BRAIN ACETYLCHOLINESTERASE ACTIVITY AT INTERVALS AFTER HEAD IRRADIATION IN THE RABBIT

by

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INTRODUCTION

The reaction of the central nervous system to ionizing radiation is recently receiving an increasing amount of attention in view of the need to determine maximum permissible exposure levels, as a result of the expanded use of radiation in medical and technological fields.

Evidence is accumulating from a large body of investigators, principally Soviet scientists, concerning the radiosensitivity of the nervous system.

The first report, based on physiological observations, of radiation-induced changes in the central nervous system occurred in 1896 (24, p. 269). Subsequent histological observations showed the nervous system to be resistant. Results were largely contradictory in nature due to inferior techniques utilized in assessing any change.

Recent evidence based on electrophysiological, morphological and conditioned reflex studies, show an alteration in the functional and structural condition of nerve tissues as a result of exposure to ionizing radiation even at levels equivalent to therapeutic doses (4, p. 264). Livanov (25, p. 4-5) cites evidence provided by Russian workers of changes in the central nervous system at doses from 25 r and above. Furthermore from the electroencephalogram measurements of Brooks (10, p. 210), some of the reactions of the central nervous system to irradiation are among the earliest manifestations of radiation effects upon the organism.

Results are still inconclusive due to the extensive variation in radiation exposure conditions used and the particular biological end-point studied.

The elucidation of the mechanism of action of radiation on the central nervous system is of utmost significance, due to its diverse regulatory function in the animal organism.

The effect of radiation energy on metabolic processes has received comparatively scant attention. Evidence linking the physiological changes brought about by radiation effects on the central nervous system with certain metabolic processes would contribute greatly to the elucidation of the nature of the radiation syndrome. The present investigation, designed to measure the effects of head X-irradiation on the cholinesterase activity of the brain, serves as a contribution to the study of the metabolic reaction of the nervous system to ionizing radiation. Such an attempt to characterize the nervous system metabolically in vivo might yield information concerning its vulnerability to injurious agents and mechanisms by which disturbances are brought about.

The major role assigned to the acetylcholine (ACh) - cholinesterase (ChE) system in nerve transmission justifies the carrying out of this study. Nachmansohn (33, p. 19-61) and his colleagues have provided substantial evidence in which the ACh-ChE system is inseparably linked with nerve activity. Acetylcholine plays an integral part in the development of the action potential and transmission at synaptic junctions, during the process of which, ACh is released from its bound form and is rapidly

hydrolyzed to choline and acetic acid by the enzyme cholinesterase.

In connection with the proposed role of ACh in the mechanism of nerve activity, an exhaustive study has been conducted on the chemistry of the cholinesterases (6, p. 16-182). Two cholinesterases were originally distinguished by Alles and Hawes (3, p. 375-390), a specific cholinesterase, or using the terminology proposed by Augustinsson and Nachmansohn (7, p. 98), acetylcholinesterase, and a non-specific cholinesterase. In the present investigation, the activity of the enzyme acetylcholinesterase (AChE) will be studied in view of its association with nerve activity. There is no substantial evidence of such an association in the case of non-specific cholinesterase (21, p. 189). In most parts of the brain the enzyme is largely AChE (29, p. 207).

The assumption is made in this investigation that the brain AChE activity is an index to the metabolism of ACh which involves its physiological role as a neurohumour. Bennett et al. (8, p. 159) and Bennett et al. (9, p. 217-218), on the basis of the absence of a correlation between ChE and brain protein and ChE and the glycolytic enzyme, lactic dehydrogenase, assumed the level of ChE in the brain to be specifically a measure of ACh metabolism rather than an index to the general metabolism of the brain or its enzyme level. Burgen and Chipman (11, p. 303-304) found a close correlation between the distribution of ACh and ChE in the brain of the dog with the exception of the cerebellar hemispheres. Burkhalter (12, p. 747-750),

using embryonic chick lung cultivated in vitro, provides evidence supporting the hypothesis that AChE activity is directly related to the amount of ACh present. Furthermore the speed of ACh hydrolysis by AChE excludes the possibility of an important gap between structures releasing ACh and those containing the enzyme.

A number of investigators (38, p. 253-255) have shown by the use of nerve stimulants and depressants that the total ACh level in the brain varies with the functional activity of the central nervous system. The level of ACh in the rat's brain increases by forty per cent during anaesthesia and decreases by forty-five per cent during convulsions. The level of AChE, which earlier has been proposed as an index to ACh metabolism, would therefore, provide a measure of the functional state of the central nervous system following irradiation.

From electrophysiological (1) and morphological studies (20, p. 14; 13, p. 284) ionizing radiation has been shown to operate selectively on different areas of the brain. Accordingly this study was designed to test the sensitivity of four anatomically and functionally different areas of the brain to radiation on the basis of a change in levels of their AChE content. Livshits (26, p. 241) ascribes the major role in the radiation reaction of the central nervous system to disturbances in the normal interaction between various divisions of this system. An analysis of changes occurring in different parts of the brain is an indispensable stage in the study of the mechanism of action of radiation sickness.

The radiation doses administered were arbitrarily chosen since there are no data in the literature concerning minimum doses to the head affecting cholinesterase activity. The doses chosen were below the threshold reported by Gerstner et al. (18, p. 643) to cause structural changes in brain tissue (2-3 kr.). Grigore'v (20, p. 19) investigated the injuring effects, on the basis of 100 per cent lethality, of different doses to various parts of the body of animals and found the minimum lethal dose to the head was in the region of 2000 r.

The purpose of this experiment may conveniently be subdivided as follows:

1. To extend previous studies on the effects of head X-irradiation on brain acetylcholinesterase activity which up till now have produced inconclusive results.

2. To determine the extent to which radiation affects the functional activity of the central nervous system, based on the assumption that AChE is an index to the metabolism of acetylcholine which plays a major role in the transmission of nerve impulses.

3. To extend previous studies on the selective action of radiation on different areas of the brain by measuring the degree to which AChE activity is altered.

4. To investigate whether any effect on the AChE of the central nervous system is reversible or phasic in nature by assaying the enzyme at three intervals post-irradiation.

5. To determine whether radiation affects the activity of AChE at doses below those reported to cause structural alterations in nerve tissue based on histological observations.

In the presentation of results and discussion, these objectives will not necessarily be dealt with in the order presented above.

EXPERIMENTAL MATERIALS AND PROCEDURE

The general approach to the problem involved the manometric determination of the acetylcholinesterase (AChE) activity of samples of the cortex, thalamus, hypothalamus and mesencephalon of the irradiated and control animals at three intervals after head X-irradiation.

Total nitrogen determinations were made on all samples in duplicate in order that enzyme activity be expressed on the basis of substrate hydrolyzed per milligram of nitrogen rather than on a wet weight basis. There is evidence of a significant increase in water content of brain tissue following irradiation at high doses (19, p. 330). While it is conceivable that the dose levels used in this study would not materially affect the moisture content, the possibility of a loss or gain of water content during the preparation of tissue samples could not be discounted. Results expressed on the basis of total nitrogen would therefore most closely approach activity in vivo.

Experimental Animals:

Rabbits were used as experimental animals since they were readily available and their brains were of a size that permitted accurate gross dissection for obtaining physiologically reproducible samples.

Twenty-four mature New Zealand white rabbits of approximately the same age and weighing from 4.0 to 5.0 kilograms, were randomly assigned to three groups constituting two treatment groups to

receive 600 r and 1200 r of X-irradiation to the head, and a control group. Each group was further subdivided into two groups of four animals for AChE assay on days one and three, post-irradiation.

Twelve young New Zealand females (offspring of the above females), 7 to 8 months old and weighing from 3.7 to 4.7 kilograms were randomly allotted to three groups representing the same two treatments and a control group. Enzyme activity was assayed 30 days post-irradiation.

The 12 young females also formed part of a separate trial and were subjected to mid-ventral loparotomies on the third day following irradiation. It could readily be assumed, however, that this departure from the routine management did not affect brain enzymatic activity on day thirty.

For the remainder, the experimental environment was maintained as constant as possible. All animals were fed a commercial rabbit family ration and housed individually in cages exposed to external temperatures in the small animal laboratory.

Radiation Exposure and Dosimetry:

Treated animals were exposed to X-irradiation under the following conditions of radiation: 250 Kvp, 15 ma., 1.00 mm. Al. and 0.25 mm. Cu. filters.

Animals were immobilized by anaesthetizing with a minimum amount of pentobarbital solution injected into the marginal ear vein and positioned in a cradle with the head resting horizontally. Lead shielding, 3.0 mm. thick, covered the body up to the second

cervical vertebra. Rays were directed from above through a cone to localize the rays to the exposed area and to minimize scattering. The dose rate measured in air with a Victoreen ionization chamber at a target to mid-cranial distance of 32 cm., was 100 r/minute.

The treated groups received a total air dose of 600 r and 1200 r. According to Gerstner et al. (18, p. 627), the tissue dose measured at the mid-cerebellum is 10 per cent or less of the air dose. On this basis the absorbed dose of the brain tissue of treated animals would be in the region of 540 r and 1180 r.

Control animals were treated in an identical manner except that the exposure to radiation was simulated.

Sampling Method and Tissue Preparation:

On the day designated, animals were sacrificed by decapitation at the first cervical vertebra.

Extreme care was taken to prevent overexcitement of the animal before sacrificing since there is substantial evidence available indicating a variation in the acetylcholine-acetylcholinesterase system with the functional state of the nervous system (38, p. 253-255).

The brain was rapidly removed and chilled to facilitate dissection. A mid-saggital section was made and samples of the cortex, thalamus, the hypothalamus and mesencephalon removed by gross dissection. Anatomical demarcations of the parts were sufficiently distinct to permit dissections with reasonable reproducibility.

A rectangular area (shown in Figure 1) weighing approximately 220 milligrams was removed from the cerebral cortex.

After mid-saggital sectioning of the brain, transverse sections were made of each half immediately rostral to the optic chiasma and between the mammillary body and the oculomotor nerve (Plane A and B, Figures 2 and 3).

The hypothalamic area comprising the preoptic area, including the optic chiasma, the tuber cinerum and the mammillary bodies was removed from the two halves.

A sample of the thalamus was dissected out in the form of a square in the region of the massa intermedia and extending laterally for 6 mm. from the mid-saggital plane.

The region of the mesencephalon removed, consisted dorsally of the corpora quadrigemina and ventrally of the narrow region extending from the oculomotor nerve to a plane of section rostral to the pons (Plane C, Figure 3).

The plane of sectioning and delineation of regions sampled was followed from atlases described by Sawyer et al. (42, p. 801-824).

Tissue samples were transferred after removal to dishes containing cold Kreb-Ringers bicarbonate solution until the completion of the dissection of a brain. The samples were weighed on a precision balance after removal of blood and excess moisture between two pieces of filter paper. The tissue samples were then transferred to a power-driven ground glass tissue homogenizer (13 x 100 mm.) surrounded by crushed ice and 2 ml. of cold

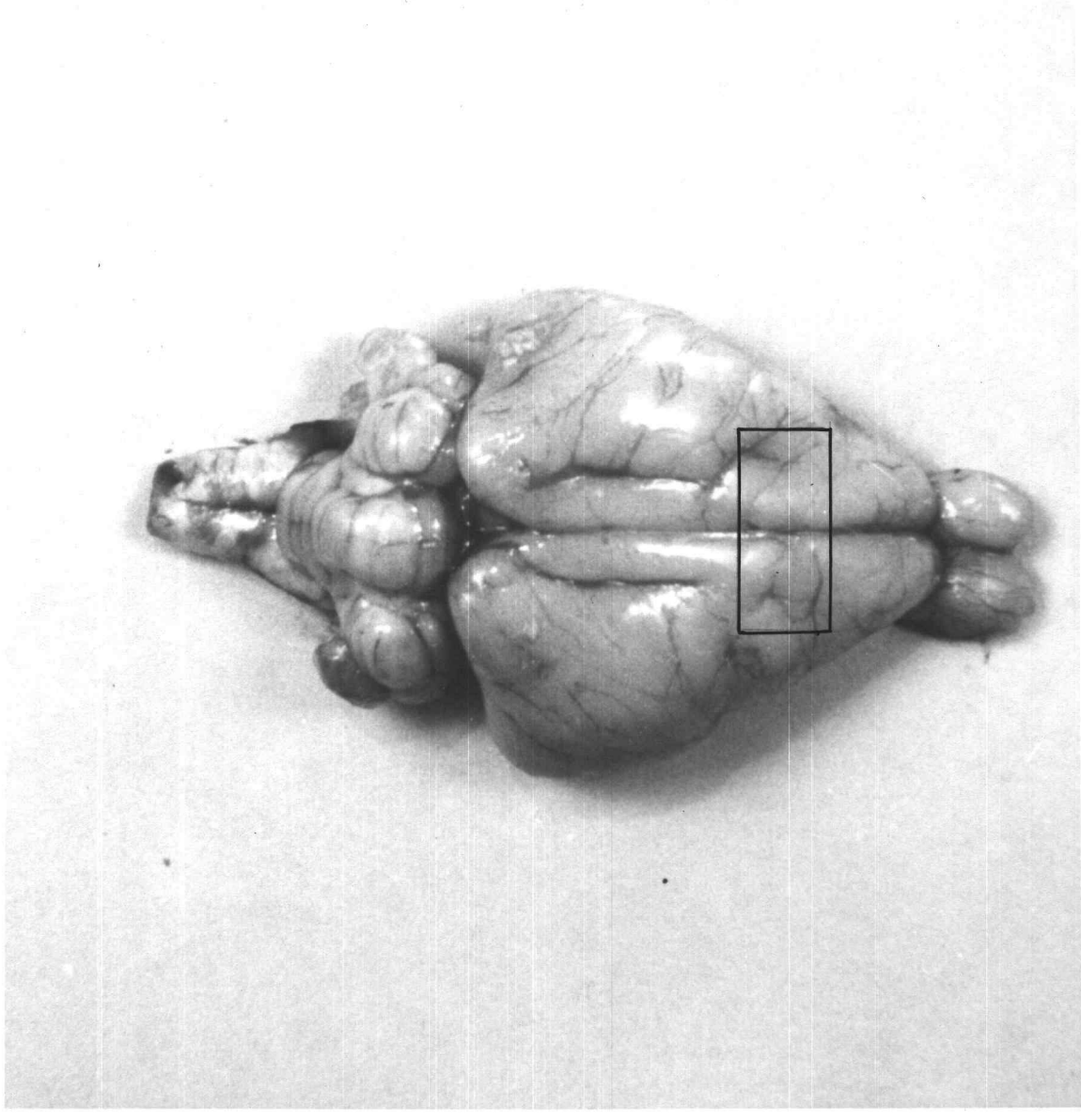

Figure 1. RABBIT'S BRAIN - LOCATION OF CORTICAL AREA SAMPLED.
(X3 Approximately)

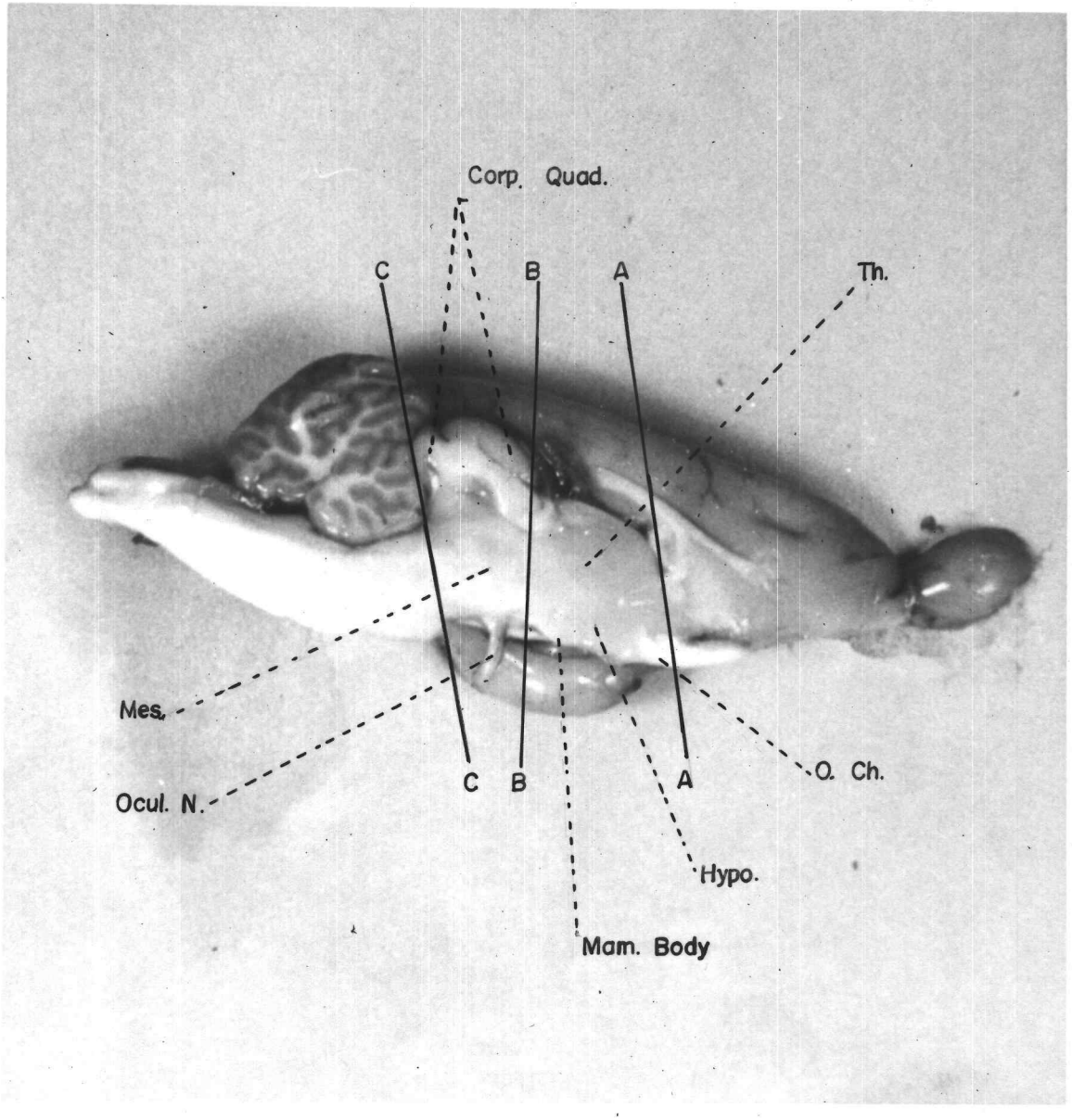

Figure 2. A MID-SAGGITAL SECTION OF THE RABBIT'S BRAIN, INDICATING FRONTAL PLANES OF SECTIONING AND LOCATION OF AREAS SAMPLED (X3 Approximately).

(Corp. Quad.: Corpora quadrigemina; Hypo.: Hypothalamus; Mam. Body: Mammillary body; Mes.: Mesencephalon; O.Ch.: Optic chiasma; Ocul. N.: Oculomotor nerve; Th.: Thalamus.)

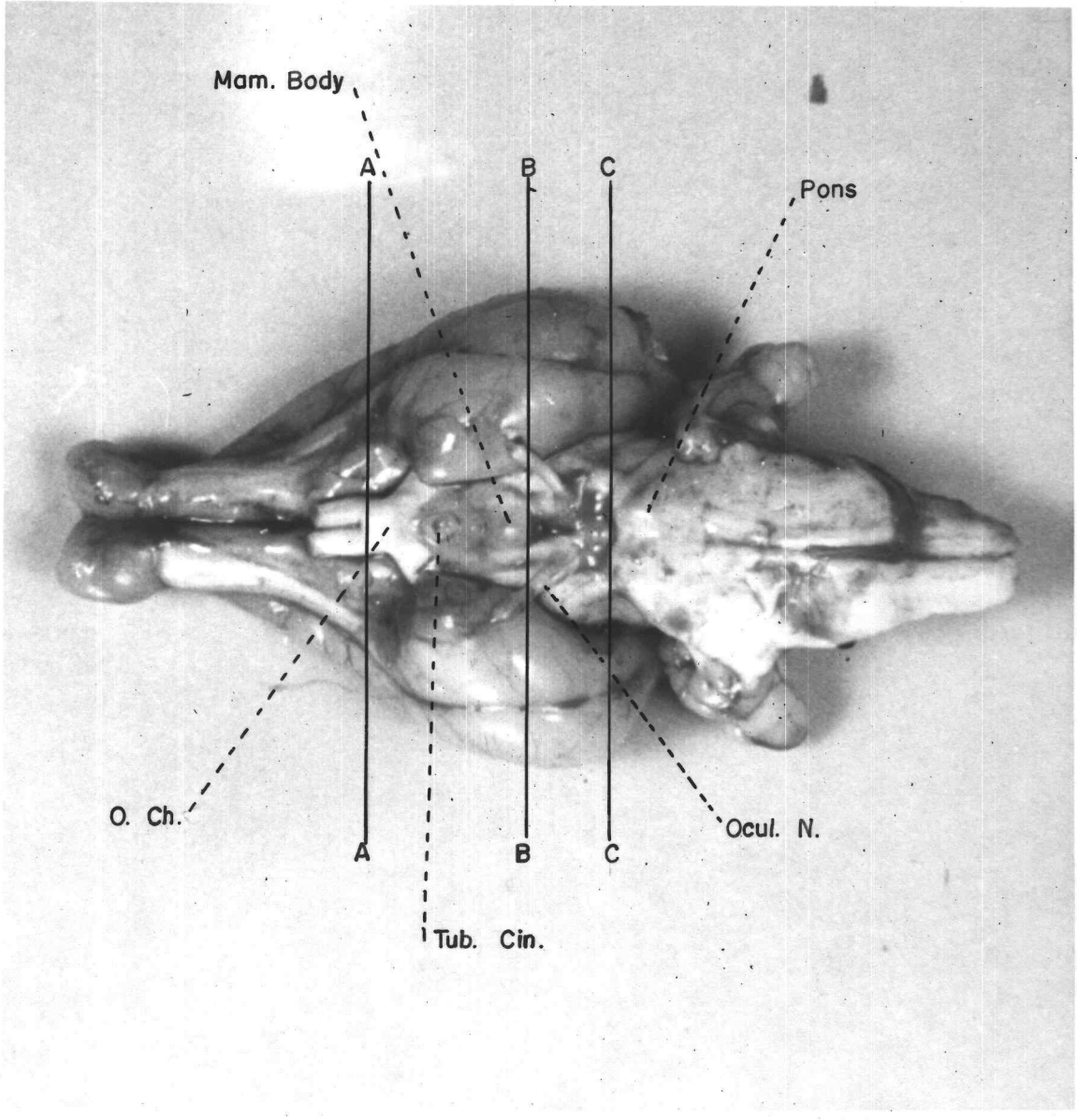

Figure 3. RABBIT'S BRAIN VIEWED VENTRALLY SHOWING FRONTAL PLANES OF SECTIONING AND ANATOMICAL DEMARCATIONS. (X3 Approximately)

(Mam. body: Mammillary body; Ovul. N.: Oculomotor nerve; O. Ch.: Optic chiasma; Tub. Cin.: Tube cinerum.)

Kreb-Ringers bicarbonate medium added. Samples were thoroughly homogenized by moving the tube vertically up and down to ensure a complete breakdown of tissue membranes.

The homogenate was washed over into chilled sample bottles with a little medium and a sufficient amount of the medium added to provide a suitable concentration of tissue per milliliter. Tissue concentrations varied on the average from 48 milligrams per milliliter in the case of the hypothalamus to 107 milligrams per milliliter in the case of the mesencephalon.

Samples were kept in the cold until ready for enzyme determinations on the same day.

Approximately 2 to $2\frac{1}{2}$ hours elapsed from removal of the brain until storage of the tissue homogenates.

Chemical Analysis:

The assay method used, was a modification of the Warburg method described by Augustinsson (5, p. 1-64). The method involves the measurement of the carbon dioxide liberated from a bicarbonate buffer by acetic acid formed as a result of the enzymatic hydrolysis of a choline ester.

Manometers and flasks were calibrated with mercury and flask constants calculated according to the method described by Umbreit et al. (43, p. 46-62).

The manometer fluid consisted of Brodie's solution prepared with a density of 1.03 (Po = 10,000 mm. = 760 mm. Hg).

Manometer ground glass joints were seated with anhydrous lanolin to ensure an airtight system.

The composition of the Kreb-Ringers bicarbonate solution used as the suspending medium for the tissue homogenate and as the buffer in the Warburg flasks, was as follows: 100 mls., 0.154 M NaCl, 30 mls., 0.149 M $NaHCO_3$ and 2 mls., 0.086 M $MgCl_2 \cdot 6H_2O$. The mixture was prepared on the day enzyme assays were made, saturated with a gas mixture of 5 per cent CO_2 and 95 per cent N and kept at 4 degrees centigrade until ready for use. Stock solutions three to five times the strength used in preparation of the buffer were stored in the refrigerator.

To demonstrate specifically acetylcholinesterase or specific cholinesterase, the reaction is carried out with acetyl-β-methyl choline (MeCh) as substrate which is hydrolyzed less rapidly but more specifically than acetylcholine (34, p. 643). Mendel et al. (31, p. 474-475) demonstrated that the specific and non-specific enzymes differ in their ability to hydrolyze particular choline esters which permits the measurement of one in the presence of the other. While both will hydrolyze acetylcholine, only the specific cholinesterase (AChE) which is associated with nerve activity, will hydrolyze acetyl-β-methyl choline. A stable stock solution (1.125 M) of the substrate, acetyl-β-methyl choline chloride (Nutritional Biochemicals Corp.) was prepared by dissolving the salt in dilute hydrochloric acid of pH 4.0. Immediately before use the pH of an aliquot of this solution was adjusted to pH 7.5 with 0.1 N NaOH and then diluted to 0.225 M with distilled water. Preliminary

trials were carried out to arrive at the optimum substrate concentration using a sample of the whole brain.

A 0.5 ml. aliquot of the tissue homogenate was pipetted into the main compartment of the flask and 2.1 mls. of the Ringers-bicarbonate solution added. Four tenths of a milliliter of the substrate (0.225 M, MeCh) was pipetted into the side bulb giving a final volume of 3.0 mls. in the manometer flasks after tipping.

The flasks were attached to the manometer and equilibrated for 15 minutes by shaking in the water bath at 25 degrees centigrade. For the first five minutes of the equilibration period, a gas mixture of five per cent CO_2 and 95 per cent N was bubbled through the system after which the stopcocks were closed and equilibration continued.

After adjusting the level of the manometer fluid to the zero mark (100 mm.) and recording the reading of the open arm, the contents of the flasks were mixed at zero time. Each manometer reading was recorded at an interval of 10 minutes for a continuous period of 30 minutes. Each series included eight to ten experiments.

The final molar concentrations of the flasks after tipping were, 0.78 M NaCl, 0.24 M $NaHCO_3$, 0.009 M $Mg_2 \cdot 6H_2O$ and 0.03 M MeCh. The final pH of the reaction mixture in a gas atmosphere of 5 per cent CO_2 was calculated to be 7.5.

Duplicate determinations were made for each tissue sample and the mean activity calculated.

In all experiments one of the flasks filled with 3 mls. of water served as a thermobarometer and readings were corrected for changes due to temperature and pressure.

The amount of CO_2 evolved as a result of spontaneous hydrolysis of the substrate was determined in a number of experiments. This figure amounted only to about $4\mu l.$ of CO_2 evolved in 30 minutes. Since this figure fell within the limits of experimental error of the method and since the analysis of the study involved a comparison of enzyme activities, this figure was disregarded in the calculations.

A slightly modified procedure of the micro-Kjeldahl, steam distillation method described by Natelson (36, p. 277-282), was followed for the determination of total nitrogen.

Aliquots of tissue homogenate (0.5 mls.) were pipetted into the micro-Kjeldahl flasks and 200 mg. of a mixture of 0.65 grams of K_2SO_4 and 0.016 grams HgO added to aid digestion followed by 1.5 ml. of concentrated sulfuric acid. The contents were digested for 20 minutes, allowed to cool and 10 ml. of distilled water added.

Flasks were individually attached to the steam distillation apparatus and 7 ml. of 50 per cent sodium hydroxide added. The contents were steam distilled for about five minutes into 4 mls. of two per cent indicating boric acid. The latter was prepared by mixing 1 ml. of 0.1 per cent bromcresol green (in alcohol) and diluting to a liter with two per cent boric acid.

The distillate was titrated with 0.011 N sulfuric acid and the milligrams nitrogen determined on the basis that 1 ml. 0.1 N sulfuric acid is equivalent to 0.14 mg. of nitrogen.

Blank determinations were made for the correction of nitrogen present in the reagents.

Duplicate determinations were carried out on all samples and the mean calculated.

Enzyme activity was expressed on the basis of micrograms of substrate hydrolyzed in 30 minutes per milligram of tissue nitrogen and denoted as Q_{MeCh}. This figure was calculated as follows:

$$Q_{MeCh} = \frac{\text{Total mm. } CO_2 \text{ evolved in 30 min. x flask constant}}{8.7 \text{ x mgs. N}}$$

(8.7 microliters of CO_2 evolved is equivalent to 1 microgram of substrate hydrolyzed.)

In the case of the hypothalamus where tissue concentrations per milliliter of homogenate were low, reaction rates declined after 10 minutes due to the high substrate-enzyme ratio. For this reason the figure for the total amount of CO_2 evolved during 30 minutes was used in expressing activity.

Experimental Design and Analysis:

In order to demonstrate adequately the effect of experimental variables on the biological endpoint studied, it is necessary that errors of measurement be small and constant with respect to time. Due to the lengthy period involved from the time the brain was excised to the time the final chemical analysis was performed, it was only possible to carry out assays on two rabbits per day on two days a week. To account for any effect of improvement of technique with time, a balanced incomplete block design was employed to effectively randomize the treatments over the length of the

experimental period. The order in which the three treatments were applied for each day group, were randomized by the use of random numbers. The block of three treatments was incomplete since only two animals were treated and assayed on any one day. The design was balanced, however, in that any two treatments occurred together with equal frequency on the same day.

Animals were alternately drawn from each day group each week for chemical assay.

The 30-day group of animals were introduced during the latter part of the study as an extension of the experiment and were therefore not randomized with the rest of the animals.

The experimental data were analyzed statistically according to the method adopted for a 3 x 3 factorial design. Significance of the dose, day and interaction effects were tested by means of the F-test. Significant differences were calculated between treatments.

RESULTS AND DISCUSSION

Mean acetylcholinesterase (AChE) levels, expressed as Q_{MeCh}, for four areas of the brain for the control and irradiated groups at one, three and thirty days post-irradiation, is presented in Table I. Total Q_{MeCh} figures for each treatment over the three intervals post-irradiation, appear in Table II.

An analysis of the data revealed a 4 - 13 per cent depression in enzyme activity in the thalamus and hypothalamus of the irradiated groups over the controls. This effect was found to be significant in the case of the hypothalamus over the three intervals ($P<0.05$). There was no significant difference in enzyme concentration of the hypothalamus between the 600 r and 1200 r groups, however, both these groups were significantly different from the controls ($P<0.01$).

Control data reveal the mesencephalon to have the highest enzyme concentration followed by the thalamus, hypothalamus and cortex. The concentration of AChE in the subcortical areas, has been shown to be localized principly in the nuclear masses. In the case of the hypothalamus, most of the activity is found (histochemically) in the paraventricular and supraoptic nuclei (17, p. 97). The preponderance of AChE activity in subcortical structures, is in agreement with studies by Rosenzweig *et al.* (39, p. 345) on rats and Pokrovskii and Ponomareva (37, p. 248) on monkeys. The latter authors observed a marked quantitative inhomogeneity in the distribution of AChE in brains of monkeys. Nachmansohn (33, p. 29-37) reports that the enzyme

Table I. THE EFFECT OF HEAD X-IRRADIATION ON THE ACETYLCHOLINESTERASE (AChE) LEVELS OF FOUR AREAS OF THE RABBIT'S BRAIN AT THREE INTERVALS POST-IRRADIATION.

Days Post-Irradiation	Treatment (Dose X-Irradiation)	AChE Activity in Terms of Q_{MeCh} = gms. Substrate Hydrolyzed per Milligram Nitrogen in 30 Minutes (Mean ± S. D.)			
		Cortex	Thalamus	Hypothalamus	Mesencephalon
1	0 (Control)	*10.41 ± 1.99	17.24 ± 1.19	13.54 ± 1.73	18.25 ± 0.87
	600 r	9.49 ± 3.08	16.33 ± 2.29	12.05 ± 2.00	17.04 ± 1.53
	1200 r	9.34 ± 2.01	15.98 ± 1.31	11.98 ± 2.22	18.38 ± 2.02
3	0 (Control)	9.93 ± 1.01	15.92 ± 0.89	13.65 ± 1.27	16.83 ± 1.46
	600 r	9.16 ± 0.83	15.17 ± 0.62	12.31 ± 1.10	17.26 ± 1.36
	1200 r	10.06 ± 2.26	14.13 ± 2.05	11.79 ± 3.27	16.61 ± 1.02
30	0 (Control)	8.08 ± 1.51	15.11 ± 1.75	12.60 ± 1.62	15.56 ± 1.50
	600 r	8.16 ± 1.56	14.72 ± 1.87	11.66 ± 1.60	17.19 ± 1.27
	1200 r	8.20 ± 1.74	13.55 ± 1.50	10.80 ± 0.42	15.31 ± 1.76

* Mean activity of samples from four rabbits determined in duplicate.

Table II. THE EFFECT OF X-IRRADIATION ON THE ACETYLCHOLINESTERASE ACTIVITY OF FOUR AREAS OF THE BRAIN IN TERMS OF Q_{MeCh}. (Pooled Day Effect)

Treatment (Dose X-Irradiation)	Cortex	Thalamus	*Hypothalamus	Mesencephalon
NIL (Control)	113.73	193.11	159.19	202.57
600 r	107.25	184.91	144.13	205.98
1200 r	110.51	174.65	138.32	201.27

*Statistically significant ($P<0.05$)

in different brain centers may vary by a factor of 10-50, while the concentration is remarkably constant for the same center and the same species. He postulates that the variation in the surface area per gram of nerve fiber in different areas is one important factor in explaining this variation.

While a decreased AChE activity has been associated with various toxicological and pathological conditions and disorders of the central nervous system, it has to date been incompletely studied in the nervous system following irradiation. Zubkova and Chernavskaya (47, p. 1114-1117), studied the effect of whole-body X-irradiation (1000 r) of rats on ChE levels at intervals from five minutes to three days in a number of tissues. The enzyme activity in the brain fell as early as five minutes and rose on the third day but still remained subnormal. Mozzhukhin and Pevsner (32, p. 34-37) reported a decline in ChE activity in the brain and other tissues of mice following total gamma-irradiation at

doses from 700 r - 10 kr. Maximum declines of about 20 per cent occurred at four days and three weeks in all tissues. Florsheim and Morton (16, p. 18) demonstrated a significant decline in brain ChE levels as early as 20 minutes following whole-body irradiation of mice at a dose level of 20,000 r.

Contrary to the above evidence, Doull and Cummings (14, p. 349) and Sabine (40, p. 278) demonstrated the absence of any effect on AChE in the brain and other tissues following lethal and sublethal doses of whole-body X-irradiation.

Egana (15) studied the effect of internal beta-irradiation (125 microcuries/100 gms. body weight) on the acetylcholine synthesis in the brain of rats. A significant increase in total acetylcholine synthesis was observed in the hypothalamus and mesencephalon during 48 hours followed by a diminuition at 72 hours. These results would indicate a concomitant change in AChE levels, assuming an inverse relationship between it and free acetylcholine.

The question whether the effect of ionizing radiation on the central nervous system and its metabolism, is primary, or a result of disturbances in the circulation, is as yet not resolved. The cholinesterase content of the blood has been widely studied in an attempt to establish a useful index to the functional condition of the organism following various pathological conditions, including radiation. The existance of acetylcholinesterase in blood erythrocytes, similar to that found in brain tissue of mammals, which is distinct from a non-specific cholinesterase found in the serum

and other body tissues, has been firmly established (3, p. 375-390; 30, p. 62).

In view of the correlation between the cholinesterase activity of blood erythrocytes and brain tissue reported by Lawson (23, p. 136-137) and others, it seems desirable to review some of the conflicting evidence of the effects of radiation on blood cholinesterase activity.

Sabine (40, p. 276-278) recorded a pronounced increase in cholinesterase activity of erythrocytes of mice in four days, following 25 - 300 r of total-body X-irradiation. The increase was followed by a depression in 9 - 20 days. Following whole-body gamma-irradiation of rats at doses from 75 - 600 r, Williams (44, p. 950) and Williams et al. (45, p. 604) noted a depression in whole blood cholinesterase levels from the third till the tenth day. Preliminary studies indicated that the changes were largely attributed to the enzyme in the erythrocytes. Lundin and Clemedson (27, p. 528-529), found no effect at 48 hours after whole-body X-irradiation (400 r) on the acetylcholinesterase content of red blood cells in guinea pigs.

The conflicting results reviewed above may be ascribed to the variation in irradiation techniques, the species of animals and method of assay used. Standardization of exposure and animal conditions would greatly facilitate comparison of work between laboratories.

The action of radiation on enzymes in vivo, are extremely difficult to analyze. The decrease in activity observed in the

present study, may be due to the destruction of the enzyme or the inactivation of the mechanism involved in its synthesis, the release of an inhibitor or the inhibition of an activator. It has also been postulated that the effect of radiation may only be on a very small fraction of the existing enzyme in vivo, the total amount of which is measured in tissue homogenates. Furthermore, the homogenate technique may introduce many artifacts and also liberate inhibitors or activators (22, p. 58-59). If radiation affected the enzyme directly, one would expect a maximum depression immediately following irradiation. The early decline observed by Zubkova and Chernavskaya (47) and Florsheim and Morton (16), lends support to this idea.

The decline in AChE activity observed in this and other experiments, is considered too small to result in a disturbance in neuronal function. It has been shown by the use of enzyme inhibitors that conduction is blocked after inactivation of at least 80 per cent of the enzyme (35, p. 151; 46, p. 154). Furthermore, according to the theory of chemical transmission, the degree of cholinesterase inhibition should be related to the appearance of cholinergic symptoms. The rabbits exposed to the larger of the two doses of radiation displayed signs of apathy and lost hair around the nose and eyes, however, there were no gross symptoms of sickness or of a neurological disturbance. Mazur and Bodansky (28, p. 269) reported the presence of cholinergic symptoms in the rabbit after more than 40 per cent inhibition of the enzyme. Natan and Aprison (35, p. 151) determined the range of physiological safety for neuronal disfunction

in the rabbit to be 50 - 100 per cent of the normal concentration of AChE. Neuronal activity in the absence of at least 50 per cent of the enzyme, testifies to the fact that AChE is present in quantities in excess of its physiological requirements.

It is significant to mention here that the degree of effect on the brain AChE activity might quite conceivably have been more severe, had the rabbits not been irradiated under anaesthesia. It is well known that by altering the initial functional state of the central nervous system before irradiation it is possible to alter the character of its response reactions (20, p. 10). It is considered unlikely, however, that the light anaesthesia administered, could have materially altered the degree of change observed, even to the extent of approaching 50 per cent inhibition.

Sabine (41, p. 281) concluded that although it is possible to inactivate cholinesterases by irradiation, it is highly improbable that the symptoms resembling cholinergic manifestations observed in animals exposed to high doses, can be ascribed to inhibition of the enzyme.

The selective action of radiation on the subcortical areas, particularly the hypothalamus and the relative resistance of the cortex, is in agreement with past results. Morphological (20, p. 14, 13; p. 284) and electrophysiological (24, p. 269-281) studies, demonstrate that maximum changes occur in the hypothalamus, mid-brain and medulla. Livanov (24, p. 279), however, cautions against the application of results obtained from the effects of

radiation on various parts of the central nervous system in lower animals to man. According to this author the role of the various parts of the nervous system in the radiation reaction and their intercentral relationships can best be evaluated from the evolutionary aspect. Abdullin (1) attributes the relative radiosensitivity of the subcortical structures to the participation of humoral-chemical processes in a considerable part of their activity. In this respect the observed radiosensitivity of the hypothalamic AChE which is reported to be concentrated in the supraoptic and paraventricular nuclei (17, p. 97), could have far-reaching consequences since these nuclei structures are associated with pituitary secretion. Abrahams and Pickford (2, p. 330-333), showed that after the injection of an anticholinesterase into the region of the supraoptic nucleus of the chloralosed dog, the release of the posterior lobe hormones of the pituitary was initiated. Acetylcholine was postulated to be the transmitter for the release of these hormones.

As stated earlier, there is substantial evidence indicating an inverse relationship between acetylcholine content and the functional activity of the brain (11, p. 253-255). A change in the functional activity of various parts of the central nervous system is of importance, since such a change may be reflected on the nervous regulation of autonomic functions and consequently on the general state of the animal. It is debatable, however, whether the small decline in enzyme concentration observed, resulted in an accumulation of acetylcholine and a concomitant change in the functional activity

of the brain. On the other hand the effect of radiation may have been more severe on the level of acetylcholine and its synthesis. A decline in acetylcholine as was observed by Egana (15) after 72 hours, could conceivably result in a change in the functional state of the brain.

The statistical analysis of the data revealed an absence of an interaction between treatments and days and the lack of a day effect in the case of the hypothalamus. This seems to indicate a consistant magnitude of effect over the three intervals at which enzyme concentration was studied and the lack of recovery after 30 days post-irradiation. The fact that enzyme activity is not restored at 30 days post-irradiation, lends further support to the hypothesis that radiation affects the enzyme directly. The recovery of rabbit brain cholinesterase activity after anticholinesterase administration has been reported to be exceedingly slow (28, p. 273). Fifty days after injection of diisopropyl fluorophosphate, the brain cholinesterase level is restored to 90 per cent of normal.

The effect of interval after irradiation at which AChE concentrations were determined, is summarized in Table III. The effect of day post-irradiation was significant over all treatments in the case of the thalamus ($P<0.01$) and mesencephalon ($P<0.05$). The significance of this fact is not entirely clear. It is conceivable that the decline in enzyme activity at 30 days post-irradiation could be due to the younger age of the animals used for assays at that interval. Control values of these animals were consistently lower than those of the one and three groups. It has been adequately demonstrated that

the concentration of AChE parallels the morphological and functional development of the central nervous system (17, p. 36).

Table III. THE EFFECT OF ASSAY INTERVAL POST-IRRADIATION ON THE ACETYLCHOLINESTERASE ACTIVITY OF FOUR AREAS OF THE BRAIN IN TERMS OF Q_{MeCh}. (Pooled Treatment Effect).

Days Post-Irradiation	Cortex	Thalamus **	Hypothalamus	Mesencephalon *
1	117.07	198.21	150.31	214.70
3	116.59	180.91	151.06	202.83
30	97.82	173.54	140.27	192.28

* Statistically significant (P 0.05)
** Statistically significant (P 0.01)

The significant decline from one to three days cannot be explained. It is quite possible, however, that the effect on the activity of AChE as a result of a change in the functional state of the central nervous system during anaesthesia, is prolonged and still evident up to the third day.

Attention is drawn to the fact that there is a noticeable increase in standard deviation with an increase in exposure dose up to three days. This is interpreted as being due to individual variation in the response of the central nervous system to radiation.

SUMMARY AND CONCLUSIONS

The present concept of the radiosensitivity of the nervous system is briefly discussed. The study of biochemical changes in the central nervous system is an important approach to the elucidation of the mechanism of action of ionizing radiation particularly when combined with morphological and electrophysiological studies.

The primary object of this investigation was to determine the effect of ionizing radiation on the levels of the enzyme acetylcholinesterase (AChE) in the brain of the rabbit. Furthermore an attempt is made to explain the attendant consequences of such an effect in terms of the concept of chemical transmission in the central nervous system.

The physiological significance of AChE is briefly discussed in connection with the presumed role of acetylcholine (ACh) in the chemical mechanism of nerve activity.

Acetylcholinesterase was assayed in four functionally important regions of the brain of two groups of irradiated rabbits receiving doses of 600 r and 1200 r of X-irradiation to the head and a control group. Enzyme concentrations were determined manometrically by means of the Warburg method at 1, 3 and 30 days post-irradiation. Activity was expressed as micrograms of substrate hydrolyzed per milligram of tissue nitrogen. The method of tissue sampling and preparation and chemical assay is described in detail.

A review of literature is presented on the effects of radiation on the AChE activity of the brain and blood.

Of the four areas sampled, namely, the cortex, thalamus, hypothalamus and mesencephalon, a significant decline in AChE activity was found only in the hypothalamus. The radiosensitivity of this area is consistent with other studies based on morphological and electrophysiological observations. It is considered likely that radiation acts directly on the enzyme and/or its mechanism of synthesis.

It is inferred that the magnitude of effect is too small to result in a disturbance of neuronal function or a change in the functional activity of the brain by way of an accumulation of acetylcholine.

The outcome of higher doses of radiation on the hypothalamic AChE which is concentrated in the nuclear masses associated with pituitary secretion, and in turn on the endocrine response of the organism, is open to speculation and warrants further investigation.

There was apparently no recovery of enzyme activity in the hypothalamus after thirty days following irradiation.

The significant effect of day and the absence of a dose effect on the AChE levels in the thalamus and mesencephalon is best explained as being due to difference in ages of the 30 day group and the effect of anaesthesia on the enzyme concentration up to three days post-irradiation.

The varied radiation exposure conditions employed by investigators, does not allow a comparison of the data accumulated from different laboratories and stresses the need for standardization of conditions.

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