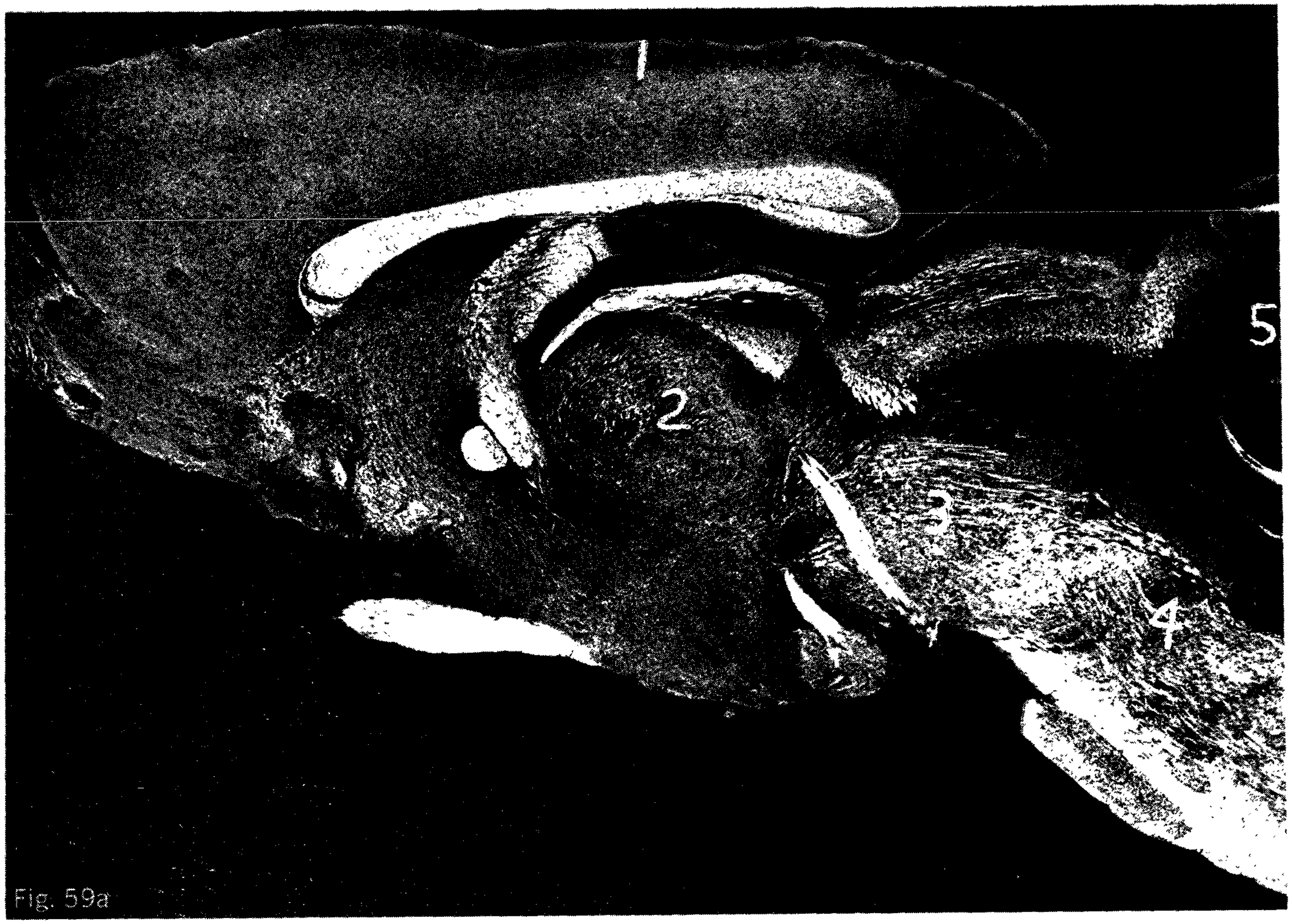
15. Van Cleave, Charles D., Irradiation and the Nervous System, Rowman and Littlefield, Inc., New York, (1963), 271.

CHAPTER II

MATERIALS AND METHODS OF PROCEDURE

Over 200 adult, female Sprague-Dawley rats ranging between 175 and 250 grams in weight were used in this study. In this weight range the brains of adult rats are fairly uniform in size and weight. Fifty rats were used to establish the normal Q_{O_2} values and the experimental format. The specific areas of the brain studied were the cerebral cortex, thalamus, mid-brain, pons-medulla, and cerebellum (Fig. 1).

The standard Warburg manometric technique as outlined in Umbreit _et al_ (1) was used in measuring the rate of oxygen uptake by the various brain tissues. The following procedure was employed. The rats were sacrificed by decapitation. The brains were removed by cutting the skull down the mid-line, and then removing the skull cap. The whole brain was lifted out as the cranial nerves and spinal cord were cut. Sections were cut into slices with a razor blade following the method of Deutsch (2), resulting in tissue thickness ranging between 300 and 500 microns. The slices were blotted quickly on cold damp filter paper, weighed on a torsion balance, and placed in single-arm Warburg flasks containing three milliliters of aerated Krebs-Ringers bicarbonate solution with 1% glucose added. The pH of the incubation media was pH-7.4. Generally, one brain supplied sufficient tissue for one flask for a given



Cortex-1 Thalamus-2 Mid-Brain-3 Pons-Medulla-4 Cerebellum-5

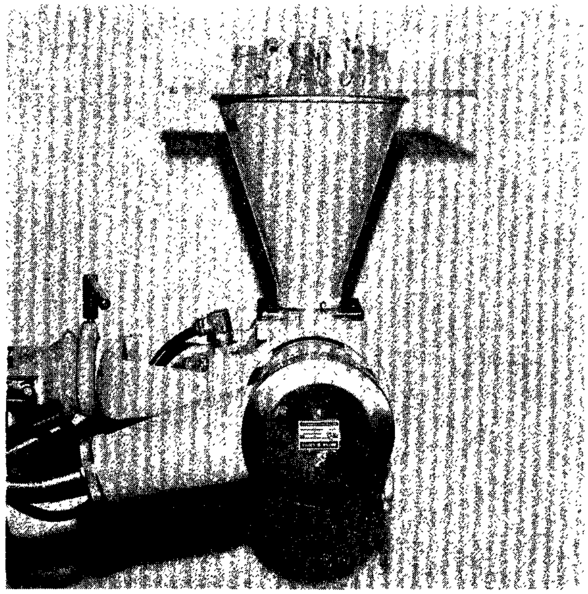
Fig. 2-1 - Showing the various brain areas studied.

brain area. The amount of tissue added to each flask ranged as follows: cortex 85-120 mgm., thalamus 35-50 mgm., mid-brain 70-95 mgm., pons-medulla 150-190 mgm., and cerebellum 140-180 mgm. During handling and slicing, the tissues were maintained in ice bowls at all times. The Krebs-Ringers bicarbonate media consisted of

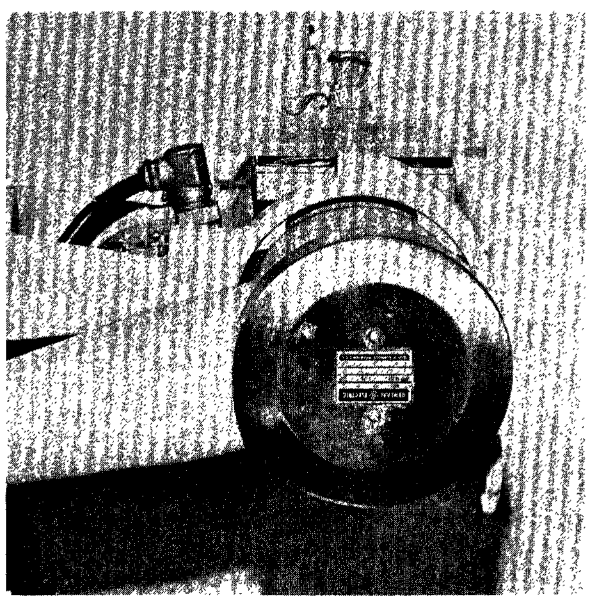
121.0	mM NaCl	1.0	mM $CaCl_2$
5.0	mM KCl	10.8	mM glucose
1.21	mM $MgSO_4$	26.2	mM $NaHCO_3$
1.25	mM KH_2PO_4		

The entire procedure from the time of decapitation through preparation of the flasks required about thirty minutes. Following loading, the flasks were placed in a water bath at 37° C and equilibrated for thrity minutes before the first reading was taken. Readings were taken every ten minutes, corrected for barometric changes and recorded as microliters of oxygen per milligram weight of tissues (wet and dry). A media control flask was run with each experiment in order to check any possible media contamination. Unfiltered irradiation was delivered by a G. E. Beryllium window X-ray unit (120 KVP, 5 ma).

The experiments were divided into a control and a test series. One control group of experiments was run to determine the Qo_2 of the various brain areas. Another control group of runs were made to determine the integrity of the tissues over a relatively long period of time (up to 8-hours post-decapitation). Finally, a sham-irradiated group of experiments was run in which the tissues were treated in the same manner as the irradiated tissues, but did not receive any irradiation. In the test, or irradiated series, one group of animals received 10 Kr at a calculated dose rate of 575 r/min., while another group received 20 Kr at a dose rate of 8,086 r/min. The apparatus used in irradiation of the flasks is shown in Figure 2. Most of the test experiments consisted of a one-hour control period followed by a two-hour post-irradiation period. Following the control period, the flasks were removed from the water bath, their mouths covered with Saranwrap and taken to the irradiation room. The sham-irradiated group was separated from the flasks to be irradiated. Following irradiation, the flasks were returned to the water bath. This entire procedure required between twenty-five and thirty-five minutes. After replacement in the bath, the flasks were allowed thirty minutes to equilibrate before readings were started again. Readings were then taken for at least two hours. From these readings, the oxygen uptake per flask was converted to ug O_2/gm. tissue wet weight/hour and recorded.



A. 10 Kr



B. 20 Kr

Fig. 2-2 -Radiation Apparatus

CHAPTER BIBLIOGRAPHY

1. Deutsch, W., "An Improvement of Warburg's Method for Outing Tissue Slices for Respiratory Experiments," Journal of Physiology, LXXXVII, (1936), 56-57.

2. Umbreit et al, Manometric Techniques, Burgess Publishing Company, Minneapolis, Minnesota, (1964).

CHAPTER III

RESULTS

It should be stated here that the data presented were those taken from experiments in which the tissues exhibited relatively constant respiratory rates during the control period.

Table I shows the Qo_2 values for the various brain areas studied. The wet weight values were converted to dry weight values by multiplying them by the factor of 5.88. It can be seen that a metabolic gradient exists along the neural axis, decreasing from the cortex to the brain stem, but excluding the cerebellum. Figure 1 contains time course curves showing mean oxygen uptake in tissues from the five separate brain areas. Each circle represents mean values for thirty rats. Again, it can be seen that a decreasing rate of respiration from the cortex to the brain stem exists. The cerebellum exhibited respiratory rates similar to that of the thalamic area.

Figure 2 shows the effects of X-irradiation on the rates of oxygen consumption of the cortical tissues. Each circle represents mean values obtained from nine to ten rats. The first reading post-irradiation was made 130 minutes after the initial reading was taken in each experiment. The broken line along the abscissa represents the time period during which the tissues were irradiated and the flasks re-equilibrated in the water bath. Cortical tissues irradiated at 10 Kr exhibited a slight

TABLE I

MEAN Q_2 VALUES FOR VARIOUS BRAIN TISSUE SLICES IN THE RAT

(Media: Krebs-Ringers-Bicarbonate Glucose Added: Temperature 37° C)

Tissue	Number of* Animals	Mean Q_2 (uls/mg dry wgt/hr.)
Cortex	30	7.76
Thalamus	30	4.70
Mid-Brain	30	3.86
Pons-Medulla	30	3.31
Cerebellum	30	5.19

*1 Flask per animal

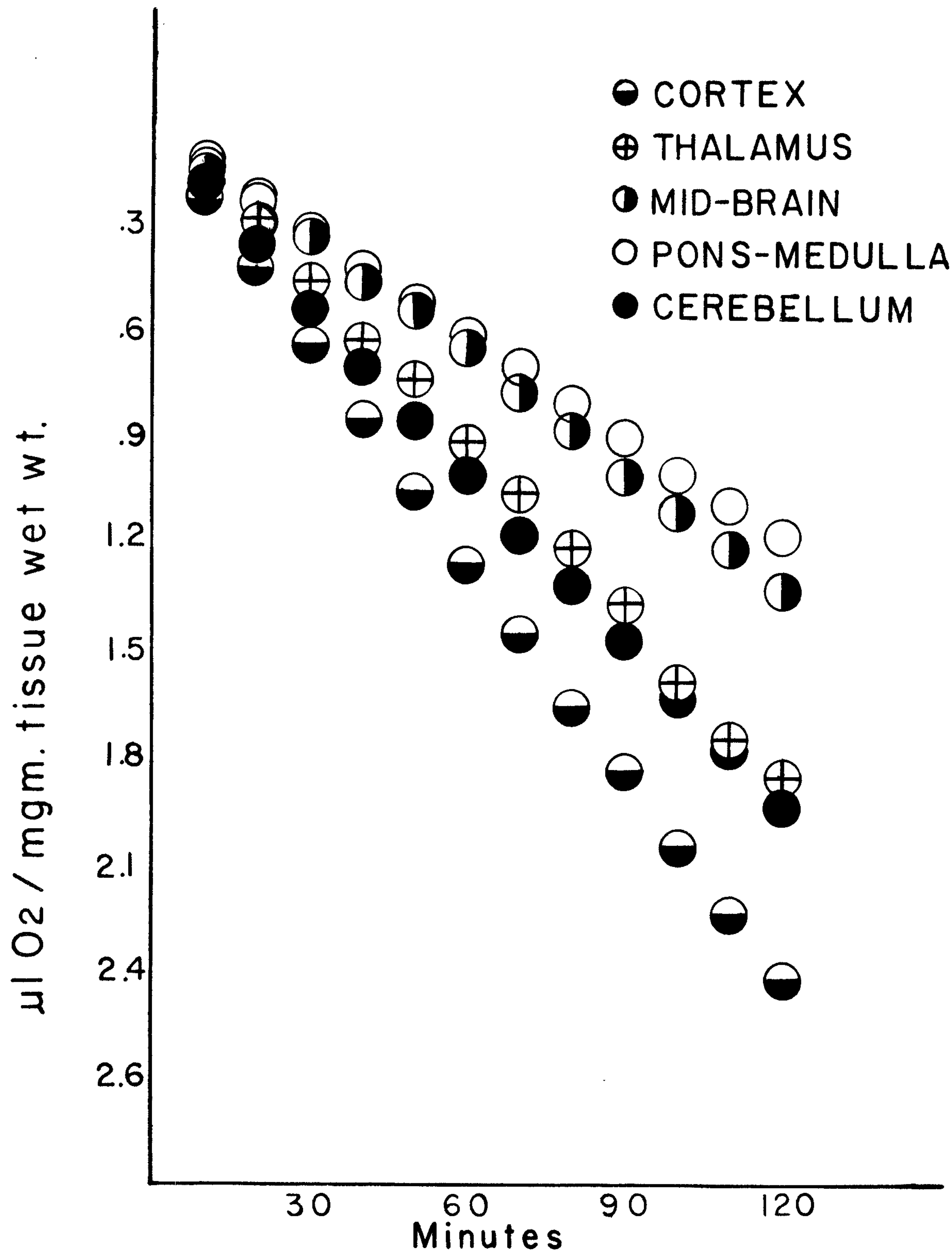


Fig. I—Mean time course curves showing oxygen uptake by various rat brain tissue slices (media-Krebs-Ringers-bicabonate-glucose added pH-7.4).

increase in the rate of oxygen uptake toward the end of the first hour of readings, while during the second hour a gradual decline in respiration occurred. The effects of 20 Kr, however, were more pronounced. An increase during the first hour followed by a sharp and sustained decline in respiration in the second hour was evident. Table II summarizes the data in Figure 2 in terms of net hourly uptake prior to and following X-irradiation. The sham-irradiated tissues exhibited a relatively steady rate of oxygen uptake throughout the length of the run. Those tissues receiving 10 Kr showed almost no change in net oxygen uptake at the end of the first hour, but a fairly significant decrease (19 per cent) was noted in half the experiments by the end of the second hour. The tissues receiving 20 Kr followed a similar directional pattern except that during the second hour the decrease in respiration was greater (29 per cent).

Figure 3 depicts time course curves showing the effects of X-irradiation on thalamic tissues. It is clear that both 10 Kr and 20 Kr had no significant effect on oxygen uptake. These findings are further borne out by the data summarized in Table III. Figure 4 and Table IV contain data indicating that 10 Kr and 20 Kr failed to alter significantly the rate as well as the net hourly oxygen uptake in mid-brain tissue slices. In Figure 5 and Table V there is an indication that 10 Kr and 20 Kr X-irradiation brought about a slight decrease in the oxygen uptake during the second hour post-radiation in pons-medullary tissue slices. No significant change occurred during the first hour following the return of the

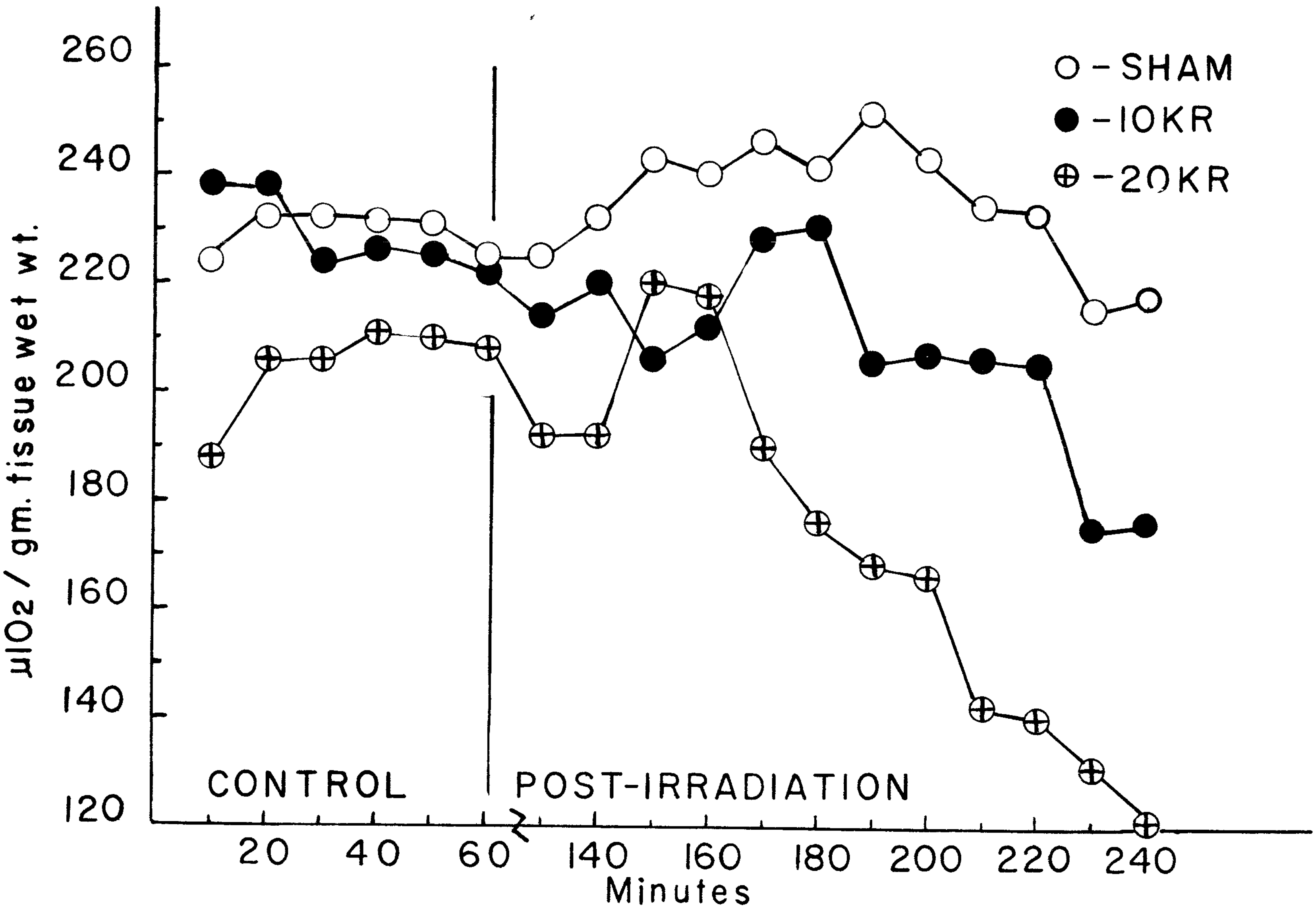


Fig. 3-2 - Effects of x-rays on the respiration of cerebral cortex tissue slices in rats.

TABLE II

EFFECTS OF X-IRRADIATION ON THE OXYGEN UPTAKE IN THE RAT CORTICAL TISSUE SLICES

A. Sham-Irradiated Tissues:

Rat No.	Oxygen Uptake (uls/gm. wet wgt./hr.) Control Period	1 Hour Post *	% Change**	2nd Hour Post	% Change
1.	1533	1393	- 9	1596	+ 4
2.	1531	1355	-11	1637	+ 7
3.	1586	1671	+ 5	1824	+15
4.	1610	1367	-16	1731	+ 8
5.	1376	1547	+12	1032	-25
6.	1264	1417	+12	1219	- 4
7.	1461	1734	+19	1469	+ 1
8.	1408	1423	+ 1	1545	+10
9.	1340	1408	+ 5	1477	+10
10.	1335	1258	- 6	867	-35
Mean	1444 ±113	1457 ±141	+ 1	1440 ±302	0
B. X-Irradiated Tissues (10 Kr):					
1.	1620	1859	+15	1284	-21
2.	1335	1245	- 7	908	-32
3.	1257	1243	- 1	614	-52
4.	1477	1384	- 6	1257	-15
5.	1511	1327	-12	1350	-11
6.	1340	1271	- 5	1305	- 3
7.	1663	1362	-18	1526	- 8
8.	1444	1355	- 6	1488	+ 3
9.	1497	1362	- 9	897	-40
10.	1478	1293	-13	1248	-16
Mean	1462 ±120	1370 ±170	- 6	1188 ±258	-19
C. X-Irradiated Tissues (20 Kr):					
1.	1190	1027	-14	546	-54
2.	1040	1106	+ 6	1090	+ 5
3.	1307	1249	- 4	1199	- 8
4.	1338	1371	+ 2	1008	-25
5.	1347	1337	- 1	1048	-22
6.	1332	1356	+ 2	1152	-14
7.	1378	1288	- 7	835	-39
8.	1190	1120	- 6	630	-47
9.	1285	995	-23	582	-55
Mean	1267 ±104	1205 ±137	- 5	899 ±242	-29

*First hour of measurement Post X-Irradiation

** Oxygen uptake first hour Post/oxygen uptake during control X 100

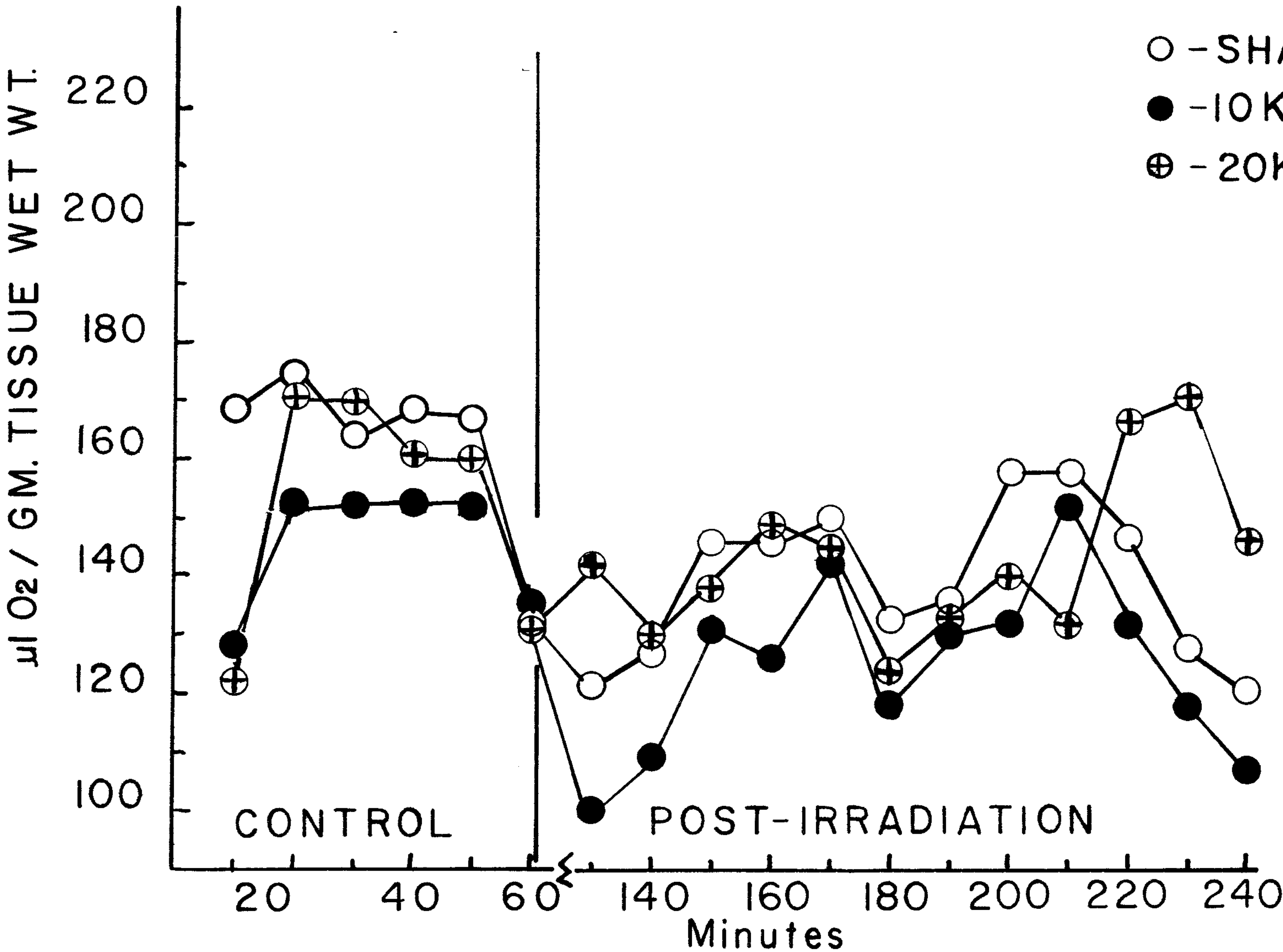


Fig. 3-3 - Effects of x-rays on the respiration of thalamic tissue slices in rats.

TABLE III

EFFECTS OF X-IRRADIATION ON THE OXYGEN UPTAKE IN RAT THALAMIC TISSUE SLICES

A. **Sham-Irradiated Tissues:**

	Oxygen Uptake (ule/gm wet wgt/hr)				
Rat No.	Control Period	1 Hour Post *	% Change**	2nd Hour Post	% Change
1.	938	846	-10	1061	+ 8
2.	1061	965	- 9	1010	- 5
3.	1015	940	- 7	955	- 6
4.	931	533	-43	1011	+ 9
5.	966	843	-13	966	0
6.	940	956	+ 2	705	-25
7.	944	832	-12	902	- 4
8.	1048	767	-27	747	-29
9.	1032	798	-23	1033	0
10.	1056	632	-40	808	-24
Mean	993 ± 70	811 ±132	-18	920 ±117	- 7
B. X-Irradiated Tissues (10 Kr):					
1.	1073	1301	+21	1021	- 5
2.	923	1117	+21	933	+ 1
3.	903	799	-12	483	-47
4.	830	705	-15	553	-33
5.	749	500	-33	899	+20
6.	856	825	- 4	707	-17
7.	914	773	-15	942	+ 3
8.	705	630	-11	880	+25
9.	780	750	- 4	660	-16
Mean	859 ±100	822 ±214	- 4	786 ±172	- 8
C. X-Irradiated Tissues (20 Kr):					
1.	1016	800	-21	832	-18
2.	789	1026	+30	868	+10
3.	900	957	+ 6	866	- 4
4.	967	1003	+ 4	902	- 7
5.	826	829	0	681	-18
6.	1312	1030	-27	862	-34
7.	833	884	+ 6	934	+12
8.	789	827	+ 5	882	+12
9.	1000	868	-13	934	- 7
Mean	937 ±156	914 ± 82	- 2	862 ± 76	- 8

*First hour of measurement Post X-Irradiation

**Oxygen uptake first hour Post/oxygen uptake during control X 100

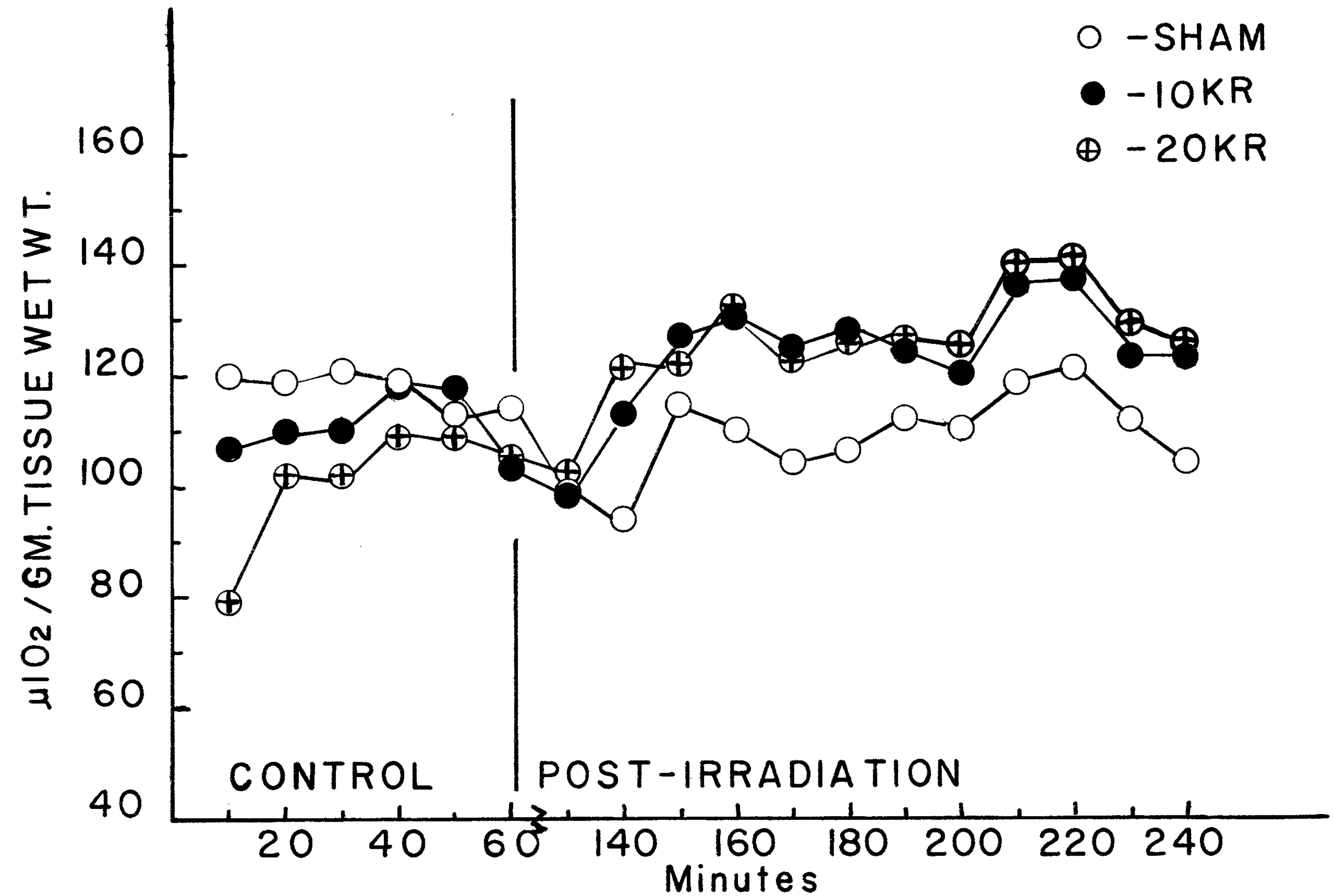


Fig. 3-4 - Effects of x-rays on the respiration of mid-brain tissue slices in rats.

TABLE IV

EFFECTS OF X-IRRADIATION ON THE OXYGEN UPTAKE IN RAT MID-BRAIN TISSUE SLICES

A. Sham-Irradiated Tissues:

	Oxygen Uptake (uls/gm wet wgt/hr)				
Rat No.	Control Period	1 Hour Post *	% Change**	2nd Hour Post	% Change
1.	766	676	-12	833	+ 9
2.	678	576	-15	564	-17
3.	728	664	- 9	770	- 6
4.	781	749	- 4	767	- 2
5.	697	710	+ 2	649	- 7
6.	638	559	-12	580	- 9
7.	656	506	-23	506	-23
8.	681	585	-14	566	-17
9.	600	500	-17	580	- 3
10.	620	615	- 1	534	-14
Mean	684 ± 57	614 ± 80	-10	635 ±108	- 7
B. X-Irradiated Tissues (10 Kr):					
1.	547	827	+51	589	+ 8
2.	601	671	+12	706	+17
3.	674	753	+12	730	+ 8
4.	789	963	+22	926	+17
5.	777	778	0	1007	+30
6.	751	618	-17	851	+13
7.	525	389	-26	448	-15
8.	757	659	-13	698	- 8
9.	676	581	-14	598	-12
10.	507	572	+13	539	+ 6
Mean	660 ±102	681 ±151	+ 3	709 ±161	+ 7
C. X-Irradiated Tissues (20 Kr):					
1.	560	724	+29	1017	+82
2.	701	723	+ 3	849	+17
3.	728	753	+ 3	645	-11
4.	701	796	+14	620	-12
5.	679	758	+12	665	- 2
6.	622	661	+ 6	583	- 6
7.	782	715	- 9	688	-12
8.	590	490	-17	714	+21
Mean	670 ±115	703 ± 76	+ 4	723 ±132	+ 8

*First hour of measurement Post X-Irradiation

**Oxygen uptake first hour Post/oxygen uptake during control X 100

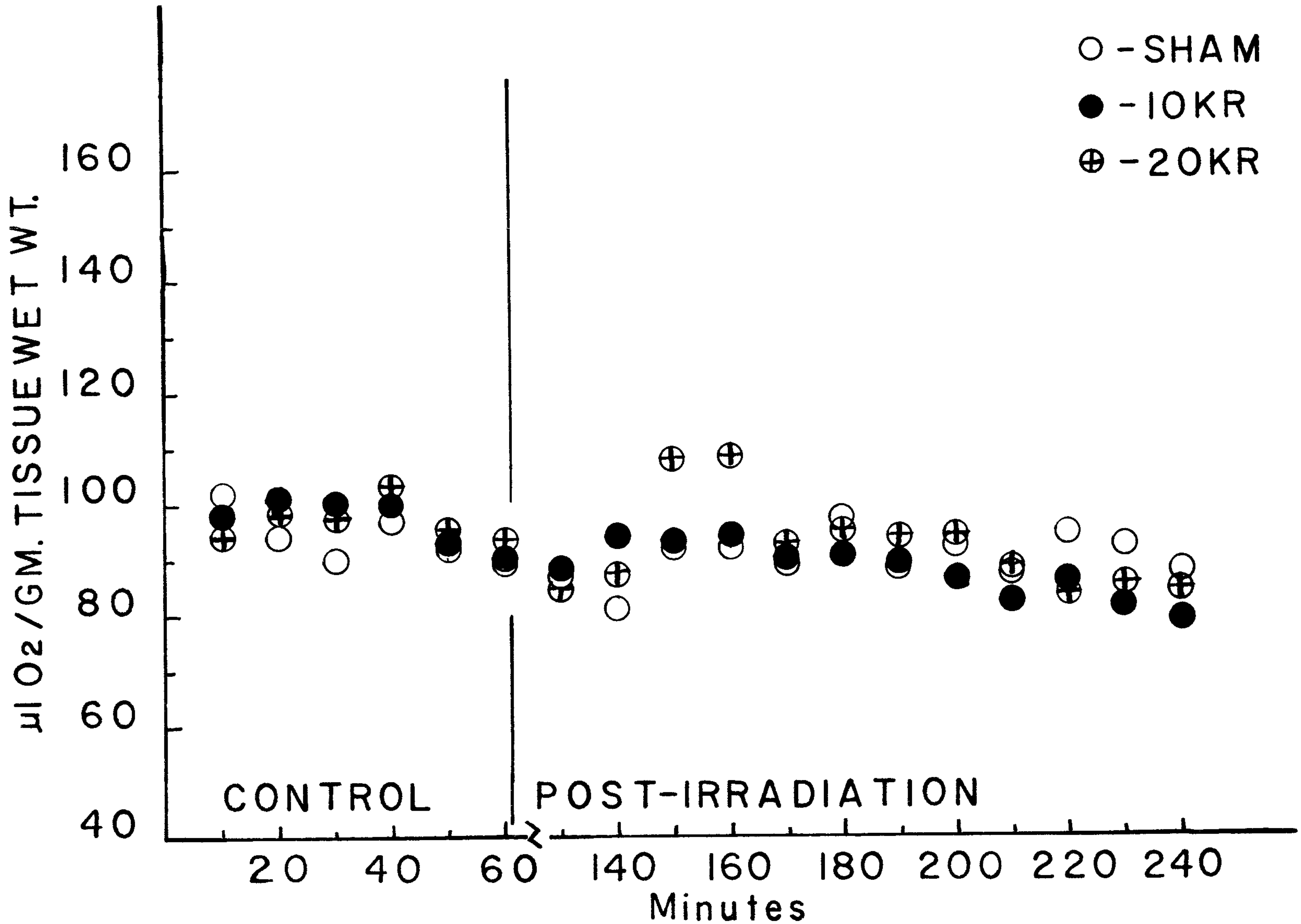


Fig. 3-5 - Effects of x-rays on the respiration of pons-medullary tissue slices in rats.

TABLE V

EFFECTS OF X-IRRADIATION ON THE OXYGEN UPTAKE IN RAT PONS—MEDULLARY TISSUE SLICES

A. Sham-Irradiated Tissues:

	Oxygen Uptake (uls/gm wet wgt/hr)				
Rat No.	Control Period	1 Hour Post *	% Change**	2nd Hour Post	% Change
1.	553	457	-17	442	-20
2.	542	488	-10	542	0
3.	564	574	+ 2	524	- 7
4.	595	585	- 2	586	- 2
5.	560	560	0	534	- 5
6.	605	563	- 7	579	- 4
7.	574	575	0	537	- 6
8.	594	587	- 1	554	- 7
9.	611	532	-13	575	- 6
10.	612	678	+11	586	- 4
Mean	581 ± 25	560 ± 41	- 4	546 ± 41	- 6
B. X-Irradiated Tissues (10 Kr):					
1.	708	660	- 7	610	-14
2.	642	713	+11	551	-14
3.	585	548	- 6	527	-10
4.	762	654	-14	527	-31
5.	762	558	-27	566	-26
6.	606	572	- 6	570	- 6
7.	533	533	0	510	- 4
8.	610	495	-19	519	-15
9.	634	595	- 6	495	-22
Mean	649 ± 64	559 ± 73	-12	541 ±35	-17
C. X-Irradiated Tissues (20 Kr):					
1.	452	431	- 5	440	- 3
2.	582	550	- 5	549	- 6
3.	493	504	+ 2	444	-10
4.	787	690	-12	594	-25
5.	754	589	-22	553	-27
6.	679	622	- 8	556	-18
7.	529	449	-15	399	-25
8.	589	617	+ 5	655	+11
Mean	608 ±128	557 ± 82	- 8	524 ± 81	-14

*First hour of measurement Post X-Irradiation

**Oxygen uptake first hour Post/oxygen uptake during control X 100

flasks to the bath post-radiation. In Figure 6 it appears that the sham-irradiated cerebellar tissue slices were reaching either a substrate depletion or a state of cellular dissolution. This finding was indicated by the fact that during the second hour the tissue respiration fell to 23 per cent below the control levels. Interestingly enough, however, the irradiated tissues failed to exhibit the same degree of inhibition of respiration.

The overall data was summarized in Table VII. It is clear that the cortex and the pons-medullary tissues were the more radiosensitive. Moreover, it may be noted that the greatest change occurred during the second hour post-equilibration. On the other hand, the thalamus and mid-brain appear to be relatively radio-insensitive to considerably high dosages of X-irradiation.

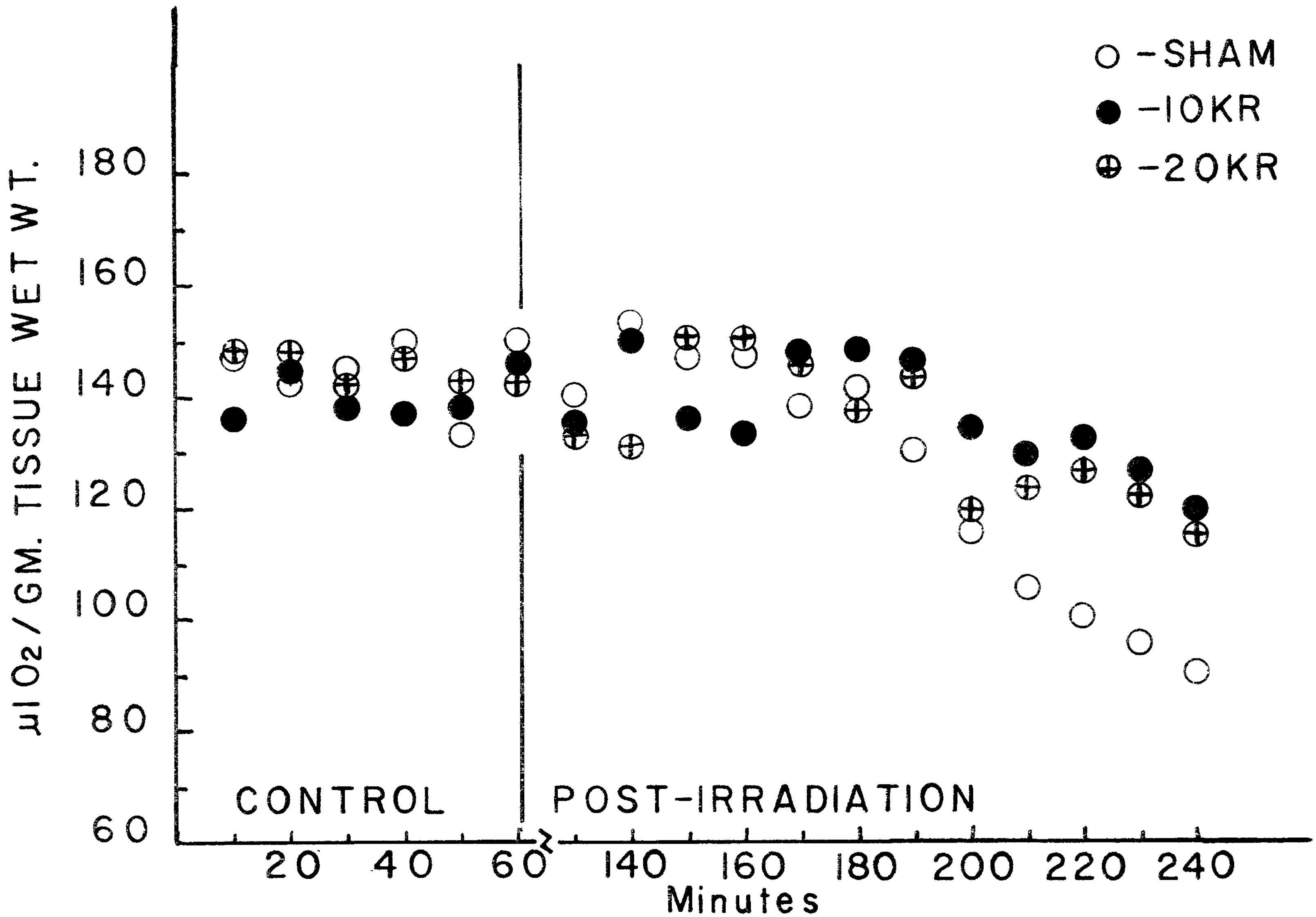


Fig. 3-6 - Effects of x-rays on the respiration of cerebellar tissue slices in rats.

TABLE VI

EFFECTS OF X-IRRADIATION ON THE OXYGEN UPTAKE IN RAT CEREBELLAR TISSUE SLICES

A. Sham-Irradiated Tissues:

Rat No.	Control Period	1 Hour Post *	% Change**	2nd Hour Post	% Change
	Oxygen Uptake (uls/gm wet wgt/hr)				
1.	623	654	+ 5	572	- 8
2.	771	663	-14	361	-53
3.	868	861	- 1	818	- 6
4.	825	830	+ 1	809	- 2
5.	885	945	+ 7	580	-34
6.	828	774	- 7	407	-51
7.	952	922	- 3	718	-25
8.	768	709	- 8	577	-25
9.	632	632	0	662	+ 4
Mean	794 ±106	777 ±111	- 2	612 ±158	-23
B. X-Irradiated Tissues (10 Kr):					
1.	810	829	+ 2	652	-20
2.	823	871	+ 6	651	-21
3.	886	858	- 3	648	-27
4.	795	712	-10	1020	+28
5.	718	760	+ 6	746	+ 4
6.	712	705	- 1	723	+ 2
7.	720	735	+ 2	846	+18
8.	806	840	+ 4	888	+10
9.	880	784	-11	938	+ 7
10.	941	955	+ 1	742	-21
Mean	819 ± 74	805 ± 77	- 2	785 ±122	- 4
C. X-Irradiated Tissues (20 Kr):					
1.	873	855	- 2	666	-24
2.	1038	1094	+ 5	1184	+14
3.	1121	1002	-11	949	-16
4.	796	822	+ 3	813	+ 2
5.	787	780	- 1	606	-23
6.	642	631	- 2	555	-14
7.	770	831	+ 8	715	- 7
8.	1042	960	- 8	718	-31
Mean	884 ±141	872 ±147	- 1	776 ±194	-12

*First hour of measurement Post X-Irradiation

**Oxygen uptake first hour Post/oxygen uptake during control X 100

TABLE VII

SUMMARY OF THE EFFECTS OF X-IRRADIATION ON RESPIRATION IN EXCISED BRAIN TISSUES

		Mean Oxygen Uptake (uls/gm wet wgt/hr)				
Tissue	No. Rats	Control Period	1st Hour Post	Per Cent Change	2nd Hour Post	Per Cent Change
1. Cortex						
A. Sham	10	1444 ± 113	1457 ± 141	0	1440 +302	0
B. 10 Kr	10	1462 ± 120	1370 ± 170	- 6	1188 ± 258	-19
C. 20 Kr	9	1267 ± 104	1205 ± 137	- 5	899 ± 242	-29
2. Thalamus						
A. Sham	10	993 ± 70	811 ± 132	-18	920 ± 117	- 7
B. 10 Kr	9	859 ± 100	822 ± 214	- 4	786 ± 172	- 8
C. 20 Kr	9	937 ± 156	914 ± 84	- 2	862 ± 76	- 8
3. Mid-Brain						
A. Sham	10	684 ± 57	614 ± 80	-10	635 ± 108	- 7
B. 10 Kr	10	660 ± 102	681 ± 151	+ 3	709 ± 161	+ 7
C. 20 Kr	8	670 ± 115	703 ± 96	+ 4	723 ± 132	+ 8
4. Pons-Medullary						
A. Sham	10	581 ± 25	560 ± 41	- 4	546 ± 41	- 6
B. 10 Kr	9	649 ± 64	559 ± 73	-12	541 ± 35	-17
C. 20 Kr	8	608 ± 128	557 ± 82	- 8	524 ± 81	-14
5. Cerebellum						
A. Sham	9	794 ± 106	777 ± 111	- 2	612 ± 158	-23
B. 10 Kr	10	819 ± 74	805 ± 77	- 2	785 ± 122	- 4
C. 20 Kr	8	884 ± 141	872 ± 147	- 1	776 ± 194	-12

CHAPTER IV

DISCUSSION

The metabolic gradient in the rat brain found in the work presented here has been observed in other animals, including cats and rats (14), dogs (7, 14) and monkeys (23). The respiratory quotients reported in this work fall in the lower range of figures given by other workers for similar brain areas. A number of factors, however, could explain the lower respiratory quotients obtained in this work. Among these factors are the type of media used, gassing the flasks (this was not done here) and the buffering system used in the media (8, 12).

It is difficult to compare the results obtained from the X-irradiated tissues with those of other workers, due to the differences in method of tissue preparation, the amount of irradiation used, and the method of irradiating the tissue, *id est*, *in vivo* versus *in vitro* radiation. Moreover, the study of whole brain respiration made it impractical to ascertain any differential effects of X-irradiation on the various brain areas. Florsheim (11) found no change in the oxygen consumption of minced mice brains either immediately or nineteen hours after 500-800 r whole body X-irradiation. Snezhko (24) reported an increased oxygen tension in the motor cortex of the *intact* rabbits after whole body doses of 900 r to 1500 r or doses to the head amounting to 1100 r to 3000 r. He concluded that the increased oxygen tension denoted a decrease in oxygen consumption. He also observed these effects to be phasic in character during a seven-hour post-irradiation period. This is not an uncommon observation in manometric determinations according to Umbreit _et al_ (25). The results presented in this study were _not_ in agreement with those reported by Egana using an internal Beta-emitter as a radiation source. Egana (9) reported an _increase_ in respiration in _all_ rat brain areas, including the cortex, hypothalamus, and diencephalon, two hourse after exposure to relatively low amount of Beta-irradiation. A number of differences exist which might explain the differences in results obtained by Egana and those reported here. The types of irradiation were different; _id est_, Beta versus X-rays; the dose rate and total dosages were different, and finally Egana used _in vivo_ irradiation as opposed to _in vitro_ irradiation of the tissues. Some workers studying the respiration of non-neutral tissues irradiated _in vitro_ reported no effects below 10 KR (1, 18, 21). Moreover, Ord and Stocken (19) stated that 10^5 and 10^6 roentgens were required before appreciable reductions (37-50%) could be detected in tissues such as the liver, muscle, kidney, spleen and thymus irradiated _in vitro._ These finding vary from those of Barron (2) who reported that in general, the respiration of tissues from the spleen, liver, kidney, thymus, adrenals, and testes were all diminished immediately after receiving

900-1000 r X-irradiation. Again, it was unfortunate for him that Barron irradiated the whole animal prior to removal of the tissues. These foregoing reports clearly indicate conflicts that need clarification with more studies and uniform techniques.

In attempting to explain the effects of ionizing radiation on the oxidative metabolism of isolated tissues, several factors have been considered. First, the effects of irradiation on specific enzyme systems have been assayed using various biochemical methods. Commarano (5) studying ascites hepatoma cells X-irradiated with 10-100 Kr found that both anerobic and aerobic glycolysis decreased in proportion to the increase in irradiation. Along this same line, Clark and Land (6) studying mitochondrial fragments in rat liver claimed that the oxidative system was more radio-resistant than the phosphorylative system. Egana (9) found that Beta-radiation brought about an increase in oxygen uptake in rat brain tissue slices but glycolysis and glucose utilization was decreased. He concluded that the tissues were using some substrate other than glucose. Barron (2) reported that the oxidation of substrates requiring sulfhydryl enzymes were diminished after 900 r whole body X-irradiation. Belonskii and Rusev (3) found at doses up to 1500 r whole body X-irradiation, the cytochrome oxidase activity increased in both the medulla and the cortex while the succinic dehydrogenase activity decreased in both tissues. At 20 Kr, the cytochrome oxidase activity increased in both areas, whereas, the succinic dehydrogenase increased in the cortex but decreased in the medulla. Egana and Velarde (10) reported that hydrogen peroxide, a product of irradiated water, stimulated respiration of control brain tissue slices at low concentrations (0.001 mM) but not the irradiated tissues. Respiration was inhibited in both control and irradiated tissues at concentrations higher than 0.004 mM. The role of the various free radicals that may be formed in irradiated water is thought to be important since nervous tissue has a high water content (80-84 per cent). No effect on specific enzyme systems were indicated by the present data. Since the data presented here showed that only the cortical and the pons- medullary tissues exhibited a decrease in respiration, it was tempting to conclude that perhaps there is a difference in sensitivity in the brain areas to oxidizing radicals.

A second consideration of the possible effects of X-Irradiation on the nervous system is the differences in morphology and microscopic anatomy of the brain areas studied. Reviews of the cellular make-up and the metabolism of the cerebrum may be found in a rairly recent volume of Kety and Elkes (17). The distribution of functional neuronal cells, glial cells, and white matter in the various areas have a profound effect on the many physiological characteristics of the tissues including oxidative metabolism. The cortex, thalamus, and cerebellar tissues contain a relatively high amount of gray matter, whereas, the mid-brain and the pons-medully contain a greater amount of glial tissue and white matter. Dixon and Meyer (7) and Himwich et al (15) have presented considerable evidence to the fact that white matter respires at a lower rate than gray matter. Moreover, Hess (13) has pointed out with substantial evidence that in white matter where oligodendroglia predominate the respiratory rate is about 40 per cent higher than in the cortical astrocytes. On the other hand, she claims that in the gray matter as a whole, over 90 per cent of the respiration may be attributed to neurons and their processes. The most difficult finding to account for was the fact that only the cortical tissues with supposedly the highest neuronal cell distribution and the pons-medullary slices which contain relatively small amounts of neuronal cells exhibited radiosensitivity. The thalamus and the mid-brain slices were virtually unaffected by the relatively high dosages of X-Irradiation. One might explain the cortical inhibition on the basis of increased enzymatic concentration, and therefore, increased number of susceptible "targets."

A third possible mode of action by radiation on tissue respiration concerns permeability changes. Such changes have been reported by Brinkman and his group (4). Ontko et al (19) studying respiration of isolated Ehrlich ascites tumour cells in mice found that following 1250 r whole body irradiation, the cells showed an increase in respiration of 56 per cent. Moreover, they found that the irradiated cells contained more nitrogen. They concluded that the increase in oxygen uptake was due either to an increase in substrate concentration by way of permeability changes or to some reaction by which stored substrate was converted into more readily oxidized form. The data presented here did not indicate physical disruption of the membranes as would be expected at the extremely high dose of 20 Kr. This, in turn, would indicate biochemical changes.

It was difficult to explain the sustained drop in oxygen uptake in the sham-irradiated cerebellar slices following re-equilibration on the basis of substrate depletion alone, according to Hosein *et al* (16). These workers found that cortical brain slices respired in the absence of *any media* at rates equal to those slices incubated in media for periods up to thirty minutes. The fact that the irradiated cerebellar slices did not exhibit such a decrease, indicates some other kind of biochemical change *id est*, an increase in oxidation or an increase in permeability.

Summarily, the most important findings in this study were as follows:

1. One can alter oxygen uptake in isolated brain tissues by X-Irradiation *in vitro*.
2. The cortex and the pons-medullary areas appear to be more radio-sensitive in terms of respiratory changes than the thalamus and mid-brain.
3. The Q_{O_2} of isolated brain tissues show a decreasing metabolic gradient from cortex to brain stem that can be altered with X-irradiation.
4. The changes in the tissue respiration observed occurred in the absence of any circulating humoral agents known to exist in irradiated animals.

5. The respiratory changes that occur in X-Irradiated brain tissues may not be explained in terms of biochemical changes alone. Permeability changes and the cellular morphology of the given areas must also be considered.

CHAPTER BIBLIOGRAPHY

1. Aurand, K. and H. Pauley, "Zui Beeinflussung der Gebeatmung durch Rontengstrahlen," Zeitschrift fur Naturforschung," IX, No. 9b, (1954), 506-507.

2. Barron, E. S. Guzman, "Effects of X-Rays on Tissue Metabolism," Biological Effects of External X and Gamma Irradiation, ed., R. E. Zirkle, McGraw-Hill, New York (1954), 412-428.

3. Belokonskii, I. and G. Rusev, "Oxidation Processes in Early Radiation Reaction," Biophysics, IV (1959), 83-87.

4. Brinkman, R., Lamberts, H. B., Wadel, J. and M. R. Zuideveld, "Contributions of the Study of Immediate and Early X-ray Reactions with Regard to Chemoprotection," International Journal of Radiation Biology, III (1961), 205-210.

5. Cammarano, P., "Protein Synthesis; Glycolysis, and Oxygen Uptake in Hepatoma Cells Irradiated In Vitro," Radiation Research, XVIII (1963), 1-11.

6. Clarke, I. D. and J. H. Lang, "The Inactivation of Mitochondrial Enzymes by X-Radiation In Vitro," Radiation Research, XXIV (1965), 142.

7. Dixon, T. F. and Alfred Myer, "Respiration of Brain," American Journal of Physiology, XXX, No. 2 (1936), 1577.

8. Durell, J. and P. J. Heald, "The Effects of Potassium Ion Concentration on Phosphate Metabolism in Cerebral Slices," Journal of Neurochemistry, IX (1962), 71-79.

9. Egana, Enrique, "Some Effects of Ionizing Radiation on the Metabolism of the Central Nervous System," International Journal of Neurology, III, No. 3-4, (1962) 631-647.

10. Egana, Enrique and Maria I. Velarde, "A Comparative Study of Normal and Beta-Irradiated Rats, The Effects of Hydrogen Peroxide on the Respiration of the Central Nervous System," Comparative Neurochemistry Symposium, Austria, (1962), 275-278.

11. Florsheim, W. and K. C. Dorenbock and M. E. Morton, "Effect of X-Ray on Radioactive Phosphorus Turnover and Oxygen Consumption of Brain," Proceedings of Society of Experimental Biology and Medicine, LXXXI (1952) 121-122.

12. Hertz, L. and M. Schou, "Univalent Cations and Respiration of Brain Cortex Slices," Biochemistry Journal, LXXXV, No. 1, (1962), 93-104.

13. Hess, Helen, H., "The Rates of Respiration of Neurons and Neuroglia in Human Cerebrum," Regional Neurochemistry, ed. by S. S. Kety and J. Elkes, Pergamon Press, New York (1964), 200-211.

14. Himwich, H. E., P. S. Ykowski and J. F. Fazekas, "A Comparative Study of Excised Cerebral Tissue of Adult and Infant Rats," American Journal of Physiology, XXXII, (1941), 293-296.

15. Himwich, H. E. and Fazekas, J.E. "Metabolism of the Brain of Infant and Adult Dogs," American Journal Physiology, CXXXII (1941), 454-465.

16. Hosein, E. A., M. Emblem, S. Rochon, and S. Morch, "Tissue Slice Respiration in The Absence of Suspension Media," Archives of Biochemistry and Biophysics, XCIX (1962), 414-417.

17. Kety, S. S., and J. Elkes. Regional Neurochemistry, Pergamon Press, New York, (1961).

18. Mendelsohn, M. L., "Response of PAH Transport and Oxygen Consumption in Rabbit Kidney Slices to Graded Doses of X-Ray and Metabolic Inhibitors," American Journal of Physiology, CLXXX (1955), 599-604

19. Ontko, J. A., R. Wells, and Moorehead, "Increased Endogenous Respiration of Ascites Tumor Cell after Radiation Exposure," Radiation Research, XXIII (1964), 135-144.

20. Ord, M. G. and L. A. Stocken, "The Biochemical Lesion in Vivo and in Vitro," Mechanisms in Radiobiology, ed. M. Errera and A. Forssberg, New York, Academic Press, (1961), 281.

21. Pauly, H. and B. Rajewsky, "The effect of X-Irradiation on Metabolism and on Enzymes," Progress in Radiobiology, ed. J. S. Smith B. E. Holmes and C. L. Smith, Charles C. Thomas (1955), 32-43.

22. Pearce, Jane and R. W. Gerard, "The Respiration of Neurons," American Journal of Physiology, CXXXIV (1942), 49-65.

23. Schmidt, Carl F., Seymour S. Kety and Harry H. Pennes, "The Gaseous Metabolism of the Brain of the Monkey," American Journal of Physiology, CIII (1945), 33-39.

24. Snezhko, A. D., "On Changes in Oxygen Uptake of Brain Tissue After X-Irradiation," Biophysics, II, (1957), 70-82.

25. Umbreit, W. W., R. H. Burris and J. F. Stauffer, Manometric Techniques, Burgess Publishing Company, Minneapolis, 1964.

CHAPTER V

SUMMARY

The changes in oxygen uptake in various brain tissues in rats X-irradiated _in vitro_ was determined manometrically. The specific areas studied were, the cerebral cortex, the thalamus, mid-brain, pons-medully and cerebellum. The dosages of X-irradiation used were 10 Kr and 20 Kr. The important findings were that (1) the Qo_2 values of isolated brain tissues show a decreasing metabolic gradient from cortex to brain stem that can be altered with X-irradiation. (2) Respiration of the cortex and pons-medullary tissues were inhibited during the second hour post-radiation following 20 Kr, whereas, the thalamic, mid-brain and cerebellar slices exhibited little change to these dosages. (3) The changes observed occurred in the absence of circulating humoral agents indicating a local effect of the ionizing radiation. The results were discussed in terms of changes in biochemical integrity, permeability, and on the basis of differences in cellular morphology of the tissues studied.

BIBLIOGRAPHY

BOOKS

Barron, E. S. Guzman, "Effects of X-Rays on Tissue Metabolism," Biological Effects of External X and Gamma Irradiation, edited by R. E. Zirkle, McGraw-Hill, New York (1954), 412-428.

Egana, Enrique, and Maria I. Velarde, "A Comparative Study of Normal and Beta-Irradiated Rats, The Effects of Hydrogen Peroxide on the Respiration of the Central Nervous System," Comparative Neurochemistry Symposium, Austria, (1962), 275-278.

Errera, Maurice, and Arne Forssberg, Mechanisms in Radiobiology, I, General Principles, Academic Press, New York, (1961) 261.

Haley, T. J., "Changes Induced in Brain Activity by Low Doses of X-Irradiation," Effects of Ionizing Radiation on the Nervous System, International Atomic Energy Commission, Christoph Reisser's Söhne, Vienna, 1962.

Hess, Helen, H., "The Rates of Respiration of Neurons and Neuroglia in Human Cerebrum," Regional Neurochemistry, edited by S. S. Kety and J. Elkes, Pergamon Press, New York (1964), 200-211.

Kety, S. S., and J. Elkes, Regional Neurochemistry, Pergamon Press, New York, (1961).

Livshits, N. N., "Physiological Effects of Nuclear Radiation on the Central Nervous System," Advances in Biological and Medical Physics, VII, Academic Press, New York, (1960), 173-248.

Miller, Dorothea Starbuck, "Effects of Low Level Radiation on Audiogenic Convulsive Seizures in Mice," Response of the Nervous System to Ionizing Radiation, edited by T. J. Haley and R.S. Snider, Academic Press, New York, 1962.

Ord, M. G., and L. A. Stocken, "Biochemical Lesion in Vivo and in Vitro," Mechanisms in Radiobiology, edited by Maurice Errera and Arne Forssberg, Academic Press, New York, (1961), 259-334.

Pauly, H. and B. Rajewsky, "The Effects of X-Irradiation on Metabolism and on Enzymes," Progress in Radiobiology, edited by J. S. Mitchell, B. E. Homes, and C. L. Smith, Charles C. Thomas, Springfield, (1955).

Umbreit, W. W., R. H. Burris, and J. F. Stauffer, Manometric Techniques, Burgess Publishing Company, Minneapolis, 1964.

Van Cleave, Charles D., Irradiation and the Nervous System, Rowman and Littlefield, Inc., New York, (1963).

ARTICLES

Aurand, K. and H. Pauley, "Zui Beeinflussung der Gebeatmung durch Rontengstrahlen," Zeitschrift fur Naturforschung, IX, No. 9B, (1954), 506-507.

Belokonskii, I. and G. Rusev, "Oxidation Processes in Early Radiation Reaction," Biophysics, IV (1959), 83-87.

Brinkman, R., Lamberts, H. B., Wadel, J. and M. R. Zuideveld, "Contributions of the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection," International Journal of Radiation Biology, III (1961), 205-210.

Cammarano, P., "Protein Synthesis; Glycolysis, and Oxygen Uptake in Hepatoma Cells Irradiated In Vitro," Radiation Research, XVIII (1963), 1-11.

Clarke, I. D., and J. H. Lang, "The Inactivation of Mitochondrial Enzymes by X-Radiation In Vitro," Radiation Research, XXIV, (1965), 142.

Deutsch, W., "An Improvement of Warburg's Method for Outing Tissue Slices for Respiratory Experiments," Journal of Physiology, LXXXVII, 56-57.

Dixon, T. F., and Alfred Myer, "Respiration of Brain," American Journal of Physiology, XXX, No. 2, (1936), 1577.

Durell, J., and P. J. Heald, "The Effects of Potassium Ion Concentration on Phosphate Metabolism in Cerebral Slices," Journal of Neurochemistry, IX, (1962), 71-79.

Egana, Enrique, "Some Effects of Ionizing Radiation on the Metabolism of the Central Nervous System." International Journal of Neurology, III, No. 3-4, (1962), 631-647.

Florsheim, W., K. C. Dorenbock, and M. E. Morton, "Effects of X-Ray on Radioactive Phosphorus Turnover and Oxygen Consumption of Brain," Proceedings of Society of Experimental Biology and Medicine, LXXXI (1952), 121-122.

Garcia, J., N. A. Buchwald, G. Bach y Rita, B. H. Feder, R. A. Koelling, "Electroencephalographic Responses to Ionizing Radiation," Science, CXL (1963), 289-291.

Garcia, J., and D. J. Kimeldorf, "Some Factors Which Influence Radiation Conditioned Behavior of Rats," Radiation Research, XII, (1960), 719-722.

Hertz, L., and M. Schou, "Univalent Cations and Respiration of Brain Cortex Slices," Biochemistry Journal, LXXXV, No. 1, (1962), 93-104.

Himwich, H. E., P. S. Ykowski, and J. F. Fazekas, "A Comparative Study of Excised Cerebral Tissue of Adult and Infant Rats," American Journal of Physiology, XXXII, (1941), 293-296.

Himwich, H. E., and Fazekas, J. E., "Metabolism of the Brain of Infant and Adult Dogs," American Journal of Physiology, CXXXII (1941), 454-465.

Hosein, E. A., M. Emblem, S. Rochon, and S. Morch, "Tissue Slice Respiration in the Absence of Suspension Media," Archives of Biochemistry and Biophysics, XCIX (1962), 414-417.

Mendelsohn, M. L., "Response of PAH Transport and Oxygen Consumption in Rabbit Kidney Slices to Graded Doses of X-Ray and Metabolic Inhibitors," American Journal of Physiology, CLXXX, (1955), 599-604.

Ontko, J. A., R. Wells, and Moorehead, "Increased Endogenous Respiration of Ascites Tumor Cell After Radiation Exposure," Radiation Research, XXIII (1964), 135-144.

Pearce, Jane, and R. W. Gerard, "The Respiration of Neurons," American Journal of Physiology, CXXXIV (1942, 49-65.

Schmidt, Carl F., Seymour S. Kety, and Harry H. Pennes, "The Gaseous Metabolism of the Brain of the Monkey," American Journal of Physiology, CIII, (1945), 33-39.

Snezhko, A. D., "On Changes in Oxygen Uptake of Brain Tissue After X-Irradiation," Biophysics, II, (1957), 70-82.

PART II

SEROTONIN CONTENT IN RAT BRAIN TISSUES X-IRRADIATED *IN VITRO*

CHAPTER VI

INTRODUCTION

Although the roles of serotonin in the nervous system are not known, it has been implicated in numerous functional and behavioral changes. Recently, Brinkman and Veninga (1, 10) and Palaic *et al* (6) have indicated that some of the damage and symptoms found in radiation injury may be due to the release of various bioamines including nor-epinephrine and 5-hydroxytryptamine (serotonin). The nature and suspected roles of these various amines in the nervous system has been recently reviewed by Freedman and Giarman (4). The neurological symptoms associated with the radiation syndrome as well as the radiation protection capacity of serotonin (8) have stimulated much work on the effects of ionizing radiation on serotonin metabolism.

Previous work on the effects of whole body irradiation on the serotonin level in the brain has been conflicting. Ershoff and Gal (3) found no significant change in the serotonin content of whole rat brains after whole body irradiation. Randic *et al* (7) also found no significant changes in the serotonin level of whole rat brains following 900 r whole body irradiation, however, in adrenalectomized rats, they did note a significant decrease in the serotonin level. Speck (9) on the other hand reported a decrease in the serotonin level of whole rat brain eighteen hours after 4500 r and immediately after 9000 r. Egana (2) measured the serotonin content of specific brain areas at various time intervals after injecting rats with P^{32}, an internal Beta-emitter (2). At two hours post-injection he noted an increased serotonin content in the hypothalamus and mid-brain while showing a slight decrease in the cerebral cortex and olfactory bulbs. Palaic et al (5) reported a decrease in the serotonin content of the brain stem after a dose of 900 r whole body X-irradiation. At the same time they found an increase in the content of the total 5-hydroxindole compounds in both the whole brain and the brain stem.

Since most of the foregoing work was carried on using whole brain tissues prepared following irradiation, it seemed feasible that a study of serotonin changes in tissues removed from specific brain areas and irradiated in vitro might be more fruitful. One important advantage of studying tissues irradiated in vitro is that various indirect factors such as circulating humoral agents and/or toxins produced elsewhere as the result of whole body irradiation are negated.

The specific aims of this study were threefold: (1) To determine the effects of ionizing radiation on the serotonin content in specific brain areas; (2) to ascertain whether or not there was a differential in sensitivity to ionizing radiation in these various areas as indicated by altered serotonin levels, and; (3) to shed some light on the relationship between serotonin content and radiation damage to the central nervous system.

CHAPTER BIBLIOGRAPHY

1. Brinkman, R. and T. S. Veninga, "Contributions to the Study of Immediate and Early X-ray Reactions with Regard to Chemoprotection, V Liberations of Serotonin (and Other Amines) in the Frog After X-Irradiation," International Journal of Radiation Biology, IV No. 3 (1962), 249-254.

2. Egana, Enrique, "Some Effects of Ionizing Radiation of the Metabolism of the Central Nervous System," International Journal of Neurology, III Nos. 3-4 (1962), 631-647.

3. Ershoff, B. H. and E. M. Gal, "Effect of Radiation on Tissue Serotonin Levels in the Rat," Society of Experimental Biology and Medicine, CVIII, (1961), 160-161.

4. Freedman, Daniel X. and Nicholas J. Giarman, "Brain Amines, Electrical Activity and Behavior," EEG and Behavior, Edited Gilbert H. Glaser, New York, Basic Books, Inc., (1963), 198-243.

5. Palaic, D., M. Randic, and Z. Supek, "5-Hydroxyindole Compounds in Rat Brains After Irradiation," Journal of Neurochemistry, XI, No. 10 (1964), 761-762.

6. Palaic, D. M. and Z Supek, "Drug-Induced Changes of the Metabolism of 5-Hydroxytryptamine in the Brain of X-Ray-Treated Rats," Journal of Neurochemistry, XII, No. 4 (1965), 329-333.

7. Randic, M., Z. Supek, and Z. Louasen, "The Influence of Whole-Body X-Irradiation on the 5-Hydroxytryptamine Content of the Brain in Normal Rats," Symposium on the Effects of Ionizing Radiation on the Nervous System, Vienna, International Atomic Energy Agency, 1961

8. Sokoloff, Boris, "The Biological Activity of Serotonin," Growth, XXVIII, (1964), 113-126.

9. Speck, Louis B., "Effects of Massive X-Irradiation on Rat Encephalogram and Brain Serotonin," Journal of Neurochemistry, IX (1962), 573-574.

10. Veninga, T. S. and R. Brinkman, "Contributions to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection. VI Random Liberation of Biogenic Amines as a Cause of Early Irradiation Effects," International Journal of Radiation Biology, V, No. 3 (1962), 283-289.

CHAPTER VII

MATERIALS AND METHODS OF PROCEDURE

Over 150 female Sprague Dawley rats ranging in weight between 175 and 250 grams were used in this study. Tissue slices were taken from the cerebral cortex, thalamus, mid-brain, pons-medulla, and cerebellum. (See Figure 1). The method of removing and slicing the tissues has been previously described in Chapter II of this thesis, page 6. Tissues from four rats were pooled in each test in order to obtain an adequate amount of tissue for analysis. The amount of tissue from each area as used for analysis was as follows: cortex 500-625 mg; thalamus 100-150 mg; mid-brain 375-450 mg; pons-medulla 500-700 mg; and, cerebellum 500-900 mg.

Prior to the serotonin determination, the tissues were homogenized in Corning tissue homogenizers. The serotonin extraction procedure used was that of Bogdanski (1) and modified by Kuntzman et al (2).

The procedure was as follows: the homogenate consisted of one part brain tissue to two parts .1N hydrochloric acid. After grinding the tissue, the homogenate was then removed to a fifty milliliter glass stoppered bottle and adjusted to pH 10 by addition of anhydrous sodium carbonate. Five milliliters of borate buffer, pH 10, was added, and then the mixture was diluted to fifteen milliliters with distilled water. To this, five grams of sodium chloride and fifteen milliliters of N-butanol were added. The sample was shaken for ten minutes, prior to being centrifuged at 2500 rpm for ten minutes. The butanol layer was then aspirated and washed with an equal volume of borate buffer. An aliquot of ten milliliters of the butanol 1 phase was then removed to another bottle containing twenty milliliters of heptane and three milliliters of .1N hydrochloric acid. This mixture was shaken for ten minutes and centrifuged at 2500 rpm for ten minutes. Two milliliters of the .1N hydrochloric acid were removed and placed in a quartz cuvette. The volume was then raised to three milliliters, with a resultant hydrochloric acid concentration of 3N.

Fluorometric determination of serotonin in the resultant extract was carried out with a Turner Fluorometer (Palo Alto, California). The fluorescence media was 3N HCl. The primary filters used were a Corning No. 7-54 unit in conjunction with a 1 mm polarizing filter No. 110-835. The secondary filter was a Corning No. 2A-12. The light source was a far ultraviolet bulb No. 110-851. Pure quartz cuvettes were used in all experiments. The foregoing filters, U-V bulbs, cuvettes and adapters were suggested specifically for the serotonin determinations by the Turner Company. The serotonin levels were expressed in terms of micrograms per gram wet tissue weight.

The experiments were divided into two series; a sham-irradiated, and an irradiated series. The sham-irradiated tissues were carried through the same procedures of handling and analysis as the irradiated tissues, except, they were not irradiated. The irradiated tissues were divided into two groups. One group received 10 Kr at an air dose rate of 575 r/minute while the second group received 20 Kr at an air dose rate of 8086 r/minute. Irradiation was delivered from a G.E. beryllium window X-ray unit (120 KVP, 5 ma).

Generally, the experimental format for each run was as follows: when the specific brain tissues were sliced, blotted and weighed, they were placed in Warburg flasks. The mouths of these flasks were then covered with Saranwrap. The procedure for irradiating the flasks has been described in Chapter II of this thesis (Figure I). Following irradiation, the flasks were returned to the laboratory and serotonin analyses were initiated. The time period required for preparing the flasks, irradiating, and extraction ranged between forty and sixty minutes. All serotonin determinations were initiated within twenty minutes post-irradiation. Special precaution for maintaining the tissues at low temperatures at all times prior to analysis was made by the use of ice baths containing the flasks.

CHAPTER BIBLIOGRAPHY

1. Bogdanski, D. F., A. Pletscher, A. B. Brodie, and Sidney Udenfriend, "Identification and Assay of Serotonin in Brain," Journal of Pharmacology and Experimental Therapeutics, CXVII, (1956) 82-92.

2. Kuntzman, Ronald, P. A. Shore, Donald Bogdanski, and Bernard B. Brodie, "Microanalytical Procedure for Fluorometric Analysis of Brain DOPA-5HTP Decarboxylase, Norepinephrine and Serotonin, and a Detailed Mapping of Decarboxylase Activity in the Brain," Journal of Neurochemistry, VI(1961), 226-232.

CHAPTER VIII

RESULTS

The data are presented in the form of bar graphs and in tabular form. Table I depicts the results of a series of fifteen runs made in order to check the method used in determining the serotonin content in this study. A stock solution of serotonin in creatinine sulfate form was made and diluted to varying concentrations. As shown in Table I, the mean recovery figures were relatively high, indicating the suitability of both the extraction method as well as the fluorometric procedures used. These values fall well within the range stated in a brochure published by the Turner Company using the fluorometric procedure outlined previously.

Figure 1 depicts in bar graph form the effects of X-Irradiation on the serotonin content of various brain tissues. Each bar represents a mean value obtained in at least ten runs (forty animals). It is clear that the thalamus and the mid-brain areas show the highest content of serotonin while the cerebral cortex and the pons-medullary slices exhibit similar but lower serotonin content. The cerebellar slices contain the least amount of serotonin. The most significant change in the serotonin levels in tissues receiving 10 Kr occurred in the thalamic tissues (nineteen per cent). Moreover, it is interesting to note a slight increase in serotonin content in the pons-medullary tissues receiving 10 Kr. The effects of 20 Kr on

TABLE I

SEROTONIN RECOVERY USING BOGDANSKI'S EXTRACTION AND FLUOROMETRIC METHODS

(Stock Solution: ug Serotonin Creatinine Sulfate/1 ml Water)

No. Tests	ug Serotonin / ml Sample	Mean Per Cent Recovery
15	.16	86
15	.25	85
15	.33	91
15	.66	85

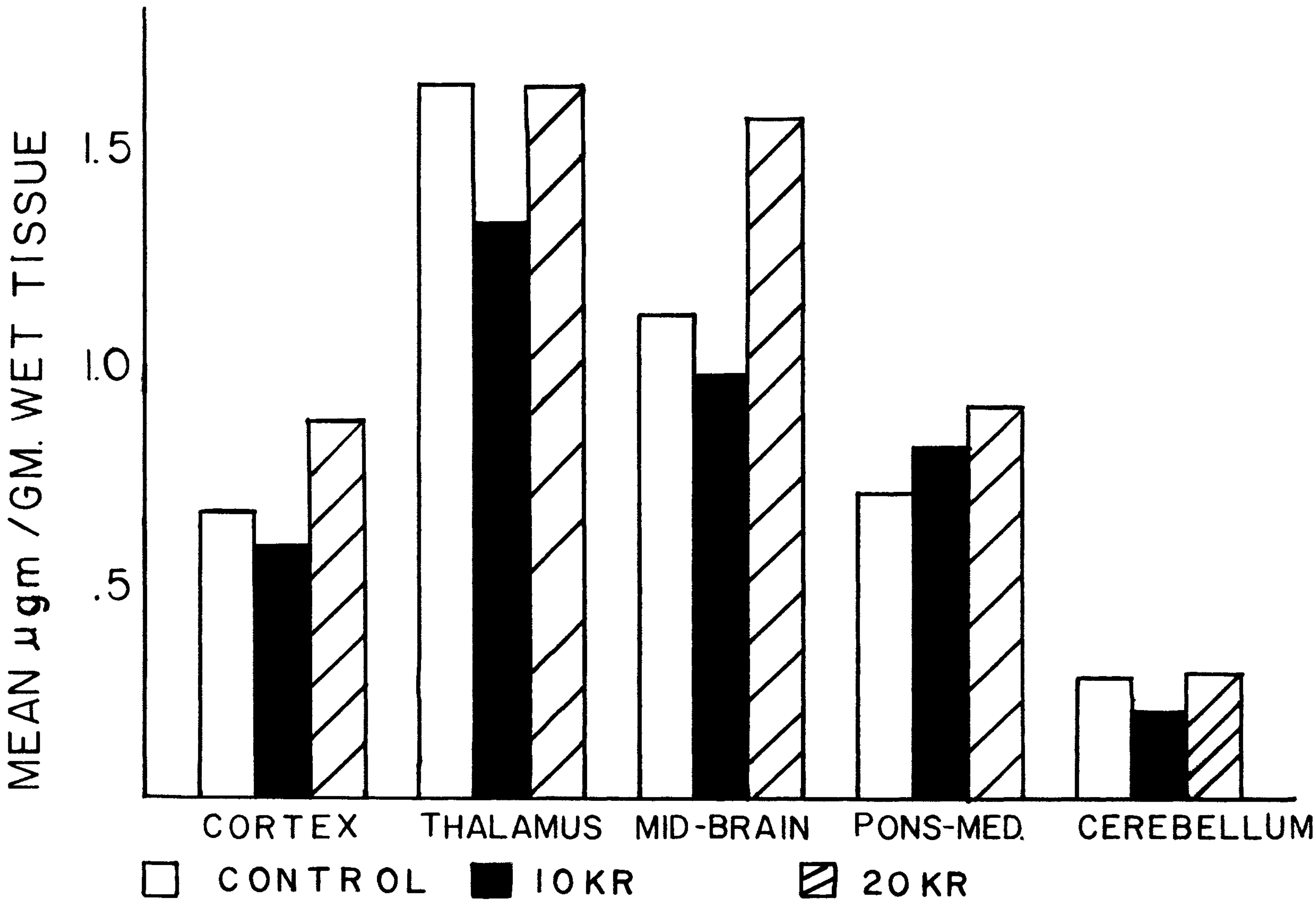


Fig.1–Effects of x-irradiation on brain tissue serotonin

the tissues were more striking. Significant increases over control levels (35 per cent, .31 percent, and 42 per cent) were noted in the pons-medulla, cortical, and mid-brain tissues, respectively, whereas, a slight increase in the serotonin level (5 per cent) was observed in the cerebellar tissues. Another interesting finding was evident in the case of the thalamus. The serotonin content of these tissues were significantly higher than was found in the tissues receiving 10 Kr, although, the level was not higher than those found in the sham-irradiated control group.

The overall results of this study are summarized in Table II. Again, the difference in effect of 10 Kr and 20 Kr on the various brain areas are striking. Generally, all of the tissues receiving 10 Kr, with the exception of the pons-medullary, exhibited slight but significant <u>decreases</u> in serotonin content. On the other hand, all of the tissues, with the exception of the thalamus and the cerebellum, exhibited significant <u>increases</u> in serotonin levels following 20 Kr. It should be stated here that the *p* values were determined by the use of the Student T test. *P* values greater than .02 were considered insignificant.

TABLE II

SUMMARY OF THE EFFECTS OF X-IRRADIATION ON SEROTONIN LEVELS IN RAT BRAIN TISSUES

Tissue	Mean Serotonin In Sham-Irradiated Tissues in ug/gm wet wgt	Mean Serotonin In X-Irradiated Tissue (ug/gm wet wgt)	Per Cent Change	P Values**
	(15 Runs)	(15 Runs)		
10 Kr	S. D.*	S. D.*		
Cortex	.659 ± .078	.595 ± .079	-10	.01
Thalamus	1.642 ± .294	1.338 ± .375	-19	.001
Mid-Brain	1.107 ± .118	.977 ± .111	-12	.02
Pons-Medulla	.697 ± .052	.809 ± .099	+16	.001
Cerebellum	.281 ± .070	.202 ± .058	-28	.02
20 Kr	(15 Runs)	(10 Runs)		
Cortex	.659 ± .078	.862 ± .138	+31	.01
Thalamus	1.642 ± .294	1.638 ± .269	- 1	n s
Mid-Brain	1.107 ± .118	1.569 ± .061	+42	.001
Pons-Medulla	.697 ± .052	.944 ± .073	+35	.001
Cerebellum	.281 ± .070	.294 ± .078	+ 5	n s

* = Standard Deviation ** = Student's T Test Values n s = Not Significant

CHAPTER IX

DISCUSSION

The distribution of serotonin in the various brain areas has been measured in many vertebrates including the cat (11), monkey (17), and rat (5). Generally, it has been found that the older phylogenetic areas such as the thalamus, hypothalamus, and mid-brain possess the highest serotonin concentration while the newer areas, such as the cerebral cortex, contain the least amount of the bioamine. The cerebellum has also been found to contain a relatively small amount of serotonin (16). The data obtained with the sham-irradiated tissues were in fair agreement with these findings.

It must be mentioned here that the procedure of removing the brain, dissecting out the areas to be studied, slicing these areas into uniform portions, extracting the serotonin and carrying out fluorometric determinations of the resultant extract is an extremely difficult task, particularly when one is using an animal as small as a rat. Speed of slicing, weighing and manipulating the tissues along with maintenance of the proper nutrient media properly buffered in a constant cold environment, were but a few of the obstacles to be overcome. Over sixty rats were used to establish the experimental conditions utilized in this study. Another factor involved was the amount of tissues used in each analysis. Such areas as the cerebral cortex cerebellum, and the pons-medulla yielded relatively large amounts of tissue for analysis. The thalamic and the mid-brain areas, however, are not large and, therefore, smaller total quantities had to suffice for serotonin analysis. As was evident in the results it was these areas that exhibited the greatest deviations from the mean. One can, therefore, see the advantage of using larger mammals such as the cat or monkey in future studies.

It is difficult to compare the data presented here with those of other workers in regard to the effects of X-irradiation on serotonin content for several reasons. First, most of the other workers made their serotonin determinations on whole brains following irradiation. In this regard, it is extremely difficult to deliver a uniform dosage to each of the brain areas due to variations in tissue density and anatomical arrangement. This is a lesser problem in the case of tissue slices of uniform thickness in a constant volume. Third, the methods of extracting and determining the serotonin differ amongst workers. The older method of Amin et al (1) was less specific for serotonin coupled with a low recovery, as compared with the more recent method of Bogdanski et al (2). Indeed, the former method was actually a bio-assay method in which the rat uterus was used as the indicator. A fourth set of major differences in experimentation involved the type of ionizing radiation used, the dosage, and the time of extraction post-radiation. Most of the literature indicates the use of X-irradiation ranging between 900 r and 12 Kr. Egana (5), however, injected p^{32}, a powerful beta emitter into animals and removed tissues at varying time intervals ranging from two hours to several days post-injection. The nature of the present study concerned the more immediate effects of ionizing radiation on serotonin levels due to the fact that the tissue slices were irradiated in vitro. It was impossible, therefore, to compare the present data with those depicting changes over two hours post-radiation. Palaic and his co-workers have probably done the most work using the newer methods of extraction in regard to irradiation on the serotonin levels in the central nervous system of animals including rats, mice, and frogs (13, 14, 15). However, they irradiated the whole animals prior to removing the brain and carrying out the tissue analysis. These workers found no immediate change in serotonin content following doses of 900 r and 4 Kr, however, in adrenalectomized animals these same doses brought about an increase in brain serotonin in mice and rats. Moreover, they reported that following 12 Kr, the serotonin level was almost doubled. Ershoff and Gal (6) earlier found no significant change in serotonin levels between irradiated and control animals following 900 r X-Irradiation. Melching et al (12) also claimed no influence of dosages below 1000 r on serotonin levels. Egana (5) reported an increase in serotonin content in tissues from the cortex, hypothalamus and the mid-brain, whereas, the least rise was noted in the cortex. The results presented here indicate only a slight but similar effect of 10 Kr on the serotonin levels in the various brain areas. In all of the tissues except the pons-medulla there was noted a slight decrease in serotonin levels. On the other hand, 20 Kr X-irradiation brought about a significant increase ranging between 31 and 42 per cent in serotonin levels in all of the brain areas with the exception of the cerebellum. The data obtained from the thalamic regions irradiated at 10 Kr and 20 Kr may not be significant due to the relatively small amount of tissue available for analysis. This resulted in a wide range of deviation in the data. In general, the data obtained at 20 Kr were in fair agreement with those of other workers with regard to the direction of change, *id est*, an increase. The data presented here, however, has an added significance in that a clearly differential effect of radiation on the various brain areas was indicated. To the knowledge of this investigator there have been no reports in regard to the effects of X-irradiation on serotonin levels of tissue slices removed from specific brain areas and irradiated *in vitro*. Moreover, it must be remembered that the effects observed here occurred in the absence of blood borne substances that have been shown to be present in whole animals immediately after irradiation. Some of the physiological changes including nervous system involvement have been explained by the presence of some of these substances in given areas (13).

In attempting to explain the changes in serotonin observed in the irradiated tissues one must consider the various factors that are concerned with (1) the formation of serotonin (2) the degradation of serotonin (3) serotonin transport (4) the release and binding of serotonin (5) and cell membrane permeability. In regard to an irradiation effect on serotonin formation and breakdown, Palaic and Supek (15) contend that irradiation interferes with the activity of enzymes engaged in the biosynthesis and degradation of serotonin (5-hydroxytryptamine). Their study involved the use of drugs (reserpine, iproniazid) that reportedly interfere with metabolism and storage of serotonin. They contend that X-irradiation provokes the liberation of serotonin only when the brain precursors are increased, for example, by the addition of 5-OH tryptophane. Palaic et al (13) earlier claimed that from their results using these same drugs, X-irradiation might affect serotonin by (a) altering monoamine oxidase activity (b) binding serotonin with tissue constituents, or (c) altering 5-hydroxytryptophane decarboxylase activity. The first two effects would lead to decreases in tissue serotonin, whereas, the last effect would bring about an increase in serotonin levels. Langerdorff et al (9) claimed that a lack of high energy phosphates coupled with a damage to the decarboxylase system necessary for the synthesis of serotonin from 5-hydroxytryptophane may be involved in the protective ability of serotonin against radiation damage. All of these findings indicate some kind of enzyme effect brought about by ionizing radiation.

Considerable work has been done in regard to the possible serotonin binding sites and mechanisms in all kinds of tissues. Veninga and De Boer (22), Brinkman and Veninga (4), Veninga and Brinkman (21), Egana (5), as well as Palaic and his co-workers (15), have all presented considerable amounts of evidence for the release of serotonin from binding sites in various tissues (uterus, intestine, nerves) as the result of X-irradiation. They propose that perhaps irradiation alters the receptor sites for serotonin.

The exact mechanism of binding and release of serotonin has not yet been elucidated. Histologically, Heller et al (8) have claimed the presence in some central nervous tissue of "serotonergic" fibers and vesicles that may account for the alterations in serotonin levels found in lesions of various areas of the central nervous system including those studied in this investigation.

In regard to the possible irradiation effect on membrane permeability, Majuro and Palade (10) and Scheline and Scott (18) using the electron microscope found that serotonin itself increased the permeability of many cells, including mast and cancer cells. If one presumes an increased cell permeability brought about by X-irradiation, then one would expect an increase in the leakage to the external media and, therefore, a decrease in the intracellular serotonin. This apparently was not evident in the data here concerning those tissues receiving 20 Kr that showed increases in serotonin content. This finding would seem to negate any permeability effect of the radiation on the isolated tissues. Preliminary runs were made to check the effects of X-irradiation on leakage of serotonin from the brain slices, however, due to the small amounts of samples involved, the data were inconclusive.

Finally, one might consider the factor of tissue density-dosage effect in the data presented here since, it has been found that the greater the surface area of a given tissue target exposed, the greater the radiation effect. In general, most of the flasks in this study contained relatively equivalent amounts of tissue with the exception of the thalamus and the mid-brain. The amount of thalamic and mid-brain tissues used per run ranged between 100 mg and 450 mg, respectively, as compared with a range of 500 mg to 900 mg used with the other brain areas. The data show that the areas showing the greatest change in serotonin (thalamus and mid-brain) presented the highest surface area per unit volume to radiation.

Summarily, the findings presented here indicate that:

1. Certain areas of the brain appear to be more radiosensitive than others on the basis of serotonin changes observed following X-irradiation;

2. One can alter the serotonin levels of various isolated brain tissues with X-irradiation in the absence of circulating humoral agents;

3. It requires a considerable amount of X-irradiation (10 Kr to 20 Kr) to bring about significant alterations in serotonin levels in isolated slices;

4. The changes in serotonin content observed in the X-irradiated tissues appear to be more of a biochemical nature rather than a physical one involving membrane damage _per se_;

5. More _in vitro_ studies should be performed involving the use of specific inhibitors to pinpoint the mechanisms involved in the radiation insult;

6. One should be most cautious in attempting to relate serotonin changes with functional changes in the nervous system brought about by ionizing radiation.

CHAPTER BIBLIOGRAPHY

1. Amin, A. H., T. B. B. Crawford and J. H. Gaddum, "The Distribution of Substance P and 5-Hydroxytryptamine in the Central Nervous System of the Dog," Journal of Physiology, CXXVI, (1954), 596-600.

2. Bogdanski, Donald F., Herbert Weissbach, and Sidney Uden Friend, "Identification and Assay of Serotonin in Brain," Journal of Pharmacology and Experimental Therapeutics, CXVII, (1956), 82-92.

3. ____________________, "The Distribution of Serotonin, 5-Hydroxytryptophane Decarboxylase and Monoamine Oxidase in Brain," Journal of Neurochemistry, I, (1957), 272-278.

4. Brinkman, R. and T. S. Veninga, "Contribution to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, V Liberation of Serotonin (and Other Amines) in the Frog After X-Irradiation," International Journal of Radiation Biology, IV, No. 3, (1963), 249-254.

5. Egana, Enrique, "Some Effects of Ionizing Radiations on the Metabolism of the Central Nervous System," International Journal of Neurology, III, No. 3-4, (1962), 631-647.

6. Ershoff, B. H. and E. M. Gal, "Effects of Radiation on Tissue Serotonin Levels in the Rat," Proceedings of the Society of Experimental Biology and Medicine, CVIII, (1961), 160-161.

7. ______________________________, R. Hellmers and A. F. Wells, "Effects of a Radioprotective Agent on Tissue Serotonin Levels in X-Irradiated Rats,", Proceedings of the Society of Experimental Biology and Medicine, CX, No. 3, (1962), 536-538.

8. Heller, A., J. A. Harvey and R. Y. Moore, "A Demonstration of a Fall in Brain Serotonin Following Central Nervous System Lesions in The Rat," Biochemical Pharmacology, XI, (1962), 859-866.

9. Langendorff, H. and H. J. Melching, "Biological Radiation Protection XXXIII Mechanism of the Action of 5-Hydroxytryptamine in Protection Against Radiation," Strahlentherapie, CX, (1959), 505-509.

10. Majno, G. and G. E. Palade, "Studies on Inflammation, One Effect of Histamine and Serotonin on Vascular Permeability: An Electron Microscope Study," Journal of Biophysics and Biochemistry Cytology, XI, (1961), 607-626.

11. McGeer, P. L., E. G. McGeer, and J. A. Wada, "Central Aromatic Amine Levels and Behavior, II Serotonin and Catecholamine Levels in Various Cat Brain Areas Following Administration of Psycoactive Drugs and Amine Precursors," Archives of Neurology, IX, No. 1, (1963), 81-88.

12. Melching, H. J., H. Langendorff and H. A. Ladner, "Dependence of Radiation Effectiveness of 5-Hydroxytryptamines on Concentration and Time Factor," Naturwisseschaften, XLV, (1958), 545.

13. Palaic, Dj., M. Randic and Z. Supek, "X-Radiation and 5-Hydroxytryptamine content in the Brain of Rats and Mice," International Journal of Radiation Biology, VI, No. 3, (1963), 241-246.

14. __________, "5-Hydroxyindol Compounds in Rat Brains After Irradiation," Journal of Neurochemistry, XI, No. 10, (1964), 761-762.

15. __________,"Drug-Induced Changes of the Metabolism of 5-Hydroxytryptamines in the Brain of X-Ray-Treated Rats," Journal of Neurochemistry, XII, No. 4, (1965), 329-333.

16. Pscheidt, G. R. and H. E. Himwich, "Reserpine, Monoamine Oxidase Inhibitors and Distribution of Biogenic Amines in Monkey Brain," BioChemical Pharmacology, XII, (1963), 65-71.

17. ______________________________, C. Morpurgo, "Studies on Nor-Epinephrine and 5-Hydroxytryptamine in Various Species - Regional Distribution in the Brain Response, Monoamine Oxidase Inhibitors, Comparison of Chemical and Biological Assay for Epinephrine," Comparative Neurochemistry, ed. D. Richter, Pergamon Press, New York (1964), 401-412.

18. Scheline, R. R., and K. G. Scott, "Mast Cell Disruption and I_{131} Distribution in the Rat," Cancer Research, XVIII, (1958), 923-927.

19. Speck, Louise B., "Effect of Massive X-Irradiation on Rat Encephalogram and Brain Serotonin," Journal of Neurochemistry, IX, (1962), 573-574.

20. Undenfriend, Sidney, Herbert Weissback and Donald F. Bogdanski, "Biochemical Finding Relating the Action of Serotonin," Annals of New York Academy of Science, LXVI, No. 3, (1957), 602-608.

21. Veninga, T. S. and R. Brinkman, "Contributions to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, VI Random Liberation of Biogenic Amines as a Cause of Early Irradiation Effects," International Journal of Radiation Biology, V, No. 3, (1962), 283-289.

22. ______________________________, and J. E. de Boer, "Contributions to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, VIII X-Ray-Induced Liberation of Histamine from the Isolated Rats Uterus," International Journal of Radiation Biology, VI, No. 6, (1963), 501-505.

CHAPTER X

SUMMARY

The changes in serotonin (5-OH tryptamine) content of various rat brain tissues X-irradiated *in vitro* at 10 Kr and 20 Kr were determined fluorimetrically. The tissue slices were taken from the cortex, thalamus, mid-brain, pons-medully, and cerebellum. It was found that (1) following 10 Kr all of the tissues except the pons-medulla showed slight (10-28 per cent) *decreases* in serotonin content; at 20 Kr, all but the thalamus and the cerebellar tissues exhibited significant *increases* in serotonin cantent (31-42 per cent), (2) the changes observed occurred in *isolated* slices and therefore, was not due to circulating humoral agents indicating localized action of the X-irradiation, (3) certain areas of the brain appear more radiosensitive than others in regard to serotonin level changes, and (4) the changes observed cannot be explained on the basis of changes in membrane permeability alone. The results were discussed on the basis of changes in serotonin formation and degradation, serotonin binding, and permeability changes.

BIBLIOGRAPHY

BOOKS

Freedman, Daniel X., and Nicholas J. Giarman, "Brain Amines, Electrical Activity and Behavior," EEG and Behavior, edited by Gilbert H. Glaser, New York, Basic Books, Inc., (1963), 198-243.

Pscheidt, G. R., C. Morpurgo, and H. E. Himwich, "Studies on Norepinephrine and 5-Hydroxytryptamine in Various Species - Regional Distribution in the Brain Reponse, Monoamine Oxidase Inhibitors, Comparison of Chemical and Biological Assay for Epinephrine," Comparative Neurochemistry, edited by D. Richter, Pergamon Press, (1964), 401-412.

Randic, M. Z. Supek, and Z. Louasen, "The Influence of Whole-Body X-Irradiation on the 5-Hydroxytryptamine Content of the Brain in Normal Rats," Symposium on the Effects of Ionizing Radiation on the Nervous System, Vienna, International Atomic Energy Agency, 1961.

ARTICLES

Amin, A. H., T. B. B. Crawford, and J. H. Gaddum, "The Distribution of Substance P and 5-Hydroxytryptamine in the Central Nervous System of the Dog," Journal of Physiology, CXXVI, (1954) 596-600.

Bogdanski, D. F., A. Pletscher, A. B. Brodie, and Sidney Udenfriend, "Identification and Assay of Serotonin in Brain," Journal of Pharmacology and Experimental Therapeutics, CXVII, (1956) 82-92.

________________, "The Distribution of Serotonin, 5-Hydroxytryptophane Decarboxylase and Monoamine Oxidase in Brain," Journal of Neurochemistry, I, (1957), 272-278.

Brinkman, R. and T. S. Veninga, "Contribution to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, Liberation of Serotonin (and Other Amines) in the Frog After X-Irradiation," International Journal of Radiation Biology, IV, No. 3, (1963), 249-254.

Egana, Enrique, "Some Effects of Ionizing Radiation of the Metabolism of the Central Nervous System," International Journal of Neurology, III, Nos. 3-4, (1962), 631-647.

Ershoff, B. H., and E. M. Gal, "Effects of Radiation on Tissue Serotonin Levels in the Rat," Society of Experimental Biology and Medicine, CVIII, (1961), 160-161.

__________, R. Hellmers, and A. F. Wells, "Effects of a Radioprotective Agent on Tissue Serotonin Levels in X-Irradiated Rats," Proceedings of Society of Experimental Biology and Medicine, CX, No. 3, (1962), 536-538.

Heller, A., J. A. Harvey, and R. Y. Moore, "A Demonstration of a Fall in Brain Serotonin Following Central Nervous System Lesions in The Rat," Biochemical Pharmacology, XI, (1962), 859-866.

Kuntzman, Ronald, P. A. Shore, Donald Bogdanski, and Bernard B. Brodie, "Microanalytical Procedure for Fluorometric Analysis of Brain DOPA-5HTP Decarboxylase, Norepinephrine and Serotonin, and a Detailed Mapping of Decarboxylase Activity in the Brain," Journal of Neurochemistry, VI, (1961), 226-232.

Langendorff, H., and H. J. Melching, "Biological Radiation Protection XXXIII Mechanism of the Action of 5-Hydroxytryptamine in Protection Against Radiation," Strahlentherapie, CX, (1959), 505-509.

Majno, G., and G. E. Palade, "Studies on Inflammation. One Effect of Histamine and Serotonin on Vascular Permeability; An Electron Microscope Study," Journal of Biophysics and Biochemistry Cytology, XI, (1961), 607-626.

McGeer, P. L., E. G. McGeer, and J. A. Wada, "Central Aromatic Amine Levels and Behavior, II Serotonin and Catecholamine Levels in Various Cat Brain Areas Following Administration of Psychoactive Drugs and Amine Precursors," Archives of Neurology, IX, No. 1, (1963), 81-88.

Melching, H. J., H. Langendorff, and H. A. Ladner, "Dependence of Radiation Effectiveness of 5-Hydroxytryptamines on Concentration and Time Factor," Naturwisseschaften, XLV, (1958), 545.

Palaic, D. M. and Z. Supek, "Drug-Induced Changes of the Metabolism of 5-Hydroxytryptamine in the Brain of X-Ray-Treated Rats," Journal of Neurochemistry, XII, No. 4, (1965), 329-333.

Palaic, Dj., M. Randic and Z. Supek, "X-Radiation and 5-Hydroxytryptamine-content in the Brain of Rats and Mice," International Journal of Radiation Biology, VI, No. 3 (1963), 241-246.

____________________, 5-Hydroxyindol Compounds in Rat Brains After Irradiation," Journal of Neurochemistry, XI, No. 10, (1964), 761-762.

Pscheidt, G. R., and H. E. Himwich, "Reserpine, Monoamine Oxidase Inhibitors and Distribution of Biogenic Amines in Monkey Brain," Biochemical Pharmacology, XII, (1963), 65-71.

Scheline, R. R., and K. G. Scott, "Mast Cell Disruption and I_{131} Distribution in the Rat," Cancer Research, XVIII, (1958), 923-927.

Sokoloff, Boris, "The Biological Activity of Serotonin," Growth, XXVIII, (1964), 113-126.

Speck, Louise B., "Effect of Massive X-Irradiation on Rat Encephalogram and Brain Serotonin," Journal of Neurochemistry, IX, (1962) 573-574.

Udenfriend, Sidney, Herbert Weissback, and Donald F. Bogdanski, "Biochemical Finding Relating the Action of Serotonin," Annals of New York Academy of Science, LXVI, No. 3, (1957), 602-608.

Veninga, T. S., and J. E. de Boer, "Contributions to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, VIII X-Ray-Induced Liberation of Histamine from the Isolated Rats Uterus," International Journal of Radiation Biology, VI, No. 6, (1963), 501-505.

__________, and R. Brinkman, "Contributions to the Study of Immediate and Early X-Ray Reactions with Regard to Chemoprotection, VI Random Liberation of Biogenic Amines as a Cause of Early Irradiation Effects," International Journal of Radiation Biology, V, No. 3, (1962), 283-289.