Rana, stage 18), together with typical Nissl-substance in peripheral areas of the neuron.

The cytoplasm of the amphibian RBCells also possessed large lipid vacuoles (2–15 μm in diameter; figure 5) and many yolk platelets (2–10 μm in diameter; figure 4). A high content of compact melanin granules (about 0.5 μm in diameter) was observed especially in the RBCells of Rana temporaria (figure 5). The melanin deposits in the RBCells of Triturus helveticus were of more complex character, 0.3–0.5 μm in diameter, each consisting of several granules of about 0.05 μm in diameter (figure 3). Subsurface cisterns were found in all stages of fish and amphibian RBCell development that were investigated – often at sites of contact between 2 neighbouring RBCells. The cell membrane showed no adherent synaptic elements.

*Discussion.* The ultrastructural development of the perikaryon of the RBCells of fishes and amphibians obviously parallels that of other neuroblasts in the vertebrate central nervous system[12–17]. In addition, the fully differentiated RBCells of both lower vertebrate groups investigated were structurally similar to the dorsal cells of the adult petromyzont spinal cord[18–20], which are regarded as persistent RBCells[17,21]. This resemblance between both types of intramedullary dorsal neurons supports the view that they are homologous features within the central nervous system of lower vertebrates.

Comparison of the rate of ultrastructural development of fish and amphibian RBCells during the hatching period revealed that structural maturity occurs earlier in the RBCells of the trout than in those of the amphibians. Corresponding evidence on neuronal functioning was obtained in enzyme-histochemical investigations, demonstrating strong enzyme activities in the trout RBCells only at hatching, while the amphibian RBCells lack any parallel development of enzyme reactions, including their degenerative period[2].

Considering these results, it may be assumed that in amphibians the primary reflex machanism has no important biological meaning, but in oviparous fishes, such as the trout, it may be involved in the control of hatching movements.

12 S. M. Billings, Z. Anat. EntwGesch. *136*, 168 (1972).
13 D. W. Caley and D. S. Maxwell, J. comp. Neurol. *133*, 17 (1968).
14 K. M. Lyser, Devl. Biol. *10*, 433 (1964).
15 E. Pannese, J. comp. Neurol. *132*, 331 (1968).
16 V. M. Tennyson, in: Developmental Neurobiology. Ed. W. Himwich. Thomas, Springfield (1970).
17 H. P. Whiting, Q. J. microsc. Sci. *89*, 359 (1948).
18 B. Bertolini, J. Ultrastruct. Res. *11*, 1 (1964).
19 R. Schultz, E. C. Berkowitz and D. C. Pease, J. Morph. *98*, 251 (1956).
20 G. Sterba and W. Naumann, Acta zool., Stockholm *51*, 159 (1970).
21 E. G. Healey, in: The biology of lampreys, vol. 2. Ed. M. W. Hardisty and I. C. Potter. Academic Press, New York 1972.

## X-ray induction of chromosome and chromatid aberrations in the same cells after treatment with hydroxyurea[1]

M. M. Reddy and M. W. Miller

*Department of Radiation Biology and Biophysics, The University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642 (USA), 9 August 1976*

*Summary.* Vicia faba root meristem cells treated for 24 h with 1.25 mM hydroxyurea and then X-irradiated contained a large number of cells with both chromosome and chromatid aberrations. X-irradiation 4 h after release from the hydroxyurea block yielded cells with almost exclusively chromatid aberrations.

The X-irradiation of eukaryotic interphase cells yields 2 major types of chromosomal aberrations: early and mid $G_1$ produce chromosome aberrations while S and $G_2$ produce chromatid aberrations. The transition from chromosome to chromatid aberrations occurs in late $G_1$, but before S[2–5]. Wolff[6] has reported that inhibitors of DNA and protein syntheses do not prevent this transition. However, the presence of chromosome and chromatid aberrations within the same cell is a rather infrequent event at various treatments[4].

We report here that when Vicia faba root tips were X-irradiated immediately after hydroxyurea (HU) synchronization and the meristematic mitotic cells scored for chromosomal anomalies a very large proportion (~40%) of cells containing both chromosome and chromatid aberrations was observed.

Seeds of Vicia faba (Kellogg Seed Company, Ventura, CA) were soaked overnight in running tap water, then planted in moist vermiculite and grown in the dark for 5 days at 19 ± 1 °C. The seedlings, with plumules and seed coats removed, were then transferred to a tank containing aerated Voth and Hawner's[7] No. 5 nurtient solution (19 ± 1 °C) for 48 h, then to a tank containing nutrient solution plus 1.25 mM HU for a period of 24 h, and subsequently returned to the culture tank containing only nutrient solution. At various times after removal from HU roots were pulse labeled (30 min) with 1 μCi/ml $^3$H-thymidine [6.7 Ci/mM, spec. act.]. Roots were X-irradiated with 400 R (150 R/min) immediately or at 4 h after the HU treatment. At different time intervals after removal from the HU solution some roots were soaked for 2–3 h in aerated 0.04% colchicine, but all were fixed in 3:1 alcohol:acetic acid. Root meristems were Feulgen stained, squashed onto microscope slides, and adhered by the method of Conger and Fairchild[8]. Autoradiographs were prepared by dipping the slides in Kodak NTB-3 liquid emulsion, drying, and storing the slides in the dark

1 Acknowledgments. This paper is based on work performed under contract with the U. S. Energy Research and Development Administration at the University of Rochester Biomedical and Environmental Research Project and has been assigned Report No. UR-3490-443. This work was also partially supported by USPHS, grants FD-00694 and GM-22680.

2 H. J. Evans and J. R. K. Savage, J. cell. Biol. *18*, 525 (1963).
3 S. Wolff and H. E. Luippold, Exp. cell. Res. *34*, 548 (1964).
4 S. Wolff, Radiation Res. *8*, 207 (1961).
5 S. Wolff, Int. Rev. Cytol. *25*, 279 (1969a).
6 S. Wolff, Mutation Res. *8*, 207 (1969b).
7 P. D. Voth and K. C. Hamner, Bot. Gaz. *102*, 169 (1940).
8 A. D. Conger and L. M. Fairchild, Stain Tech. *28*, 281 (1953).

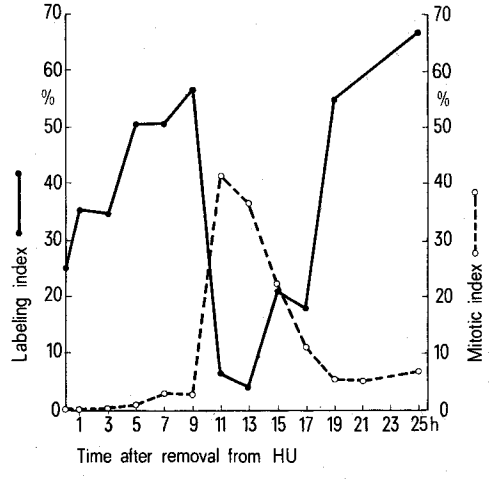
Frequencies and $^3$H-thymidine labeling patterns of radiation induced chromosome and chromatid aberrations from different 1.35 mM hydroxyurea regimens

| Regimen | Approximate fixation times (h) post HU | Total number of mitotic cells observed | Number with chromosomal aberrations (% of mitotic cells in parentheses) Total | Only chromosome | Both chromosome and chromatid | Only chromatid** |
|---|---|---|---|---|---|---|
| 24 h HU | 9–17 | 390 | 25 (6.4) | 11* (2.8) | 0 | 14 (3.6) |
| 24 h HU + 400 R | 38–71 | 232 | 171 (73.7) | 45* (19.4) | 96* (41.4) | 30 (12.9) |
| 24 h HU + 4 h $H_2O$ + 400 R | 5–27 | 147 | 146 (99.3) | 3* (2.0) | 0 | 143 (97.3) |

Label pattern: * unlabeled metaphase, ** approximately 60% $^3$H-labeled.

at 4 °C for 3 weeks before development. The slides were scored for $^3$H-labeling index, mitotic index, and chromosomal anomalies.

The figure, similar to that obtained by Hall et al.[9], shows the $^3$H-labeling and mitotic indices at different times after removal of the seedlings from the 24 h HU treatment. The HU treatment did not completely arrest DNA synthesis as evidenced by the presence of $^3$H-labeled nuclei immediately at the end of the HU treatment; the 0 h labeling index was obtained by maintaining the roots in HU for $23^1/_2$ h and then a further $^1/_2$ h in HU plus $^3$H-TdR. There were no cells in mitosis until the 5–9 h intervals; after which the mitotic index rose steeply to a peak (~40%) at 11–13 h and then gradually declined. The effect of 24 h HU alone on the production of chromosome and chromatid aberrations was slight. The majority of the cells (94%) had no chromosomal anomaly; the remaining cells contained chromatid exchanges, chromosome deletions and some gaps (table). The radiation regimens produced considerable delays in the onset of mitosis; for example, for the 24 h HU + 400 R regimen mitotic cells were obtained from 2 h colchicine collection intervals beginning at 38 h post-HU treatment and extending through 71 h. For these long intervals over which fixations were accomplished the mitotic index was very low and many roots and intervals were needed to obtain cells for scoring. Within regimens there was not an apparent change in the frequency or type of chromosomal anomalies with fixation intervals.




Mitotic and $^3$H-thymidine label indexes with time after removal from a 24 h exposure to hydroxyurea (HU); 3 slides per point, 1000 cells per slide.

Slightly more than 40% of the mitotic cells from the 24 h HU + 400 R regimen had chromosome and chromatid aberrations within the same cell; all were unlabeled, as were any cells with solely chromosomal aberrations, thus indicating they were in $G_1$ at the time of X-irradiation. When the roots were released from the 24 h HU treatment for only 4 h very few chromosome aberrations were scored, and no cells were observed containing both chromatid and chromosome aberrations.

The transition from chromosome to chromatid aberrations occurs a) in a short period of time[10] in comparison to a DNA synthetic period (S) of 6 h in Vicia[11], and b) at the end of $G_1$ but before S[3–5, 12–14]. The transition does not appear to be the result of separation of the DNA double helix into 2 single strands[15].

Autoradiographic studies have demonstrated that DNA synthesis in eukaryotic cells often does not occur synchronously in all chromosomes of a complement; the chromosomes do either not initiate, or terminate DNA synthesis at the same time, or both[16–19]. Thus, on an individual chromosomal DNA synthesis basis the transition from $G_1$ to S may be variable. Lin and Davidson[20] have further suggested that the timing of terminal DNA replication is determined independently in each chromosome. The transition from chromosomeness to chromatidness occurs before the onset of DNA synthesis, i.e., in late $G_1$. Our data suggest that a) there is a substantial subpopulation (~40%) of cells which appear with a 24 h HU treatment whose chromosomes are in a state of transition from chromosomeness to chromatidness, and b) these cells can be resolved by the application of X-rays.

9 E. J. Hall, J. M. Brown and J. Cavanah, Radiation Res. *35*, 622 (1968).
10 K. Sax and K. Mather, J. Genet. *37*, 483 (1939).
11 A. Howard and S. R. Pelc, Heredity *6* (Suppl.), 261 (1952).
12 T. C. Hsu, W. C. Dewey and R. M. Humphrey, Exp. cell. Res. *27*, 441 (1962).
13 V. Monesi, M. Crippa and R. Zito-Bignami, Chromosome *21*, 369 (1967).
14 F. Vogal and M. Vrba, Mutation Res. *4*, 874 (1967).
15 J. A. Heddle and J. E. Trosko, Exp. cell. Res. *42*, 171 (1966).
16 L. Gavosto, P. M. Pegoraro and G. Rovera, Exp. cell. Res. *49*, 340 (1968).
17 C. W. Gilbert, S. Muldal, L. G. Lajtha and J. Rowley, Nature *195*, 869 (1962).
18 J. German, J. cell. Biol. *20*, 37 (1964).
19 N. Takagi and A. A. Sandberg, Cytogenetics *7*, 135 (1968).
20 M. S. Lin and R. L. Davidson, Somatic Cell Genetics *1*, 111 (1975).