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QUANTITATIVE STUDIES ON THE RADIOSENSITIVITY OF SINGLE CELLS IN THE NERVOUS TISSUE

by

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The central nervous system, formerly considered fairly radioresistant, is known to respond to even extremely low doses of ionizing radiation. Physiologic and chemical changes in brain tissue have been reported and effects on constituents and enzymes at the cellular level have been demonstrated by histochemical techniques (HALEY et coll. 1962).

The specific response of the CNS to ionizing radiation comprises several phases; an acute stage (the first weeks after irradiation) which is followed by more or less complete recovery. A second stage starts in the fourth or fifth month after irradiation and lasts for years (ARNOLD et coll. 1954). Unless the irradiation dose is quite high the acute stage passes with unspecific or absent morphologic changes.

The present study was designed to elucidate the chemical events in nerve cells during the acute (first weeks) reaction to ionizing radiation before any morphologic changes have set in. Since glial cells (BROWNSON et coll. 1963) react to ionizing radiation differently from nerve cells, the problem can only be approached via isolated nerve cells. We applied a single dose of irradiation to investigate the reversible changes of the acute stage.

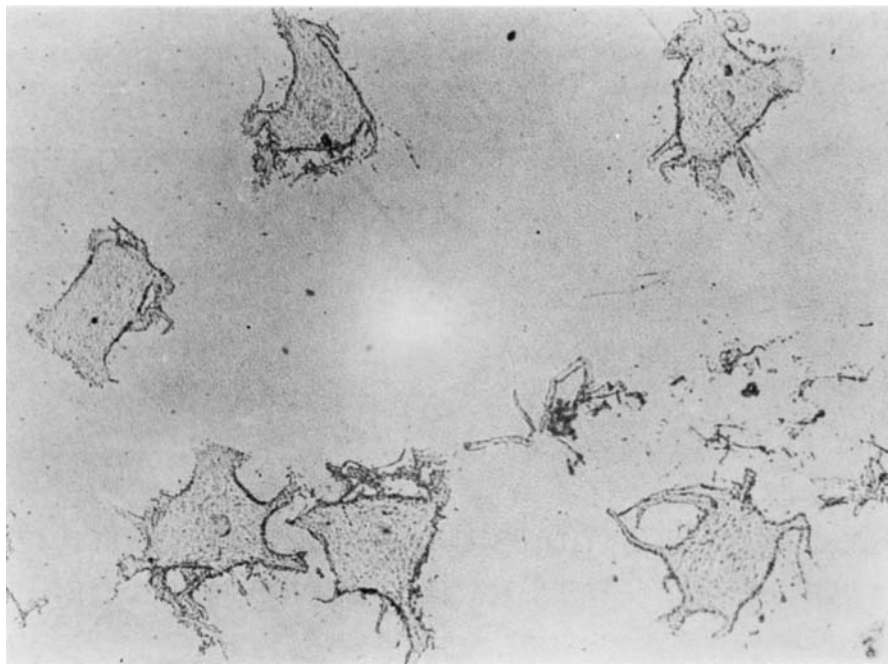

Fig. 1. Isolated nerve cells from the Deiters' nucleus prepared on aluminium foils for mass determination.

The caudal part of the brain of albino rabbits was irradiated and measurements on neurons from the lateral vestibular nucleus (N. Deiters) were performed at intervals ranging from 3 hours to 3 weeks after single exposures of 2 000 and 3 000 R. The total organic mass, the succinoxidase activity, the membrane permeability for potassium and the RNA content were recorded.

Material and Methods

Albino rabbits were used, and the brain stem area was irradiated with roentgen rays under the following conditions: 200 kV, 0.9 mm Cu HVL, 310 R/min and 6 cm×4 cm field. The animals were given a light barbiturate anaesthesia. Single surface doses of 3 000 R and 2 000 R were given and the actual dose given to the brain stem was estimated to be about 90 per cent of the surface dose.

The animals were killed 3 hours and 1 to 15 and 20 days after irradiation.

Slices of the brain stem were cut and a flake of tissue was taken from that part of the nucleus which contains Deiters' giant nerve cells. The single nerve cells were then separated by hand dissection.

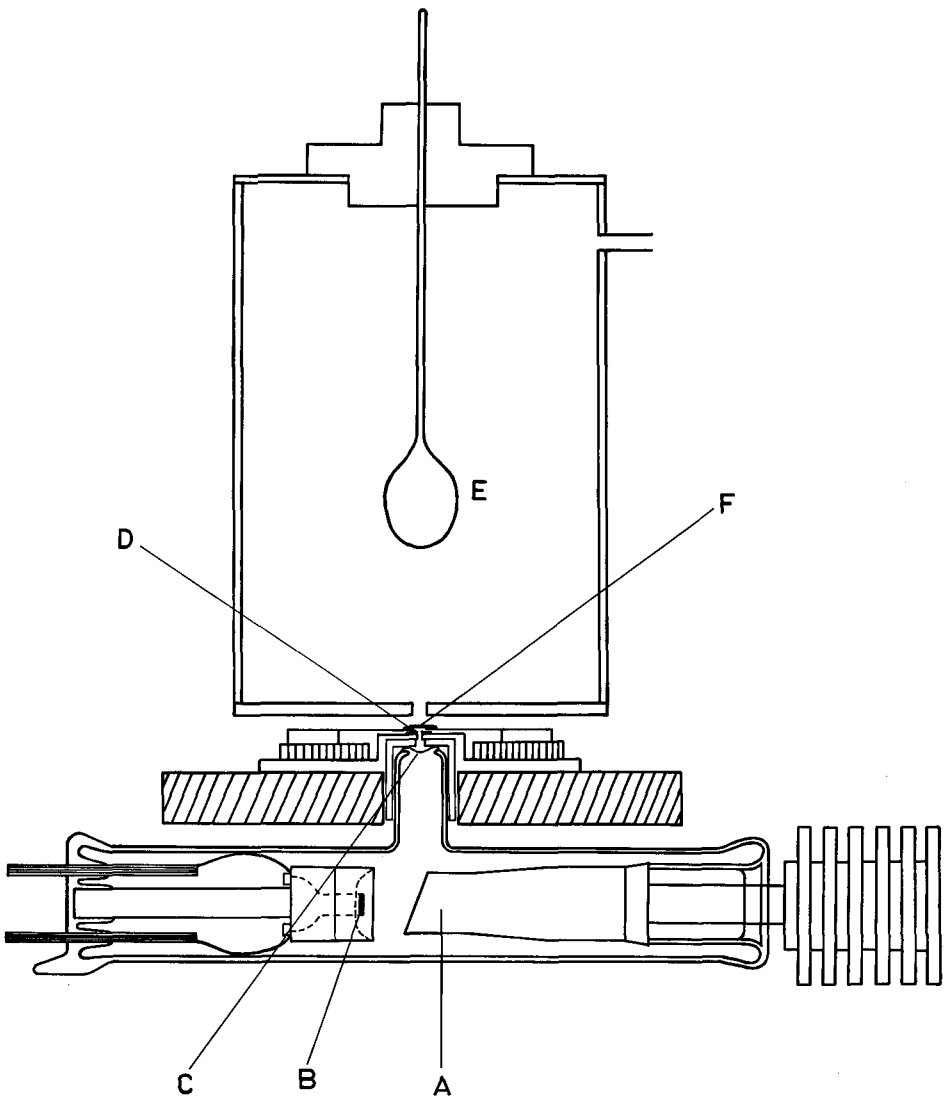

Fig. 2. Roentgen tube, specimen mount and proportional detector. A — anode, B — filament, C — 9 μ Al window, D — specimen stage with aperture, E — high voltage electrode, F — specimen.

The total organic mass, succinoxidase activity, potassium permeability and RNA-content were then determined in the following way.

Mass determination. The cells were placed on aluminium foils where they were dried (Fig. 1). The analysis was performed with an integrating roentgen

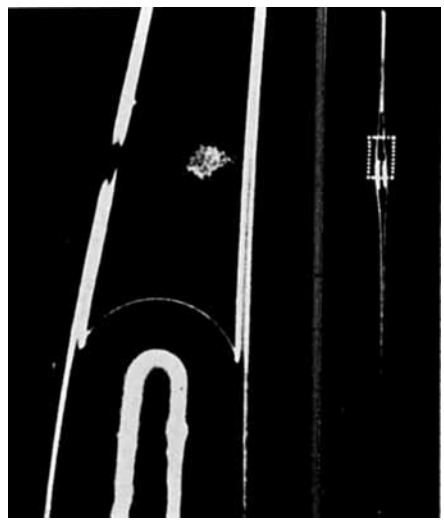

Fig. 3. Nerve cell within microdiver for respiration analysis. To the left, enlargement of the area indicated to the right in the picture. (From HAMBERGER 1963.)

absorption technique. A special roentgen tube operating at 3 kV is used. The soft radiation leaves the tube through a 9 μ aluminium window and is collimated by a small aperture, 100 to 200 μ in diameter, on top of which the specimen is placed. Passing the specimen, the cell, and, partly absorbed, the radiation is detected by a windowless proportional counter. The total organic mass is then calculated from the absorption value (ROSENGREN 1959, HYDÉN & ROSENGREN 1962). Fig. 2 shows a cross section of the apparatus.

Measurements of succinoxidase activity. The neurons were transferred to a drop of incubation medium and each cell was introduced in a microdiver (Figs 3 and 4a). The succinoxidase activity was determined by measuring the oxygen consumption according to ZEUTHEN (1953), and expressed as 10^{-4} μl O_2 per sample per hour (HAMBERGER 1963).

Potassium determination. The specimen is hit by roentgen rays from a Cosslett-Nixon X-ray microscope which can produce a high energy over a small spot. A set of electron microscope apertures has been used as scatter trap. On top of the last aperture the specimen is placed on a mylar foil. Secondary roentgen rays, the fluorescent radiation, is generated spherically. The energy of the fluorescent radiation is due to the elements present in the specimen and the intensity of each wavelength is proportional to the amount present in the specimen. A sector of the fluorescent radiation is taken up by a proportional counter, and together with a multichannel pulse height analyzer different energies of the radiation can be

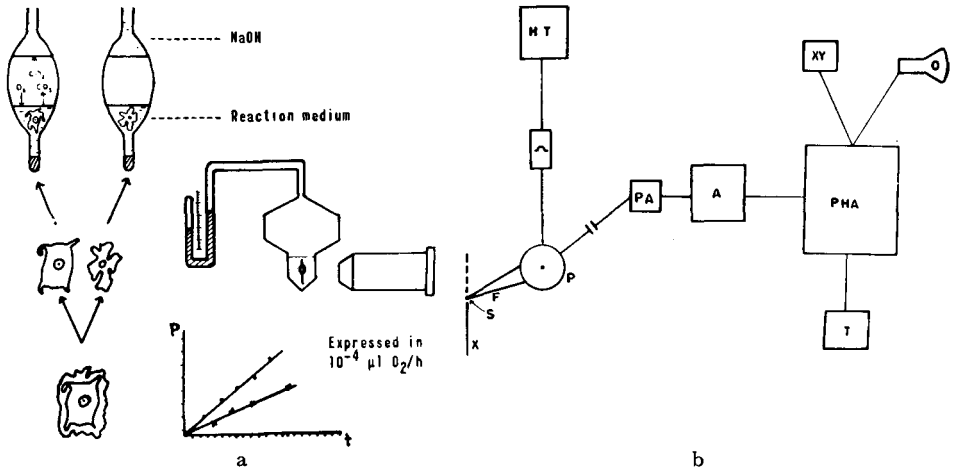

Fig. 4. a) Schematic demonstration of the micro-diver technique. b) Schematic demonstration of roentgen fluorescence micro-analysis of potassium. X — primary roentgen beam, S — specimen, P — proportional counter, F — fluorescent radiation, HT — high tension, pA — praecemplifier, A — amplifier, PHA pulse-height analyser, xy — xy recorder, O — oscilloscope, T — typewriter.

sorted out. A curve with different peaks is seen on an oscilloscope (Fig. 4b). Each peak represents one element and the height of the peak is proportional to the amount. By using an internal standard the system is independent on variations in the primary beam. Microdrops containing K and ^{131}I have been used as reference systems. The volume of the drops have been determined by their known concentrations of ^{131}I with a scintillation counter. Amounts down to 10^{-11} g K have been determined (Long & Röckert 1963).

RNA determination. The method of Edström (1953) was used. The cells were extracted with ribonuclease solution. The extracts were evaporated to dryness and redissolved in buffered glycerol to form lens-shaped droplets which were photographed at 257 Å. The plates were scanned and the RNA calculated from the curve obtained. The procedure is shown in Fig. 5.

Results

The results at 3 000 R are represented by the curves in Fig. 6. There was an increase in the total mass the first day, and a considerable increase occurred after 5 to 6 days. The results from the 12th and 13th day indicated a return towards the normal level. The means for the groups irradiated with 2 000 R and 1 000 R showed no significant difference from the control mean.

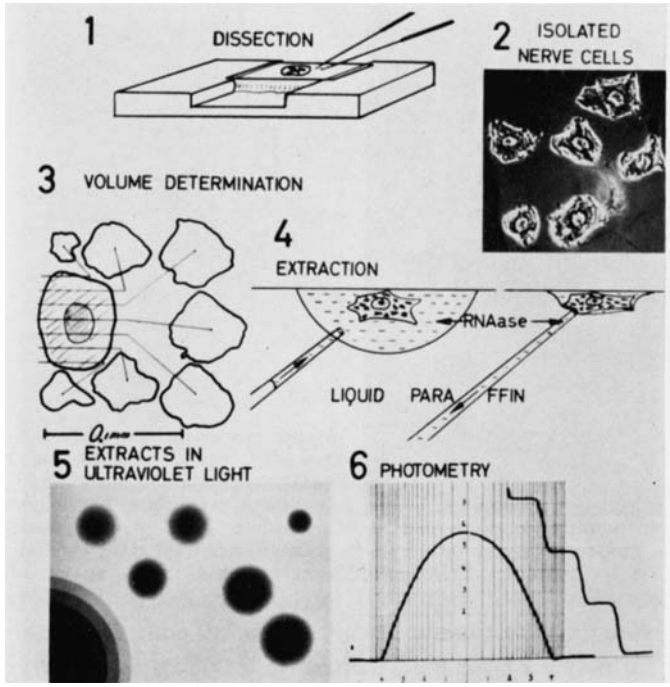

Fig. 5. Schematization of the technique for RNA analysis.

The succinoxidase activity after 3 000 R showed that the neurons developed an increase in respiration as early as a few hours after exposure to irradiation. The relatively moderate change was followed by a more slowly rising level, producing a peak of over 200 per cent increase 9 days after irradiation. The effect of 2 000 R was considerably smaller.

As can be seen from the curve, the potassium permeability increased so that the cell contained less potassium as early as 2 days after irradiation. The peak value occurred 4 days later. The cells gradually recovered but did not reach the level of the controls during the period studied.

The RNA-determination showed no impressive deviation from the normal value, but a slight increase after 5 days.

Discussion

The largest groups of animals received an irradiation dose of 3 000 R. This level was chosen after preliminary trials, mainly with lower doses. As histologically demonstrated by BERG & LINDGREN (1958) for delayed radiation lesions, the

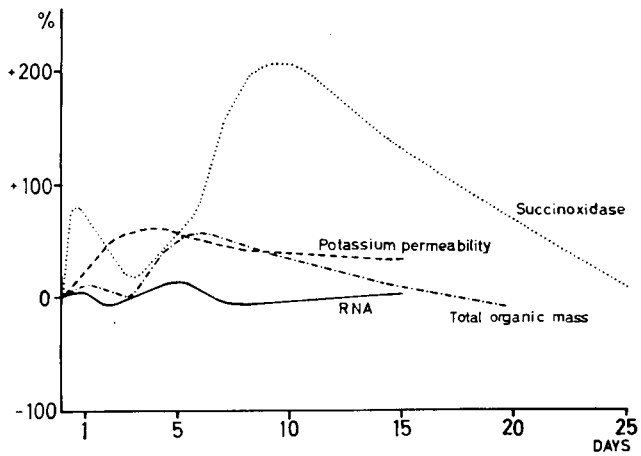

Fig. 6. Total organic mass, RNA content, succinoxidase activity and potassium permeability of Deiters' cell. Results in per cent of control correlated to time after local irradiation with 3 000 R (HALLÉN et coll. 1967).

measurable effects are small below 2 000 R, and a more definite change from the controls would be desirable, especially owing to the variation in response that may occur in one and the same animal. Even at this dose no obvious functional disturbances were encountered. The morphologic changes in the nerve cell bodies were also insignificant during the first months (ARNOLD et coll. 1954, SCHÜMMELFELDER 1962 and ZEMAN et coll. 1962).

The increase of the total cell mass is in accordance with DEVIKS' (1962) observation that protein synthesis is increased after exposure to radiation. The high level of respiration may also be due to the accelerated protein synthesis, partly involving mitochondria. Mitochondria are, however, morphologically and chemically very radiostable cell components and qualitative changes are probable only during the very early stage of increased succinoxidase activity. The prompt and rapidly reversed increase in enzymatic activity in this stage is difficult to rationalize.

The effects on potassium amounts indicate an action on cellular membranes. The technique used here does not distinguish between cells having a low potassium content in vivo and cells losing their potassium more rapidly in vitro. The intracellular potassium concentration is normally high after the radiation. An increase in extracellular potentials of motoneurons following irradiation has been reported (SATO et coll. 1962) and it is interesting that this effect reached its

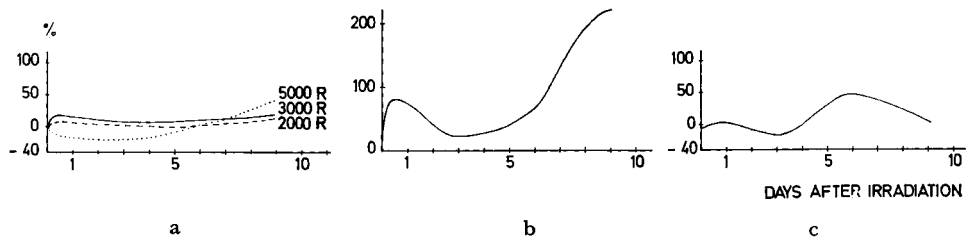

Fig. 7. Succinoxidase activity versus time after irradiation a) Brain stem homogenate. b) Nerve cells after 3 000 R. c) Glial cells after 3 000 R.

maximum about a week after the irradiation and then gradually decreased, but that the potentials still remained higher than the control values.

In a recent paper, SEYMOUR et coll. (1967) suggest that the effect of radiation of the frog sciatie nerve is probably leading to increased influx of sodium and efflux of potassium. Our findings strongly support this suggestion since the measured efflux of potassium was demonstrated in isolated neurons.

We have also made some studies of the succinoxidase activity in brain stem homogenates after various irradiation doses. Fig. 7 shows that the specific nerve cell response could possibly be reflected in the results of the homogenate but there is a considerable modification, probably because the changes in the glial cell respiration is quite different from those in the nerve cells.

We have found a threshold for measurable effects after a single surface dose of about 3 000 R, which corresponds to the histologic changes after delayed radiation lesions demonstrated by BERG & LINDGREN (1958).

After using a correcting factor according to DU SAULT (1958) for the small volume irradiated in these experiments, the single dose in the brain stem could be referred to a clinical fractionated treatment course of 6 000 R in 30 days. There also seems to be a threshold for radiation damage at this dose level, which agrees with the histologic and chemical experimental observations mentioned here.

SUMMARY

The total organic material, the RNA content, the succinoxidase activity and the potassium content of isolated Deiters' giant nerve cells were studied in rabbits after irradiation of the brain stem area. Succinoxidase activity in brain stem homogenates was also studied. The cell response is discussed in relation to other types of cell stimulation and other radiation experiments with nerve cells. The dose level for measurable effects is discussed in connection with histologic findings after delayed radiation lesions.

ZUSAMMENFASSUNG

Das ganze organische Material, das RNA-Gehalt, die Aktivität der Succinoxydase und das Kaliumgehalt isolierter Deiters-Riesennervenzellen wurden in Kaninchen nach Bestrahlung des Gehirnstammes studiert. Auch die Aktivität der Succinoxydase in den Homogenaten des Gehirnstammes wurde studiert. Die Zellenreaktion wird im Verhältnis zu verschiedener Arten von Zellenreizmitteln und experimenteller Bestrahlung von Nervenzellen diskutiert. Die Schwellendosis messbarer Effekte wird in Relation zu den histologischen Befunden bei Spätbestrahlungsläsionen gestellt.

RÉSUMÉ

Les auteurs ont étudié sur des lapins, après irradiation du tronc cérébral, le contenu organique total, la teneur en ARN, l'activité de la succinoxydase et la teneur en potassium de cellules nerveuses géantes isolées de Deiters. Ils ont aussi étudié l'activité de la succinoxydase d'homogénates de tronc cérébral. Ils comparent les effets cellulaires de cette irradiation aux effets d'autres types de stimulation cellulaire et d'autres expérimentations d'irradiation de cellules nerveuses. Ils étudient les quantités de dose nécessaires pour produire des effets mesurables et les rapprochent des constatations histologiques que l'on trouve dans les radiolésions retardées.

REFERENCES

ARNOLD A., BAILEY P. and LAUGHLIN J. S.: Effects of betatron radiations on the brain of primates. Neurology 4 (1954), 165.

BERG N. and LINDGREN M.: Time-dose relationship and morphology of delayed radiation lesions of the brain in rabbits. Acta radiol. (1958) Suppl. No. 167.

BROWNSON R. H., SUTER D. B. and DILLER D. A.: Acute brain damage induced by low dosage X-irradiation. Neurology 13 (1963), 181.

DEVIK F.: Cellulaere strålevirkninger. (In Norwegian.) Nord. Med. 67 (1962), 533.

DU SAULT L. A.: The time-dose relationship in radiotherapy. *In*: Progress in radiation therapy. Vol. 1, p. 100. Edited by F. Buschke. Grune & Stratton, New York 1958.

EDSTRÖM J.-E.: Ribonucleic acid mass and concentration in individual nerve cells. A new method for quantitative determinations. Biochem. biophys. Acta 12 (1953), 361.

HALEY T. J. and SNIDER R. S. (editors): Response of the nervous system to ionizing radiation. First Internat. Symposium. Academic Press, New York and London 1962.

HALLÉN O., HAMBERGER A., ROSENGREN B. and RÖCKERT H.: Quantitative response of neurons to X-irradiation: total organic mass, succinoxidase activity, potassium permeability and RNA content in isolated cells. J. Neuropath. exp. Neurol. 26 (1967), 327.

HAMBERGER A.: Difference between isolated neuronal and vascular glia with respect to respiratory activity. Acta Physiol. scand. (1963) Suppl. No. 203.

HYDÉN H. and ROSENGREN B.: A comparison of two integrating X-ray absorption methods for the determination of total cell mass. Biochem. biophys. Acta 60 (1962), 638.

LONG J. and RÖCKERT H.: X-ray fluorescence microanalysis and determination of potassium in nerve cells. *In*: Third Internat. Symposium on X-ray Optics and X-ray Micro-Analysis, p. 513. Academic Press, New York 1963.

ROSENGREN B.: Determination of cell mass by direct X-ray absorption. Acta radiol. (1959) Suppl. No. 178.

SATO M., AUSTIN G. M. and STAHL W. R.: Delayed radiation effects on neuronal activity in the spinal cord of the cat. *In*: Effects of ionizing radiation on the nervous system. A Symposium, pp. 93—110. Proc. IAEA, Vienna 1962.

SCHÜMMELFELDER N.: Sequence of X-radiation damage in mouse cerebellum. *In*: Response of the nervous system to ionizing radiation, p. 191. Edited by T. J. Haley and R. S. Snider. Academic Press, New York and London 1962.

SEYMOUR R. and DAWSON K. B.: Variation in the response and threshold to electrical excitation of X-irradiated isolated frog nerve with dose and dose-rate. Int. J. Radiat. Biol. 12 (1967), 1.

ZEMAN W., CURTIS H. J., SCARPELLI D. G. and KLEINFELD R.: Chemical and enzymatic changes in nerve cells irradiated with high energy deuteron microbeams. *In*: Response of the nervous system to ionizing radiation, p. 429. Edited by T. J. Haley and R. S. Snider. Academic Press, New York and London 1962.

ZEUTHEN E.: Growth as related to the cell cycle in single-cell cultures of *Tetrahymena piriformis*. J. Embryol. exp. Morph. 1 (1953), 239.