

Uptake of Adenine-8-C¹⁴ and Orotic-6-C¹⁴ Acid into Nuclear DNA of Non-Dividing Cells in the Adult Feline Neuraxis.* BY HAROLD KOENIG. With the technical assistance of Barbara Rich. (From the Department of Neurology and Psychiatry, Northwestern University Medical School and Veterans Administration Research Hospital, Chicago.)†

A biochemical investigation (1, 2) of the incorporation of adenine-8-C¹⁴ and orotic-6-C¹⁴ acid into the nucleic acids of the adult feline neuraxis showed rapid uptake of these precursors into pentose nucleic acid (PNA) in both white and gray matter. Deoxypentose nucleic acid (DNA) exhibited little, if any, uptake in short term experiments. In chronic experiments, however, the DNA fraction acquired high specific activity; the specific activity of DNA in white matter often approached that of PNA. A collateral autoradiographic study (2, 3) has substantiated and expanded these findings; radioactivity has been demonstrated in the nuclear DNA of a number of cell types in the central nervous system of mature cats given labelled precursors by intrathecal injection. Messier *et al.* (4), in an autoradiographic study, showed the incorporation of tritium-labelled thymidine into nuclei of nerve and glial cells in the brain of the young adult mouse. This does not appear to be equivalent to the incorporation described in this report, since in their experiments radioactivity was evident only rarely in non-dividing glia and neurons. For the most part, nuclear blackening occurred in autoradiograms over dividing cells present in or originating from the mantle cell layer. The latter is a feature of the embryonic neuraxis which persists into adulthood in the mouse, but not in the cat.

Materials and Methods

Adenine-8-C¹⁴ (7 μ c/mg.) and orotic-6-C¹⁴ acid (3 μ c/mg.) were used in single doses of 7 to 14 μ c

and in multiple doses totaling 30 μ c. These were injected into the cisterna magna of anesthetized adult cats weighing 1800 to 3000 gm. and the cats sacrificed from 2 to 51 days after injection. A total of nine cats were studied. The central nervous system was fixed in neutral 10 per cent formalin, dehydrated in graded alcohols, and imbedded in paraffin. Sections of spinal cord, medulla, pons, cerebellum and cerebrum were cut at 10 μ . They were mounted on slides, deparaffinized, soaked in cold 0.2 N HClO₄ followed by chloroform-methanol (2:1) and coated with 1 per cent collodion. Control sections were digested in ribonuclease (0.1 mg./ml. in phosphate buffer of pH 6.0 for 24 hours at 37°) for removal of PNA; some ribonuclease-extracted sections were incubated further in deoxyribonuclease (0.1 mg./ml. in 0.005 M MgSO₄·7H₂O containing 0.1 per cent gelatin for 24 hours at 37°C.) for removal of DNA. At first a stripping film technique (5) was used for autoradiography. Later, it was replaced by a slide dipping procedure employing melted Ilford G 5 emulsion (6). After development of the autoradiograms, sections were usually stained with a solution of safranin O and thionine (0.5 per cent safranin O, 0.005 per cent thionine in 0.1 M acetate buffer at pH 5 for 10 minutes).

RESULTS

In animals surviving 2 or more days following the intrathecal injection of labelled precursors, a heavy blackening was found in the autoradiograms over nerve cells, oligodendrocytes in white and gray matter, ependyma, choroidal plexus, cells in walls of blood vessels, leptomeningeal cells, and Schwann cells of nerve roots. See Figs. 1, 3, 4. Resting, non-reactive astrocytes and microglia and satellite cells of sensory ganglia contained little, if any, radioactivity. Virtually all of the radioactivity of the nerve cells was abolished by incubation of sections in ribonuclease. However, appreciable numbers of the other cells still showed

* This investigation was supported in part by the Atomic Energy Commission (Contract No. 11-1-(89)).

† Received for publication, May 21, 1958.

nuclear blackening of considerable intensity. See Figs. 2, 5, 6. No nuclear blackening was observed in autoradiograms over control sections that had been incubated successively, in ribonuclease and deoxyribonuclease. Thus, the nuclear images in autoradiograms over ribonuclease-digested sections can be attributed to radioactivity in DNA. At 2 days following an injection of labelled adenine, about 1 to 2 per cent of the oligodendrocytes in white matter showed autoradiographic evidence of ribonuclease-resistant radioactivity in their nuclei. By the ninth postinjection day, as many as 20 to 40 per cent of the interfascicular oligodendrocytes, principally those with small basophilic nuclei, exhibited evidence of ribonuclease-resistant nuclear radioactivity. Such radioactivity appeared to be uncommon in the neuraxis of cats killed within a day of injection, but this conclusion is still tentative.

DISCUSSION

These results indicate that adenine-8-C¹⁴, a precursor of the purine moieties of nucleic acids (7), and orotic-6-C¹⁴ acid, a precursor of the pyrimidine moieties of nucleic acids (8), can be taken up into the nuclear DNA of several cell types in the adult feline neuraxis, although at a considerably slower rate than into PNA. The large number of oligodendrocytes and other cells showing radioactivity in DNA within 9 days of injection makes it unlikely that these cells are synthesizing DNA preliminary to cell division. Indeed, mitotic figures are not seen in the neuraxis of the adult cat. That such large numbers of cells could be engaged in DNA synthesis leading to polyploidy also seems implausible. Polyploidy does not occur among interfascicular oligodendrocytes in the human neuraxis (9). However, the possible presence of polyploidy in glia and other cells of the feline neuraxis requires investigation.

The conditions of these experiments seem to be favorable for the demonstration of a slow turnover

of DNA. The labelled precursors are rapidly incorporated into PNA and are retained with little apparent loss for considerable periods of time, particularly when labelled adenine is used (1). This suggests rapid local degradation of PNA, with equally rapid reincorporation of degraded components. If this explanation is correct, a pool of radioactive precursors derived from the turnover of PNA would be constantly available to DNA; in time even a sluggish uptake into DNA would be demonstrable. Another explanation of these results is that there may be a stable precursor pool from which both PNA and DNA draw during turnover, PNA at a high rate and DNA at a low rate. Data concerning turnover of the labelled precursors and their derivatives in the acid-soluble pool of the brain would assist in choosing between these alternatives. These experiments indicate that DNA in the central nervous system may be metabolically less stable than is generally believed (10). The labelling of nuclear DNA in mesodermal elements, *i.e.*, cells in the walls of cerebrospinal blood vessels, suggests that a slow turnover of DNA may occur in other tissues.

BIBLIOGRAPHY

1. Koenig, H., *Proc. Soc. Exp. Biol. and Med.*, 1958, **97**, 255.
2. Koenig, H., *Progress in Neurobiology*, (J. Nurnberger and S. Korey, editors), New York, Hoeber, **4**, in press.
3. Koenig, H., data in preparation.
4. Messier, B., Leblond, C. P., and Smart, J., *Exp. Cell Research*, 1958, **14**, 224.
5. Doniach, I., and Pelc, S. R., *Brit. J. Radiol.*, 1950 **23**, 184.
6. Messier, B., and Leblond, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1957, **96**, 7.
7. Brown G. B., Roll, P. M., Plentl A. A., and Cavalieri, L. F., *J. Biol. Chem.*, 1948, **172**, 469.
8. Weed, L. L., and Wilson, P. W., *J. Biol. Chem.* 1951, **189**, 435.
9. Lapham, L. W., personal communication.
10. Sinsheimer, R. L., *Science*, 1957, **125**, 1123.

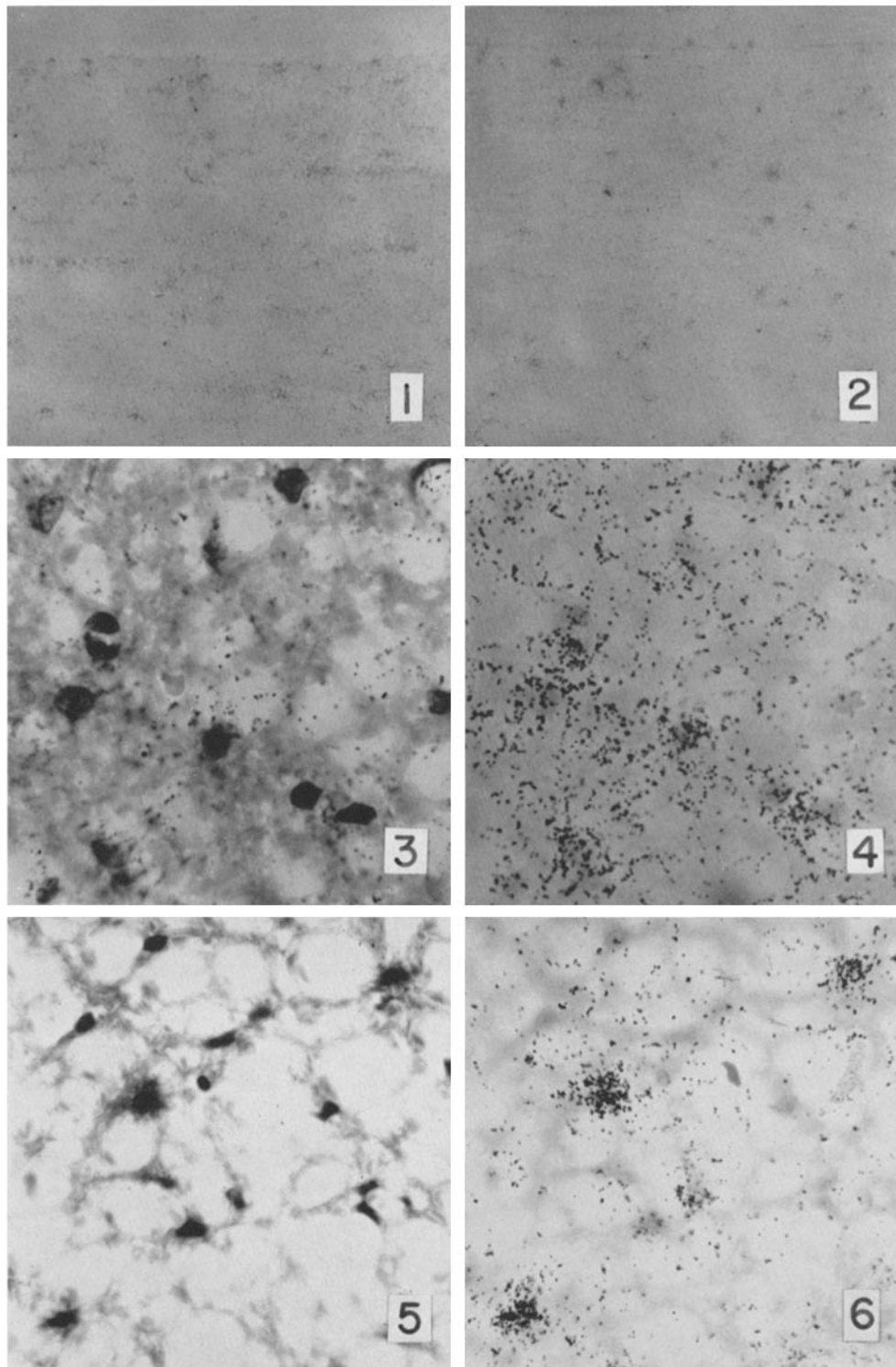
EXPLANATION OF PLATE 327

FIG. 1. Autoradiogram of a longitudinal section of spinal white matter from a cat (CA3) which received 7 μ c. of adenine-8- C^{14} intracisternally 9 days prior to sacrifice. Note the clusters of reduced silver grains which are present over interfascicular oligodendrocytes and also the more diffusely distributed silver grains. Unstained. $\times 120$.

FIG. 2. Autoradiogram of a comparable section which had been incubated in ribonuclease. Note the considerable number of "cluster" images and the marked reduction in diffuse blackening. Unstained. $\times 120$.

FIGS. 3 and 4. Higher magnification of transverse section of spinal white matter from the same experiment. FIG. 3 is a photomicrograph of the section stained with safranine O-thionine and photographed with a green filter. FIG. 4 is the overlying autoradiogram photographed with a red filter to reduce the contrast of the stain in the subjacent section. Note the nuclear and cytoplasmic location of radioactivity in oligodendrocytes. $\times 670$.

FIGS. 5 and 6. Comparable section which had been incubated in ribonuclease. FIG. 5 is a photomicrograph of the stained section. FIG. 6 is the overlying autoradiogram. The radioactivity is largely restricted to the oligodendroglial nuclei and is attributed to labelling of DNA. Conditions for photomicrography were the same as for FIGS. 3 and 4. $\times 520$.



(Koenig: Uptake of adenine-8-C¹⁴ and orotic-6-C¹⁴ acid)