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EXPERIMENTAL RADIATION CATARACT

II. Cataract in the Rabbit Following Single Exposure to Fast Neutrons

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RADIATION cataracts which have occurred in cyclotron workers and in persons exposed to the atomic bombs are said to have been caused, in part at least, by the fast-neutron component of the radiation.[1] It is therefore of considerable practical moment to know the cataractogenic dose of neutrons as exactly as possible under a variety of conditions and to compare the neutron effects on the lens with those on other tissues and with the effects of other forms of radiation.

The biologic action of neutrons has been studied in a variety of tissues, ranging from such relatively simple systems as hatching Drosophila eggs and germinating wheat seedlings[2] to carcinoma in human beings.[3] It is generally conceded that neutrons have much the same qualitative effect on tissue as do x-rays and other ionizing radiations,[4] but the effects produced by different amounts of neutron radiation do not necessarily vary in a way comparable to those induced by different

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1. Abelson, P. H., and Kruger, P. G.: Cyclotron-Induced Radiation Cataracts, Science **110**:655, 1949. Krause, A. C., and Bond, J. O.: Neutron Cataracts, Am. J. Ophth. **34**:25, 1951.

2. A relatively recent review of the biologic effects of neutrons on a variety of tissues may be found in Aebersold, P. C., and Lawrence, J. H.: Physiological Effects of Neutron Rays, Ann. Rev. Physiol. **4**:25, 1942.

3. The most exhaustive study of neutron therapy of malignant neoplasms is that of Stone, R. S.: Neutron Therapy and Specific Ionization, Am. J. Roentgenol. **59**:771, 1948.

4. Thus, both neutrons and x-rays produce an inhibition of mitosis, epilation, epidermolysis, and comparable blood and lymphatic changes. But Spear and Tansley (Spear, F. G., and Tansley, K.: Action of Neutrons on Developing Rat Retina, Brit. J. Radiol. **17**:374, 1944) found a greater amount of cell damage from neutrons, and Marshak (Marshak, A.: Proc. Nat. Acad. Sc. **28**:29, 1942) found more chromosomal damage in the resting cells with neutrons than with x-rays. Moreover, Mitchell (Mitchell, J. C.: Brit. J. Radiol. **20**:368, 1947) found that the biologic effectiveness of neutrons as compared with that of gamma rays was much greater for 48-hour exposures than for 1-hour exposures, indicating a difference in the modus operandi of the two types of radiation. The latter author suggested that proliferating cells are much more sensitive to gamma radiation during a short interval in the prophase of mitosis, but, on account of the high specific ionization of the recoil protons, the proliferating cells show a much more uniform sensitivity to neutrons throughout both the resting stage and the mitotic cycle.

amounts of x-rays or gamma rays. These differences are due in part to the greater ion density of the recoil particles resulting from the neutrons. But even when allowances are made for this difference in distribution and amount of ionization, there still appear to be discrepancies in the effectivity of neutrons and of x-rays. Thus, when a comparison is based on reps (roentgen equivalent physical), whereby tissue ionization is equalized by definition, the ratio of neutron to x-ray (or gamma-ray) effectivity varies from less than 1, in the case of gene mutations,[5] to over 30, in the case of the survival time of mice.[6] Since all the factors responsible for this divergence in tissue susceptibility are not currently understood, the cataractogenic properties of neutrons cannot be reliably predicted on the basis of present x-ray studies and must be determined by experimentation.

The most thorough study of neutron cataract to date has been that of Titus Evans [7] on the mouse, but it should be noted that the first observation on the cataractogenic property of neutrons was made by Horn [8] on a no more evolved species than Amblystoma larvae.

Evans found that cataracts were produced in mice with neutron doses which were compatible with life and emphasized especially that the cataractogenic properties of neutrons, in comparison with those of x-rays, increased significantly with chronic exposures. Evans' conclusions are especially noteworthy, since they were made before it became known that cataracts were developing in cyclotron workers.

Heretofore, practically all the biologic studies of neutron effects have been necessarily limited by an arbitrary method of dosimetry. With few exceptions,[9] when some other indirect means was employed, air ionization chambers (usually the Victoreen dosimeter) have been used.

In this country, the N unit has been adopted, which by definition is the amount of ionization induced by the neutrons that is equal to 1 r induced by x-rays in the ionization chamber. But it has been apparent to all investigators that a ratio of r : N readings with such an ionization chamber will not indicate the relative amounts of ionization in tissues; nor will the correction factor of 2 proposed for the difference between air and tissue [10] have general validity for all energies. Thus, it is probably not justifiable to attach absolute values to the results unless the exposures were

5. Lea, D. E.: Actions of Radiation on Living Cells, New York, The Macmillan Company, p. 149.

6. Mitchell, J. S.: Experiments on Mechanism of Biologic Action of Fast Neutrons: Using Summation Method for Lethal Effects in Mice; with Section of Dosimetry of Fast Neutrons, Brit. J. Radiol. **20**:368, 1947. Henshaw, P. S.; Riley, E. F., and Stapleton, G. E.: Plutonium Project: Biologic Effects of Pile Radiation, Radiology **49**:349, 1947.

7. Evans, T. C.: Effects of Small Daily Doses of Fast Neutrons on Mice, Radiology **50**:811, 1948; Nucleonics **4**:2 (March) 1949.

8. Horn, E. C.: Relative Biological Effectiveness of Roentgen Rays and Neutrons on Regeneration of Forelimb of Amblystoma Larvae, Am. J. Roentgenol. **46**:727, 1941.

9. Zimmer, K. G.: Dosimetrische und strahlenbiologische Versuche mit schnellen Neutronen, Strahlentherapie **63**:517, 1938. Gray, L. H., and Read, J.: Measurement of Neutron Dose: Biological Experiment, Nature **144**:509, 1939. Zirkle, R. E., and Aebersold, P. C.: Relative Effectiveness of X-Rays and Fast Neutrons in Retarding Growth, Proc. Nat. Acad. Sc. **22**:34, 1936. Investigators who have used methods analogous to those in the present experiment are to be cited subsequently.

10. Lawrence, J. H., and Lawrence, E. O.: Biological Action of Neutron Rays, Proc. Nat. Acad. Sc. **22**:124, 1936. Lawrence, E. O.: Biological Action of Neutron Rays, Radiology **29**:313, 1937. Evans, R. D.: Radioactivity Units and Standards, Nucleonics **2**:32 (Oct.) 1947.

made with neutrons of the same energy and measurements were made with one and the same ionization chamber. It is known, for instance, that two ionization chambers calibrated to give comparable readings for equal x-ray exposures may vary considerably for equal neutron exposures.[11] While experiments using the same neutron source and ionization chamber will yield valuable information within any one series, it is obviously desirable to have a more absolute method of dosimetry for universal comparisons.

Recently, several types of apparatus have become available whereby the neutron flux can be ascertained at the source more readily than has been the case with the cyclotron, thus greatly facilitating the problem of accurate dosimetry. Two of these were used in the present experiments. One is the Cockcroft-Walton Accelerator,[12] in which deuterons bombard a tritium target,[13] with the emission of alpha particles and 14-mev neutrons in a 1:1 ratio. Thus, by measuring the alpha particles, one has a direct measure of the neutron flux. The other type of apparatus used in the present experiments is the so-called Fast Reactor,[14] utilizing the neutrons from plutonium fission, the emission being varied by the power yield. In contrast to the monoenergetic neutrons of the Accelerator, those of the Fast Reactor have a spectrum with a peak energy of 300 kev and will be collectively referred to as fission neutrons. The purpose of running duplicate experiments with the Accelerator and the Reactor was to determine the difference in cataractogenic properties of neutrons of widely different energies. In both types of apparatus, the dosage was controlled by varying the time of exposure and the distance from the target, with the addition of the power control in the case of the Fast Reactor. The doses could then be expressed in terms of the absolute units of neutrons per square centimeter reaching the lens.

While this type of apparatus gives promise of replacing the cyclotron for much biologic work in which neutrons are desired, few such studies have been made with them so far, and none on their cataractogenic properties. Using the Cockcroft-Walton accelerator, Carter[15] found in preliminary studies that 2×10^{10} neutrons/cm² had an effect in causing reduction in size of the thymus and spleen comparable to that of 144 (± 20) r of 250-kv. x-rays, while with the 1-mev neutrons of the electrostatic generator 2×10^{10} neutrons/cm² had the same effect as 49 to 58 r. To date, there do not appear to be any other publications of biologic studies using this method of dosimetry.

MATERIALS AND METHODS

French silver rabbits were used in all the experiments. This was the available species most closely related to the chinchilla rabbits used in the previous x-ray study.[16] All the rabbits were within a week of being 10 weeks old at the time of exposure. For purposes of protection of

11. Abelson, P.: Neutron Interactions with Tissue, prepared for the Division of Medical Sciences, National Research Council, 1950.

12. Manley, J. H.; Haworth, L. J., and Luebke, E. A.: Developments in Ion-Accelerating Tubes, Rev. Scient. Instruments **12**:587, 1941.

13. Graves, E. R.; Rodrigues, A. A.; Goldblatt, M., and Meyer, D. I.: Preparation and Use of Tritium and Deuterium Targets, Rev. Scient. Instruments **20**:579, 1949.

14. Los Alamos Fast Reactor, Rev. Scient. Instruments **18**:688, 1947.

15. Carter, R.: Unpublished data.

16. Cogan, D. G., and Donaldson, D. D.: Experimental Radiation Cataracts: Cataracts in the Rabbit Following Single X-Ray Exposure, A. M. A. Arch. Ophth. **45**:508 (May) 1951.

the animal's body from the radiation, it was necessary in the experiments with high-energy neutrons to use an animal the size of a rabbit, rather than such small animals as the mouse and rat, which have been used in many previous studies. The employment of paraffin blocks of sufficient thickness to shield the animal's body from 14-mev neutrons would have necessitated removing the animal so far from the target that the exposure times would have been impractically long. Instead, the rabbit was placed with head so much nearer the source that the body was protected effectively by the attenuation of the dose with distance, the dose varying, of course, inversely as the square of the distance. In this way, it was possible with rabbits to give doses to the eyes many times the equivalent lethal dose applied to the whole body. After the exposures the animals were examined for clinical evidence of epilation, keratoconjunctivitis, and cataract in the same manner as previously described in the x-ray study.

Examinations of the eyes were made at weekly intervals except immediately after the exposures, when they were made at semiweekly intervals. When the animal died or was intentionally killed, the eyes were enucleated, and flat preparations of the lens capsules were made according to the technique previously described.[16] White blood cell counts and differential counts were done at intervals of 1, 3, 5, 10, and 20 days after the exposure on all rabbits which were expected to receive more than one-tenth lethal dose of body radiation.

As the technique of exposure to the Cockcroft-Walton Accelerator was necessarily different from that to the Fast Reactor, the two will be described separately. In both sets, however, the range of doses delivered to the lens was between 1×10^9 and 1×10^{12} neutrons/cm.^{2}

The Cockcroft-Walton-Accelerator neutron source consists essentially of a tritium target (zirconium saturated with tritium) bombarded by deuterons. The target of the Accelerator used in the present study was enclosed in a thin-walled spherical metal shell having a radius of 2.03 cm. The neutrons were emitted uniformly in all directions with a source strength that varied during the exposure but reached a peak of 5×10^9 neutrons/sec. The rabbits were secured firmly to a board in an upside-down position, with head held effectively immobile by side supports.[17] The rabbits were then arranged about the target so that the eyes were as close as possible to, and the bodies were as far away as possible from, the target. In this way four or five rabbits were exposed at a time. The dose delivered to each eye varied according to the source strength, time of exposure, and distance. The dose delivered was equal to neutrons produced by the source divided by $4\pi r^2$, where r is the distance from the target to the lens. The duration of the exposure varied from six hours, for the more heavily irradiated animals, to less than one hour, for the rabbits least heavily irradiated.

The neutron source produces no primary gamma rays. The only gamma rays are those produced by inelastic scattering of neutrons in the target material. This target material is kept to a minimum, so that less than 1% of the neutrons suffer collisions on their way out through it. Consequently, the neutron source may be considered a pure neutron source, free from gamma rays.

The Fast Reactor[18] consists essentially of a nuclear reacting assembly. It is enclosed in a heavy concrete housing having portals for exposure, the exit of one of these portals being 158 cm. from the source. For our purposes, an aluminum tube was fitted to one of the exit portals, so that the total distance from the pile to the end of the tube was 203 cm. For the exposure, the rabbit's eye was firmly placed against this exit opening and the animal immobilized as previously described. Only one eye of each animal was used, and only one animal was exposed at a time. Accurate calibrations were available which gave neutrons/cm.^{2}/sec. at the distance used as a function of operating power of the Reactor. The dose was adjusted by varying the operating power of the Reactor and the time of exposure. The maximum power of the pile was 25 kw., and the longest time of exposure was 70 minutes. For all exposures involving less than 1×10^{11} neutrons/cm.,^{2} the exposures were five to seven minutes.

The spectrum of the neutrons has been measured by Nereson,[19] using proton recoils in a photographic plate and a cloud chamber. The most probable energy is that previously mentioned, about 300 kev. Gamma rays in the neutron beam are estimated by countermeasurements to be about 1 r per 10^9 neutrons/cm.^{2}

17. Dr. Clarence Lushbaugh designed the rabbit holder and made many helpful suggestions.
18. Dr. David Hall and Dr. Clarke Williams furnished and operated the Fast Reactor.
19. Nereson, N.: Personal communication to the authors.

RESULTS

Except for the rabbit receiving the maximal radiation to the eye (1×10^{12} neutrons/cm.^{2}), none of the animals died of the syndrome of radiation sickness. The blood counts during the three weeks following irradiation showed no drop in comparison with the preirradiation levels (average 7,800 cells/cm^2 with a variation between 4,600 and 14,000), and there was no detectable shift in the granulocyte-lymphocyte ratio. Several animals did die, however, as a result of radiation nasopharyngitis, and many died of coccidioidosis.

The ocular lesions resulting from the neutron irradiation were essentially similar to those following x-irradiation. The prime abnormalities consisted of epilation, keratoconjunctivitis, and cataracts. The threshold dose for both the epilation and the keratoconjunctivitis was of the order of 1.5×10^{11} neutrons/cm.^{2}, while that for the cataracts, to be discussed subsequently, was considerably less. The epilation varied from a minimum of loss of hair for several centimeters about the eyes to complete epilation of the head. The keratoconjunctivitis varied according to the dose from a mild and completely reversible redness of the eyes and irregularity of the corneal surface to ulceration and spontaneous perforation of the cornea.

The early lens changes were of two types, distinguishable on the bases of morphology, time of onset, duration, and probable pathogenesis. For purposes of identification, they may be called the vacuolar and the granular type.

The vacuolar type consisted of vacuoles appearing first at either end of the posterior suture and on progression extending toward and across the midline to form a rope of vacuoles just anterior to the posterior capsule. While predominantly situated in the region of the posterior suture line, they may be scattered for some distance about the suture and rarely may be unassociated with the suture.

When present, these vacuoles were the first changes to be found in the lenses after irradiation and appeared to be a more conspicuous feature of the neutron-induced cataracts than had been the case with x-ray-induced cataracts. Their time of onset and duration varied widely (Charts 1 and 2). With the higher doses (e. g., 1×10^{12}/cm.^{2}) the vacuoles appeared within a few hours after the irradiation, whereas with intermediate doses (e. g., 1×10^{10}/cm.^{2}) they did not appear for two weeks; with lower doses they were absent altogether, although other lens changes did occur. The duration of the purely vacuolar type of change was difficult to determine with the higher doses because it overlapped the granular type of opacification, which by itself may have produced vacuolation, but with the intermediate doses the vacuolar changes were characteristically transitory, the duration varying with the dose from a few days to a few weeks. On disappearance of the vacuoles the lenses appeared entirely normal.

The granular type of change appeared as black dots when seen ophthalmoscopically or as white punctate granules when seen biomicroscopically. They were to be found both centrally, especially condensed along the posterior suture line, and at the periphery, as a corona coming from the equator. Their appearance and further course were the same as has been described and pictured for the x-ray-induced changes.[16]

The time of onset of these granular opacities was in some measure a function of dosage (Charts 1 and 2), varying from a matter of a few days, for the higher doses, to months, for the lower doses, but in any case being longer than that for the

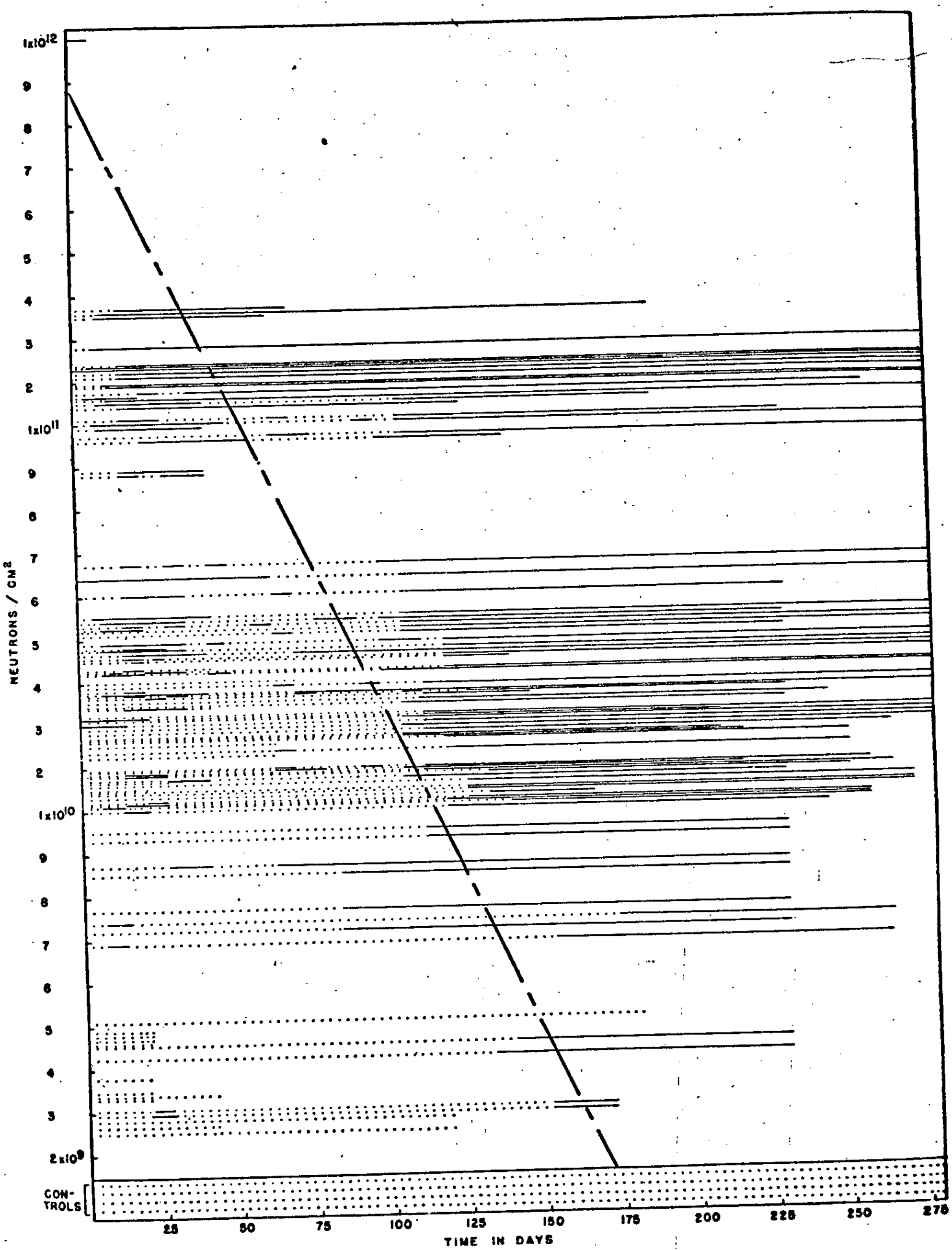

Chart 1.—Lens changes occurring in eyes which had been exposed to 14-mev neutrons (Cockcroft-Walton Accelerator). Each horizontal line represents the period of observation on a single eye. The dotted portions indicate periods when the lenses were normal, and the solid portions, periods when abnormalities were to be found in the lenses. No attempt was made to indicate the qualitative changes in the lenses, but it may be stated that the transitory changes were exclusively vacuolar, while the permanent changes were entirely granular in the lower-dose range and were a mixture of vacuolar and granular in the higher-dose range. Termination of a line indicates the end of the observation on that eye through spontaneous death or purposeful disposal of the animal. The oblique line represents the approximate time of onset of the permanent lens changes.

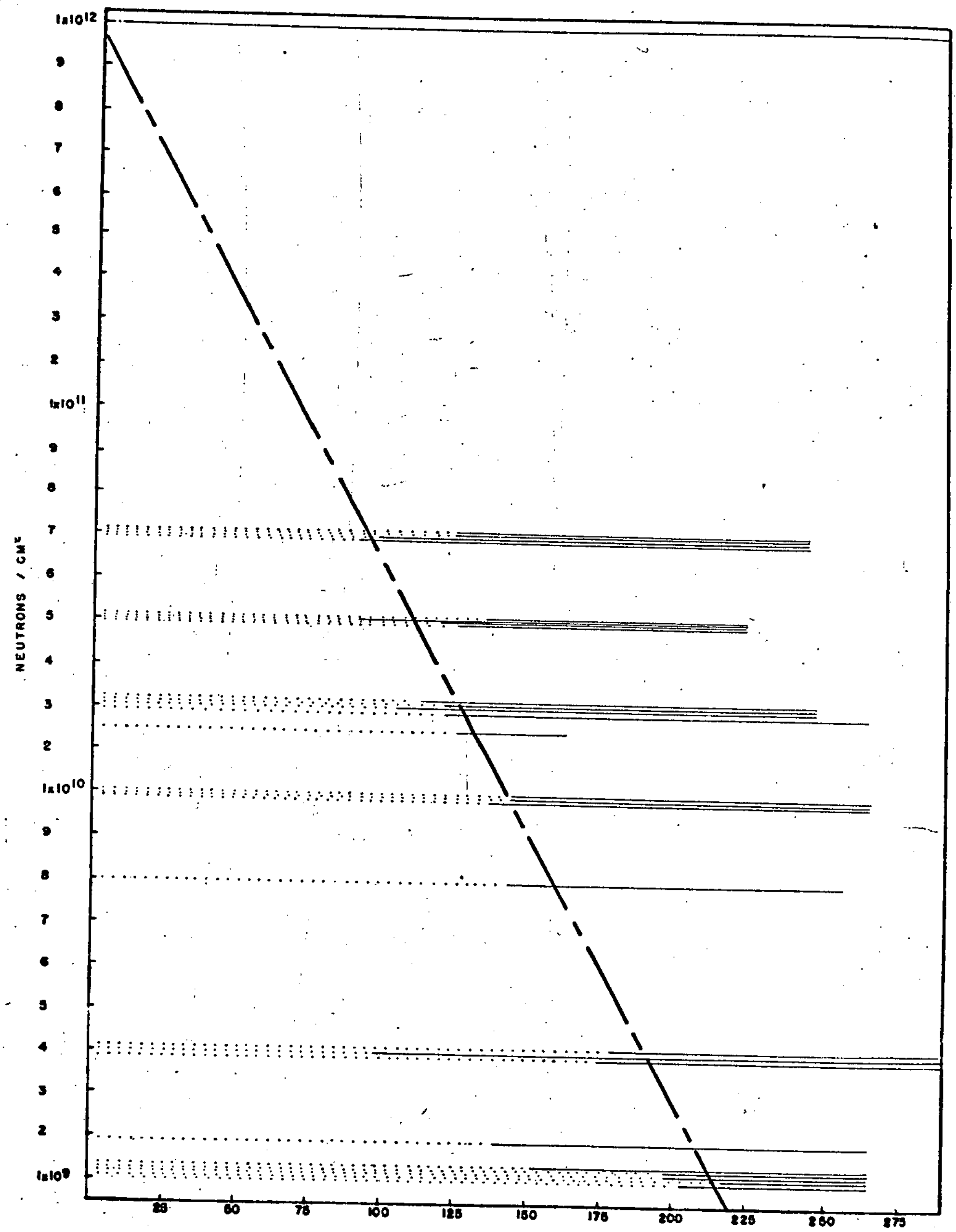

Chart 2.—Lens changes occurring in eyes which had been exposed to fission neutrons (Fast Reactor). The designations are the same as those indicated in Chart 1.

vacuoles. The distribution was also a function of dosage, for with the higher doses peripheral and central changes occurred approximately synchronously, while with the lower doses (i. e., less than 1×10^{10} neutrons/cm.^{2}) central changes were to be found exclusively. Unlike the vacuolar changes, the granular changes were never reversible. Once present, they were always present, although with the lower doses they showed no gross change and were stationary for months, or possibly for the life of the animal.

Histologic studies on the neutron-irradiated lenses were carried out similarly to those described in the x-ray experiments.[16] The findings were entirely similar, consisting of fragmentation and anisocytosis of the anterior lens epithelium and migration of cells beneath the posterior capsule, with varying amounts of cortical and cellular lysis. Because of the reported observation that neutron irradiation was more damaging to resting cells,[20] a search was made to determine whether or not the damage to the lens epithelium was less spotty than it had been with x-rays, but no convincing evidence was obtained that this was the case.

The pathogenesis of the granular type of opacity appeared clear-cut. The opacities were made up of cells which had actively migrated or had been displaced to the posterior pole of the lens. They were presumably cells which had been damaged by the irradiation and, while not being killed, had lost their ability to differentiate normally into lens fibers at the equator. If the dose had been relatively low and the damage not severe, the cast-off cells remained as the only residuum of an otherwise normally growing lens. If the damage had been severe, on the other hand, the number of cells, and therefore the granular opacities, continued to pile up both in the center and in the periphery of the lens. The pathogenesis of the vacuolar type of opacity is much less clear-cut. It antedated the granular opacities and was, therefore, not related to the migration of cells. The fact that it was noted within a few hours after the severe irradiation (1×10^{12} neutron/cm.^{2}) suggested that it resulted from direct denaturation of the cortical material of the lens, perhaps analogous to irradiation denaturation of protein systems in vitro.[21] But this does not account for the predilection of the vacuoles for the posterior suture line or for the latent period of several days when intermediate doses were used. A further possibility which has been suggested in the case of x-ray cataracts, and which is difficult to confirm or deny, is that the vacuoles resulted from cytolysis at the equator of the lens or from abnormal permeability of the lens capsule and that the resultant vacuoles had been passively squeezed to the region of the posterior longitudinal fissure as the place of least mechanical resistance.

The course of events, the dosage, and the morphology of the cataracts were in every way the same for the irradiation with neutrons of 14-mev energy as for that with neutrons of fission energy, except that there were none of the transitory changes noted in the lenses which had received the neutrons of lower energy (Chart 2).

COMMENT

Using the latent period and the morphology of the cataracts as the basis for comparison, one may approximate equivalent cataractogenic properties of neutrons and x-rays for acute exposures by comparing the data reported in this paper with

20. Marshak, A.: Relative Effects of X-Rays and Neutrons on Chromosomes in Different Parts of the "Resting Stage," Proc. Nat. Acad. Sc. **28**:29, 1942.

21. Failla, G.: Biological Effects of Ionizing Radiation, J. Applied Physics **12**:279 (April) 1941.

those previously obtained for x-rays.[16] When 500 r (1.2 mev) was taken as an arbitrary value, the latent period for x-ray cataracts was found to be 125 days; approximately equivalent latent periods for neutron cataracts were found to be 8×10^9 particles/cm.^{2} for 14-mev neutrons and 3×10^{10} particles/cm.^{2} for fission neutrons. Similarly, epilation and keratoconjunctivitis occurred with 1.5×10^{11} particles/cm.^{2} (14 mev) comparable to those changes occurring with 1,500 r of x-rays (1 mev).[22]

The latent period for cataract development was slightly longer in the case of fission neutrons than in the case of 14-mev neutrons, suggesting a somewhat slighter cataractogenic property. But the difference was of questionable significance in view of the considerable biologic variation. It is, however, in the direction that might be expected on the basis of ionization.

It should be noted that neither in the x-ray studies previously reported nor in the neutron studies here reported has the absolute threshold for cataract production been determined. In both the cataractogenic properties were underestimated in setting up the experiments, so that insufficient exposures were made in the low range. Such exposures are, however, being currently undertaken and will be reported at a subsequent date, after an observation period sufficiently long for the results to be conclusive. At the present time, however, it may be stated that the thresholds for lens opacities in both studies are considerably lower than previous evidence would have led one to believe, being 250 r (1.2 mev) or less in the case of x-rays and 2×10^9 particles/cm.^{2} or less in the case of neutrons.

The present experiments were concerned with acute exposures only, and it should be emphasized that the results do not indicate what the hazard is for chronic exposure or what the relation of x-ray to neutron dosage will be for chronic exposures. Evans[7] has shown, however, that the cataractogenic properties of neutrons, in comparison with those of x-rays, increase considerably with chronic exposure, an occurrence which has held for other biologic effects as well.[6] From a separate study now in progress, we hope to have data on the cataractogenic properties of chronic exposure to neutrons as measured by the number of neutrons per square centimeter incident on the lens.

SUMMARY AND CONCLUSIONS

The production of radiation cataracts and other ocular effects in rabbits has been studied, with the use of single exposures to relatively pure neutron sources of 14 mev and fission energy.

The course of events following neutron irradiation is basically similar to that following x-ray exposures, and effectivity as regards cataract production obtained with 8×10^9 neutrons/cm.^{2} (14 mev) or with 3×10^{10} neutrons/cm.^{2} (fission energy) is comparable to that obtained with 500 r (1.2 mev). While the morphologic changes in the lenses with neutrons and x-rays showed the same differences when heavy and when light doses were used, irradiation with 14-mev neutrons produced more of the transient vacuoles than did the x-irradiation or the fission-neutron irradiation.

The threshold dose for production of lens opacities by neutrons is less than 2×10^9 neutrons/cm.^{2} incident on the lens.

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22. The epilating dose of fission neutrons was approximately the same, but the determinations were less reliable, as the periocular regions were not uniformly exposed.