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Effect of Head X-Irradiation on the Uptake of Radiophosphorus by Rat Brains.* (29608)

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Our primary purpose has been the development of a practical, reliable, quantitative technique for production of increased permeability of the so-called blood-brain barrier (BBB). Such a method would permit not only the study of the physiological effects of increased BBB permeability *per se* but also that of drugs which normally do not penetrate the brain in appreciable amounts. One successful method developed by Purpura *et al* (1) involves freezing of a local area of the brain. The major limitation of this technique is the inability to adapt it to large surface areas and to subcortical sites. A possible practical method circumventing this difficulty utilizes ionizing radiation to produce large scale increases in barrier permeability.

The blood-brain barrier (BBB) now constitutes a functional concept rather than an anatomical entity. It is derived in part from the knowledge that a variety of substances (*e.g.,* metallic cations, phosphates) pass from the blood stream into the brain tissue at very slow rates compared with their entrance into other tissues; in muscle these ions equilibrate in a fraction of the time necessary for equilibration in brain. The mechanisms or structures responsible for this disparity between tissues are unknown and are currently the subject of speculation [reviews by Dobbing (2), Tschirgi(3)].

Numerous investigators have studied the effect of ionizing radiation on uptake and incorporation of a variety of dyes and isotopically labelled compounds(4,5,6,7,8). However, the contradictory results obtained preclude definitive statements as to the effect of radiation on BBB permeability. Explanations for these conflicting reports include: (1) differences in radiation dose; (2) species differences; (3) time of test relative to irradiation; (4) nature of test substance; and (5) differences in treatments of controls.

Radiation has been reported to increase uptake of P^{32} by brain, and to have no effect on uptake. Florsheim and Morton(5) studied the effects of doses up to 20 Krads on *in vitro* and *in vivo* uptake of P^{32} by mouse brain and noted no significant differences between control and experimental animals 24 hours postirradiation. Stajic and co-workers(8), using counting and autoradiographic procedures, confirmed the findings of Florsheim for the 24-hour postirradiation period but found that 20 Krad irradiation to

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part or all of the head resulted in increased uptake 72 hours later.

These apparently discrepant results made further investigation desirable, particularly as to the basic causes responsible for the contradictory results.

The following report relates the temporal and qualitative parameters of irradiation to alterations in BBB permeability to P^{32}. We have shown that manipulation of these factors may result in increased permeability or no change in permeability; in no case was decreased permeability noted.

Methods. Adult male Holtzman-strain white rats (200-300 g) were randomly selected, marked for identification, and divided into 4 groups (fed control, fasted control, fed irradiated, and fasted irradiated). In the initial series of experiments, 3 animals in each group were given parenteral injections of neutral isotonic $Na_3P^{32}O_4$ (50 μc/100 g body weight) immediately prior to irradiation. A total of 225 animals was used in the first experimental series. Groups were sacrificed at 24, 48 and 96 hours after 20 Krads of X-irradiation.

Six animals were irradiated simultaneously in special plastic restraining cages, thus avoiding the necessity of using anesthesia. Only the heads were irradiated; the remainder of the body was shielded with ¼-inch lead sheeting. The shielding procedure still permitted about 3% of the total dose to the head to reach the body. Dose was determined at the time of each experiment by means of a Victoreen condenser rate meter and 250 r probe. The probe head was placed in a plastic phantom approximating the head of a rat to determine tissue dose more accurately. Radiation characteristics of the therapy X-ray unit were 250 kv, 16 Ma, 2 mm Al filtration. At a distance of 30 cm, dose rate was 360 r/min.

In the 2 subsequent series of experiments, the same basic procedure was followed except that in the second experiment the animals (59) received the isotope 24 hours after irradiation and were sacrificed 24 hours later, and in the third experiment 103 animals were injected with the isotope 48 hours after start of irradiation and were sacrificed 24 hours after injection. Additional experiments were performed to determine the degree of contamination of brain radioactivity by blood P^{32}. In these experiments, the brains were perfused with physiological saline and the results compared with an equal group of non-perfused brains.

Animals were lightly anesthetized with ether. By cardiac puncture 4-6 ml of blood were withdrawn into heparinized syringes. The samples were centrifuged to separate formed elements from plasma. Aliquots of the plasma were treated with 20% trichloroacetic acid (TCA) and centrifuged to remove precipitated proteins. Ether washes (2 vol. twice) removed the TCA and the remaining protein free plasma sample was then analyzed for total phosphorus and radioactivity. Immediately after blood withdrawal, the rats were decapitated, the brains removed and rinsed, then weighed and homogenized in sufficient distilled water to make a final volume of 10 ml. Cell debris was removed by centrifugation and aliquots of the supernatant were analyzed for total phosphorus by a modified Fiske-SubbaRow method(9). In the processing sequence, control animals alternated with experimental animals.

Duplicate determinations were made on each plasma and brain sample for phosphorus content and radioactivity. Counts were estimated from duplicate planchets for all samples in a gas-flow end window counter having an efficiency of 30%. All necessary corrections for comparative purposes have been made and results have been expressed as specific activity (counts per minute P^{32}/mg P).

Results and discussion. The results are shown in Tables I-V. The values of plasma and brain P^{32} and phosphorus are not included in these Tables. We agree with Herlin(10), who claims that considerable individual differences in absorption rate of intraperitoneally-injected P^{32} and in excretion and metabolism of phosphorus lead to such marked differences in brain and blood values that neither one alone can be used to derive meaningful information. As the brain phosphorus metabolism depends in part on the

plasma phosphorus pool, and as there is constant exchange of phosphorus between the 2 compartments, it is evident that the specific activity (SA), *i.e.*, ratio of P^{32} counts to P in the brain, reflects the P^{32} to P ratio in the plasma. If one assumes constancy of the ratio in the plasma, one would expect an identical ratio to obtain in the brain after complete equilibration (after infinite time). The steepness of the curve representing the time course of the brain specific activity reflects the rate of phosphorus exchange. The time course and peak value of specific activity of the plasma depend on amount of P^{32} injected, absorption rate, clearance into the various organs (including the brain), and

TABLE I. Specific Activity of Plasma and Brain, and Specific Activity Ratios (SA Brain/SA Plasma) for Exp 1. Irradiation with doses indicated (Krad) at 0 hr, P^{32} injection at 0 hr, sacrifice 24 hr after irradiation.

Condition	Krad	No. rats	SA plasma	SA brain	SAR
Fed	0	20	1935 ± 709	338 ± 103	.188 ± .051
"	10	16	2716 ± 730	405 ± 133	.152 ± .037
"	20	3	1336 ± 104	232 ± 29	.175 ± .011
Fasted	0	18	3340 ± 1245	424 ± 137	.126 ± .021
"	10	12	3867 ± 1225	527 ± 198	.134 ± .021
"	15	4	2522 ± 280	267 ± 26	.107 ± .041

TABLE II. As in Table I. Exp 2. Irradiation at 0 hr, P^{32} injection at 0 hr, sacrifice 48 hr after irradiation.

Condition	Krad	No. rats	SA plasma	SA brain	SAR
Fed	0	17	1803 ± 1291	420 ± 166	.281 ± .091
"	10	19	1976 ± 859	450 ± 149	.238 ± .032
"	15	11	1522 ± 562	339 ± 144	.217 ± .031
"	20	11	1932 ± 609	410 ± 141	.206 ± .034
Fasted	0	22	2520 ± 934	547 ± 274	.219 ± .071
"	10	16	2773 ± 1093	642 ± 243	.242 ± .079
"	15	4	1525 ± 164	324 ± 51	.214 ± .039
"	20	11	1912 ± 435	416 ± 65	.225 ± .047

TABLE III. As in Table I. Exp 3. Irradiation at 0 hr, P^{32} injection at 0 hr, sacrifice 96 hr after irradiation.

Condition	Krad	No. rats	SA plasma	SA brain	SAR
Fed	0	18	762 ± 562	352 ± 158	.390 ± .095
"	10	3	1934 ± 512	736 ± 178	.386 ± .123
Fasted	0	16	1916 ± 1002	511 ± 264	.375 ± .088
"	10	11	2159 ± 672	644 ± 184	.306 ± .047

TABLE IV. As in Table I. Exp 4. Irradiation at 0 hr, P^{32} injection at 24 hr, sacrifice 48 hr after irradiation.

Condition	Krad	No. rats	SA plasma	SA brain	SAR
Fed	0	14	1720 ± 474	305 ± 65	.183 ± .011
"	20	15	2344 ± 339	399 ± 106	.169 ± .029
Fasted	0	15	2532 ± 565	347 ± 66	.138 ± .044
"	20	15	2488 ± 632	371 ± 93	.150 ± .018

TABLE V. As in Table I. Exp 5. Irradiation at 0 hr, P^{32} injection at 48 hr, sacrifice 72 hr after irradiation.

Condition	Krad	No. rats	SA plasma	SA brain	SAR
Fed	0	20	1935 ± 709	338 ± 103	.188 ± .051
Fasted	0	18	2136 ± 480	375 ± 108	.126 ± .023
"	20	19	2827 ± 1251	506 ± 115	.196 ± .056

rate of dilution by exogenous non-radioactive phosphorus. The last factor is of particular importance when comparing irradiated with non-irradiated animals fed *ad lib*. Irradiated rats cease to eat and thus cease to dilute endogenous stores of radioactive phosphorus with nonlabelled phosphorus in food. In the fed animals, the SA of the blood continually drops and the specific activity ratio, SAR (brain SA/plasma SA) becomes larger as compared to fasted animals (Tables I-V). Because of this variable, it is of paramount importance to compare fasted irradiated rats with fasted non-irradiated controls.

From Tables I, II, and III, it is seen that fasted controls and irradiated animals did not differ significantly in SAR. This indicates that during the first day after irradiation, the brain transfer rate of phosphorus is not appreciably changed. Lack of differences in the 48- and 96-hour group also suggests that irradiation has failed to affect phosphorus transfer (Tables II, III). However, at this time after administration of the tracer, the SA differences between plasma and brain are reduced to the point that relatively small differences in rate between controls and experimental animals may be masked by experimental variability.

A highly significant difference between fasted irradiated animals and faster controls was found in the group in which the tracer was administered 48 hours after irradiation (Table V), and the animals were sacrificed 24 hours later. Since fed groups earlier showed discrepancies attributable to decreased food intake of irradiated animals, *i.e.*, decreased intake of phosphate compared with the fed non-irradiated rats, the latter group was not investigated in the final series. The mean SAR in the irradiated animals is considerably higher than in the fasted controls. This strongly suggests an increase of phosphate exchange occurring 2 to 3 days after irradiation. A trend toward an increase of the SAR is also seen in the animals in the group which received the tracer 24 hours after irradiation (Table IV). This suggests that the beginning of accelerated phosphate exchange may be placed as early as 24 hours post-irradiation.

Conclusions. Whole head irradiation in rats with 20 Krad increases P^{32} uptake by the brain. These findings confirm the results of Stajic *et al*(8), and strongly suggest that ionizing radiation enhances the permeability of the BBB to phosphate. By introducing the time parameter in our study, we also were able to approximate the time of onset of the permeability change. It begins after a delay of at least 24 hours after exposure to irradiation. This delay time explains the negative findings of Florsheim *et al*(5), who could not have detected the delayed effect because of the temporal arrangement of their experiments. It now must be determined if ionizing radiation of the whole brain or its parts increases the permeability to other molecules, particularly to biogenic amines, and whether whole head irradiation affects the permeability of the brain in toto or whether there are loci of predilection in their reaction to X-ray treatment.

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