

short day plant *Pharbitis nil*. The onset of light as well as the onset of darkness induce rhythmic sensitivities to red light. Only the on-rhythm shows in addition to this a rhythmic sensitivity to the length of the dark period, leading to a step-wise increase in flowering with variable dark period lengths. They conclude, therefore, that on- and off-rhythms are physiologically different. In our department investigations are under progress to characterize the on- and off-rhythms of *D. pseudoobscura* and to find out whether the two rhythms are qualitatively different<sup>5</sup>.

The validity of the on- and off-rhythm concept is further stressed by experiments on *Drosophila* emergence, in which more complicated programmes such as repeated cycles of pulses are given. Even in this case the experimental results are in fairly good agreement with the predictions of superimposed on- and off-rhythms<sup>6</sup>. Another point which is under investigation and will be published elsewhere should be mentioned: short light periods (2.5 min) still contain the information of both the on- and off-signal<sup>7</sup>. An important paper by WEVER<sup>8</sup> will be discussed in respect to this work and to similar experiments on the petal movement of *Kalanchoe* in another place.<sup>9</sup>

**Zusammenfassung.** Bei *Drosophila pseudoobscura* synchronisiert ein einmaliger Übergang von Dauerdunkel zu Dauerlicht und von Dauerlicht zu Dauerdunkel das Schlüpfen der Fliegen aus dem Puparium (Figur 2). Die Ergebnisse von Licht-Puls- und Dunkel-Puls-Experimenten lassen sich als Überlagerung solcher einfachen Stufeneffekte erklären.

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<sup>5</sup> M. K. CHANDRASHEKAR, unpublished.

<sup>6</sup> D. HENGST, unpublished.

<sup>7</sup> H. W. HONEGGER, unpublished.

<sup>8</sup> R. WEVER, Z. vergl. Physiol. 57, 1 (1965).

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## Electrolyte Content of the Cerebral Cortex in Developing Rats after Prenatal X-Radiation<sup>1</sup>

Prenatal X-radiation alters the functional development of the central nervous system (CNS) as shown by enhanced appearance of the maximal seizure pattern<sup>2</sup>, by abnormal electrocorticograms and encephalograms<sup>3,4</sup>, increased susceptibility to spontaneous<sup>5</sup> and audiogenic<sup>6</sup> seizures. These effects of prenatal X-radiation may reflect anatomical, neurochemical, and physiological changes during CNS development.

In view of the role of the ionic environment in the development of CNS activity, the present study was designed to investigate electrolyte content in the cerebral cortex of rats irradiated in utero.

At 14 days of gestation, pregnant rats were exposed to a single dose of 100 r whole body X-radiation at a rate of 19 r/min. A 180 kV 15 mA X-ray machine was used. The filters were 0.5 mm Cu and 1.0 mm Al. The animals were placed in individual open-ended lucite cylinders rotated on a movable table 59 cm from the X-ray source. A Victoreen R-meter was used for dose calibrations. As controls, pregnant rats sham-irradiated at 14 days of gestation were used.

Litters of 6 rats were used. 2 rats from each litter were sacrificed by decapitation at 9, 23, and 44 days after birth. Samples of cerebral cortex from 8 controls and 8 irradiated animals were used for determinations of Na, K, and Cl content. Cerebral cortex samples were dried at 105°C to constant weight, and water content was calculated from the difference in wet and dry weights. The dried tissue was ground, extracted in 1 N HNO<sub>3</sub> for 48 h at 56°C, and Na and K contents were determined with a Li internal standard flame photometer. Cl was measured by the electrometric titration method of COTLOVE et al.<sup>7</sup>. To determine significance of differences between control and irradiated rats, the *t* test for non-paired data was applied<sup>8</sup>.

Water content decreased with age in both controls and irradiated animals; differences were not observed be-

tween the 2 experimental groups at any age period studied (Figure).

In control animals, K progressively increased and Na and Cl contents progressively decreased with age. This is in agreement with other studies by VERNADAKIS and WOODBURY<sup>9</sup>. In irradiated animals, K content remained generally constant with age, except at 9 days where it was significantly higher than in controls. Na and Cl contents progressively decreased with age. At days 23 and 44 these ions were significantly lower in the irradiated than those in appropriate controls, whereas at 9 days significant differences were not observed.

The changes induced by prenatal X-radiation on electrolyte content cannot be attributed to water changes between control and irradiated animals, but rather reflect changes in cellular brain compartments. BRIZZEE and JACOBS<sup>10</sup> have shown that the glial index (number of glia divided by number of neurons) increases with age. VERNADAKIS and WOODBURY<sup>11</sup> have reported that during

<sup>1</sup> This work was supported by contract AT(11-1)-34, Project 82, from the U.S. Atomic Energy Commission.

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<sup>3</sup> J. T. EAYRS, In *Regional Neurochemistry* (Ed., S. S. KETY and J. ELKES; Pergamon Press, New York 1961), p. 423.

<sup>4</sup> M. BERRY, B. G. CLENDENNIN, and J. T. EAYRS, Electroenceph. clin. Neurophysiol. 15, 91 (1963).

<sup>5</sup> M. R. SIKOV, J. S. MEYER, C. F. RESTA, and J. E. LOFSTROM, Radiat. Res. 12, 472 (1960).

<sup>6</sup> J. WERBOFF, J. DEN BROEDER, J. HAVLENA, and M. R. SIKOV, Expl. Neurol. 4, 189 (1961).

<sup>7</sup> E. H. COTLOVE, H. V. TRANHAM, and R. L. BOWMAN, J. Lab. clin. Med. 57, 461 (1958).

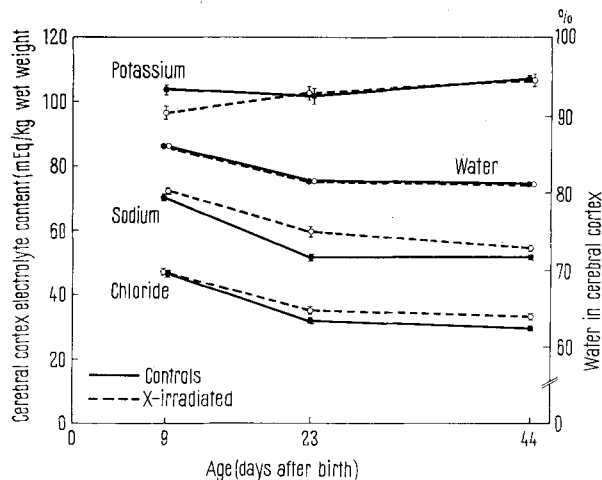
<sup>8</sup> R. A. FISHER, *Statistical Methods for Research Workers* (Hafner, New York 1950).

<sup>9</sup> A. VERNADAKIS and D. M. WOODBURY, Am. J. Physiol. 203, 748 (1962).

<sup>10</sup> K. R. BRIZZEE and L. A. JACOBS, Growth 23, 337 (1959).

<sup>11</sup> A. VERNADAKIS and D. M. WOODBURY, Arch. Neurol. 12, 284 (1965).

brain maturation the volume of the glial compartment increases, and those of the interstitial and neuronal compartments decrease. According to these authors, the increase in the glial compartment and decrease in interstitial compartment account for the decrease in Na and Cl, since



Changes in water and Na, K, and Cl content of the cerebral cortex in offspring of rats exposed to 100 r whole-body X-radiation at 14 days of gestation. Points with bracketed lines represent means and standard errors (standard errors for water values are less than 0.1% and are not shown in the Figure). *Abscissa* is age in days after birth, *left-hand ordinate* is electrolyte content (mEq/kg wet weight) and *right-hand ordinate* is % water.

the concentrations of these ions are lower in the glial than in the interstitial compartments. The greater decrease in these ions in the irradiated groups suggests a further increase in the glial compartment induced by prenatal X-radiation. Further evidence for an increase in glial cells after prenatal X-radiation has been reported by Hicks and D'Amato<sup>12</sup>. These authors found a marked increase in glial cells in the outer parts of the cortex of animals irradiated at 18 days of gestation with 30 r.

Changes induced by prenatal X-radiation in the electrolyte distribution may lead to an altered extraneuronal environment which together with cytoarchitectural alterations<sup>12</sup> could influence the functional development of the CNS.

*Zusammenfassung.* Bei in utero röntgenbestrahlten Rattenembryonen wurden die Elektrolyte im Gehirncortex bestimmt. Während der K-Gehalt unverändert blieb, war derjenige von Na und Cl bei 9 Tage alten Tieren erniedrigt. Zytologische Veränderungen, durch pränatale Bestrahlung verursacht, waren mit diesen Befunden korreliert.

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<sup>12</sup> S. P. Hicks and C. J. D'Amato, *Science* 141, 903 (1963).

## On the Origin of Homovanillic Acid in the Cerebrospinal Fluid

In the cerebrospinal fluid of man, homovanillic acid (HVA; 3-methoxy-4-hydroxyphenylacetic acid) occurs normally<sup>1</sup> and appears also after i.v. injection of <sup>14</sup>C-dihydroxyphenylalanine (DOPA)<sup>2</sup>. The origin of the acid in the cerebrospinal fluid is not clear. A possible source may be the blood which probably takes up HVA formed in the extracerebral tissues like liver, heart, etc. HVA may, however, also originate in the central nervous system which is known to transform DOPA into dopamine and subsequently into acidic metabolites. Therefore, the concentration of HVA in the cerebrospinal fluid might be an indicator of the metabolic activity of the central nervous system.

In order to clarify the origin of HVA in the cerebrospinal fluid, cats were administered either L-<sup>14</sup>C-DOPA or <sup>3</sup>H-HVA and the radioactivity of the HVA fraction was compared in blood, cerebrospinal fluid and brain.

*Experimental.* In cats of 1.8–2.5 kg, fasted for 16 h and anaesthetized with Nembutal, L-<sup>14</sup>C-DOPA<sup>3</sup> (labelled in 2-position of the side chain; specific activity 103.5  $\mu$ C/mg) or <sup>3</sup>H-HVA<sup>3</sup> (labelled in 5-position of the ring; specific activity 284  $\mu$ C/mg) were administered i.v. At various time intervals thereafter, arterial blood was withdrawn and supplemented with 1/10 vol versene (5%). At the end of each experiment, cerebrospinal fluid was obtained by occipital puncture. Plasma and cerebrospinal fluid were deproteinized by addition of equal volumes of per-

chloric acid, 7 and 3.5% respectively. The total brains were homogenized in 7% perchloric acid.

The determination of the unconjugated HVA fraction was carried out according to a previously described procedure<sup>4</sup>. Thereby, the supernatants of plasma, cerebrospinal fluid and brain homogenates were passed through columns of Dowex 50 · 4 in order to remove the amines and the amino acids. The effluent containing the phenolcarboxylic acids was adjusted to pH 1.5, saturated with NaCl and extracted 5 times with a double volume of peroxide-free ethylether. After evaporation of the ether (previously dried with Na<sub>2</sub>SO<sub>4</sub>), the residue was dissolved in 0.75 cm<sup>3</sup> methanol-water (3:1) and submitted to paper chromatography (Whatman No. 1; solvent system: propionic acid-benzene-H<sub>2</sub>O, 2:2:1). The radioactivity of the HVA spot was measured with a Packard radiochromatogram scanner and expressed in % of the values in the plasma after 10 min (=100%). Recoveries of <sup>3</sup>H-HVA carried through the whole procedure were about 40–50%.

*Results.* (1) The radioactivity of the HVA fraction of the plasma which appears subsequent to i.v. injection of <sup>14</sup>C-DOPA progressively declines between 10 and 120 min,

<sup>1</sup> N.-E. ANDÉN, B.-E. ROOS, and B. WERDINIUS, *Life Sci.* 448 (1963).

<sup>2</sup> A. PLETSCHER, G. BARTHOLINI, and R. TISSOT, in preparation.

<sup>3</sup> Synthesized by Dr. J. WÜRSCH, Department of Physics and Physical Chemistry, F. Hoffmann-La Roche & Co. Ltd., Basel.

<sup>4</sup> K. F. GEY and A. PLETSCHER, *Biochem. J.* 92, 300 (1964).