Incorporation of thymidine into onion root meristematic cell nuclei in presence of hydroxyurea and its role in recovery of mitotic activity*

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Abstract

Hydroxyurea treatment of onion roots induced mitotic block which was released by transfer of bulbs to water, and also to some extent by addition of cold or ^{3}H-thymidine to hydroxyurea solutions. In presence of hydroxyurea there was noted very intense incorporation of ^{3}H-thymidine into cell nuclei, giving labelling index of 40—70%. However, all the mitotic figures appearing in presence of hydroxyurea and ^{3}H-thymidine were unlabelled. On the other hand, labelled mitotic figures were obtained when roots incubated with ^{3}H-thymidine in presence of hydroxyurea had been transferred to water. Incorporation of ^{3}H-uridine was unaffected by hydroxyurea. The results show that hydroxyurea arrests onion root meristematic cells, either in the S phase and the G_2 phase. Enhanced incorporation of ^{3}H-thymidine in the presence of hydroxyurea, and release by added thymidine of the mitotic block indicate that hydroxyurea induces in onion root meristematic cells a particular shortage of thymidylate.

INTRODUCTION

Hydroxyurea is a potent inhibitor of DNA synthesis in bacteria (Rosenkranz et al., 1966; Jyssum, 1973), in yeasts (Slater, 1973), in protozoa (Buetow and Mego, 1967; Injeyan et al., 1974), in mammalian cells in vitro (Mohler, 1964; Sinclair, 1965, 1967; Manso-Martinez and Frank, 1972; Miyamoto et al., 1976) and in mammalian cells in situ (Schwartz et al., 1965; Gillette

et al., 1970; R a j e w s k y et al., 1971 a, b; R a b e s et al., 1974; N e c a s and N e u w i r t, 1976). Moreover, hydroxyurea has proved to arrest duplication of viral DNA (R o s e n k r a n z et al., 1973; M a g n u s s o n, 1973).

Also plant cells and tissues are sensitive to hydroxyurea. So, effects of hydroxyurea were investigated in algae (H e i l p o r n - P o h l and L i m b o s h - R o l i n, 1969; W a n k a et al., 1972), in *Haplopappus gracilis, Compositae* (K i h l m a n et al., 1966), in root meristems of *Vicia faba* (K i h l m a n et al., 1966; H a l l et al., 1968; O d m a r k, 1971) and *Allium sativum* (B r u l f e r t and D e y s s o n, 1973) in *Nicotiana tabacum* tissue culture (R e n n e r t, 1977 a; M a c i e j e w s k a - P o t a p c z y k o w a et al., 1970; B i l e c k a, 1975 a, b), in *Helianthus annuus* tissue culture (R e n n e r t, 1977 b) and in *Cucumis sativum* seeds and leaves (R e n n e r t and K n y p l, 1971, 1972).

The primary biochemical effect of hydroxyurea is inhibition of ribonucleoside diphosphate reductase (A d a m s and L i n d s a y, 1967; Y o u n g et al., 1967; K r a k o f f et al., 1968; M o o r e, 1969; S i n h a and S n u s t a d, 1972; T h e i s s and F i s c h e r, 1976). In this way, hydroxyurea-treated cells become depleted of deoxyribonucleotides indispensable for DNA synthesis, what makes S phase cells particularly sensitive to action of hydroxyurea (S i n c l a i r, 1965, 1967).

The present study was designed to examine incorporation of ^{3}H-thymidine in onion root meristems in presence of hydroxyurea, and its effects on the meristematic mitotic activity.

MATERIALS AND METHODS

R o o t   c u l t u r e. Onion bulbs (*Allium cepa* L., var. Wolska-Szepietowski) were obtained from the Gardening Institute, Skierniewice. Growth of roots was induced by placing the bulbs in necks of conical 200 ml flasks with boiled and filtered spring water. When roots were 0.5—1 cm long, the bulbs were placed (in groups of six) on nylon netting over 2.8 l glass crystallizers. The water was intensively aerated, and changed every day. After four days, most roots reached 4—5 cm. Shorter and longer roots were removed before subsequent treatment with hydroxyurea. The cultures were grown in darkness at room temperature.

C h e m i c a l s   a n d   r a d i o c h e m i c a l s. Hydroxyurea (HU) was purchased from Calbiochem (San Diego). Cold thymidine (TdR) was from British Drug Houses. ^{3}H-Thymidine-(5-C^{3}H$_3$), 19.69 Ci/mmole, 1 mCl/ml, 12.3 µg/ml, and ^{3}H-uridine, 21.5 Ci/mmole, 1 mCi/ml, were purchased from Institute for Research, Preparation and Application of Radioisotopes, Prague (Czechoslovakia). ^{3}H-Thymidine and ^{3}H-uridine were diluted with boiled spring water to activity of 5 µCi/ml.

Incubation of roots. Each onion bulb was mounted at the top of a glass vessel with 40 ml of 2.6 mM (0.2 mg/l) hydroxyurea solution. Next, it was replaced by: (A), water (24 hrs HU — 48 hrs H_2O); (B), ^{3}H-thymidine (5 μCl/ml) in 2.6 mM hydroxyurea (24 hrs HU — 24 hrs ^{3}H-TdR+HU); (C) and (D), O.3 mM and 0.03 mM cold thymidine in 2.6 mM hydroxyurea (24 hrs HU — 24 hrs TdR+HU); (E) and (F), ^{3}H-thymidine (5 μCi/ml) in 2.6 mM hydroxyurea for 6 hrs, and subsequently 2.6 mM hydroxyurea alone (24 hrs HU — 6 hrs ^{3}H-TdR+HU — 18 hrs HU) or water (24 hrs HU — 6 hrs ^{3}H-TdR+HU — 18 hrs H_2O); (G), ^{3}H-thymidine in water for 6 hrs and next water (24 hrs HU — 6 hrs ^{3}H-TdR+H_2O — 18 hrs H_2O). Each experimental treatment was performed twice. At time points as indicated, two roots were collected from each bulb for examination.

Autoradiography and microscope examination. The roots were fixed'with aceto-alcohol (1:3 v/v) and stained with 0.2% acetoorceine. Squash preparations were made. Autoradiography was performed by the striping film method, using Kodak AR-10 plates. The preparations were exposed for 3 weeks at 4°C. The global mitotic index, the index of labelled mitoses and index of labelled nuclei were scored by counting 1000 cells from each root.

RESULTS

Incubation of onion in 2.6 mM hydroxyurea (0.2 mg/ml) for 24 hrs proved to reduce the mitotic index in meristems to below 1%, while in untreated controls. it amounted to 14—24% (Fig. 1). The hydroxyurea--induced mitotic block was released by transfer of roots to water, and a distinct peak of mitotic activity, indicative of synchronization of meristematic cells, exceeding considerably the mitotic index in untreated roots, was found after removal of hydroxyurea (Fig. 1).

It claims attention that mitotic activity proved to be also induced in presence of hydroxyurea by addition of 0.03 and 0.3 mM cold thymidine (Fig. 2) or 0.25 μM ^{3}H-thymidine (Fig. 3, 4a), while in meristems treated in parallel with hydroxyurea in absence of exogenous thymidine, the mitotic index still remained below 1% (Fig. 2).

Furthermore, autoradiography has revealed intense incorporation of ^{3}H-thymidine into many cell nuclei when roots were incubated with it in the presence of hydroxyurea, resulting in the labelling index of 40—70% (Fig. 3, 4a-c). It has to be emphasized that labelling of individual nuclei was much more intense in presence of hydroxyurea (Plate I, photos 4, 6) than in controls (Plate I, photos 3, 5).

In spite of the intense labelling of nuclei and prolongation of incubation for further 24 hrs, all the mitotic figures observed in the presence

of hydroxyurea supplemented with ^{3}H-thymidine did not contain any label (Plate II, photos 1, 2). On the other hand, labelled mitotic figures were obtained only when roots preincubated with ^{3}H-thymidine in the
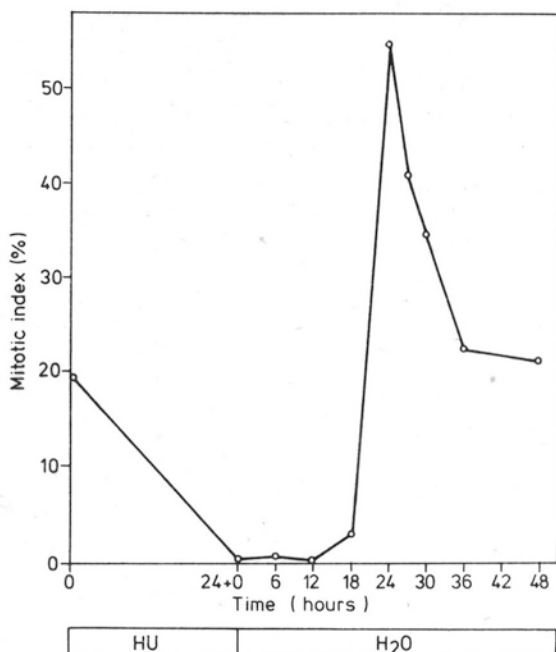

Fig. 1. Synchronization of meristematic mitotic activity in onion roots transferred to water after 24 hrs incubation with 2.6 mM hydroxyurea
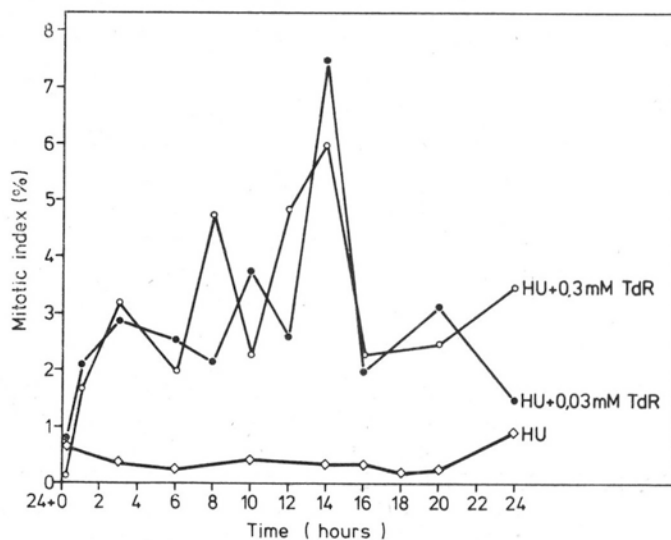

Fig. 2. Release of mitotic block in onion root meristems after transfer of roots incubated for 24 hrs with 2.6 mM hydroxyurea to 0.03 mM or 0.3 mM thymidine in 2.6 mM hydroxyurea
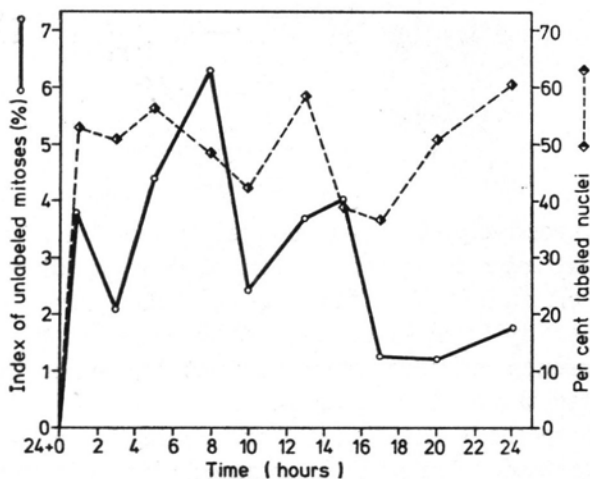
Fig. 3. Index of unlabelled mitoses and index of labelled nuclei after transfer of onion roots, incubated for 24 hrs with 2.6 mM hydroxyurea, to solutions of ³H-thymidine (0.25 µM, 60 ng/ml, 5 µCi/ml) in 2.6 mM hydroxyurea. No labelled mitoses were found

presence of hydroxyurea had been transferred to water (Plate II, photos 3—5). First labelled divisions appeared under these conditions 12 hrs after removal of hydroxyurea.
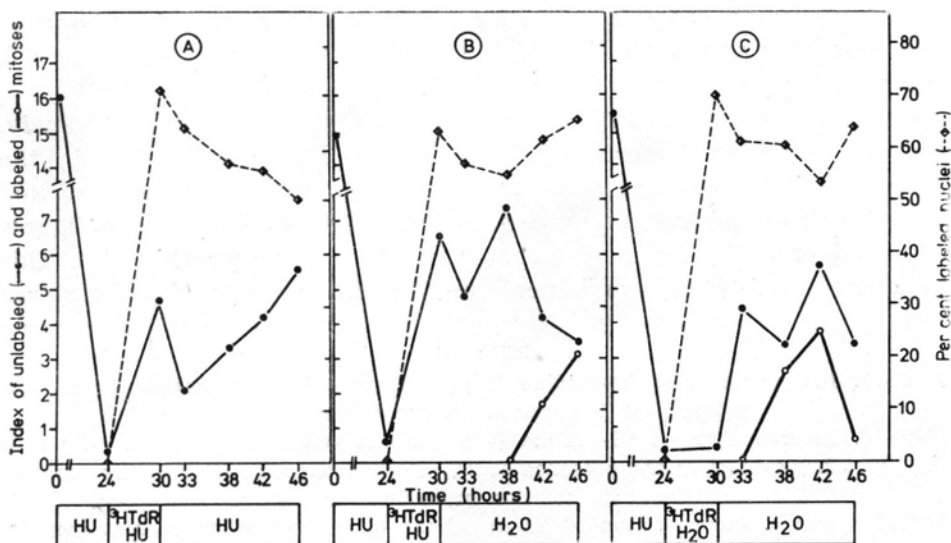

Fig. 4. Indices of labelled nuclei, unlabelled mitoses and labelled mitoses in onion root meristems treated for 24 hrs with 2.6 mM hydroxyurea, and subsequently transferred to: (a), HU with ³H-TdR for 6 hrs, and then HU alone; (b), HU with ³H-TdR for 6 hrs, and then water; (c), ³H-TdR in water for 6 hrs, and then water alone

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Uptake of ³H-uridine by the meristematic cells has proved to be unaffected by hydroxyurea (Plate I, photos 1, 2).

DISCUSSION

Rather unexpectively, during prolonged incubation with hydroxyurea there was noted intensive incorporation of ³H-thymidine to onion root meristematic cell nuclei (Plate I, photos 4, 6). Occurence of not labelled cells besides heavily labelled ones indicated that the ³H-thymidine uptake in the presence of hydroxyurea was not due to repair DNA synthesis, but reflected the S-phase process.

It has been recently established that mammalian cells in culture, in spite of extreme reduction of DNA synthesis by hydroxyurea, preserve the ability to synthetize short fragments of DNA (C o y l e and S t r a u s s, 1970; B r o w n and W h e a t l e y, 1974; F u j i w a r a, 1975; M a r t i n et al., 1976; W a l t e r s et al., 1976), and the same applies to duplication of viral DNA (M a g n u s s o n, 1973). This phenomenon is considered to reflect relatively low sensitivity to shortage of deoxyribonucleotides of the DNA polymerase acting at first stages of DNA synthesis, while the gap-filling DNA polymerase is highly sensitive (M a r t i n, 1976). Moreover, W a l t e r s et al. (1976) have shown that hydroxyurea permits G_1-phase cells to enter the S phase at the same time and rate as the untreated controls, while previously, hydroxyurea was believed to arrest cells at the G_1 side of the G_1/S boundary (S i n c l a i r, 1965, 1967; T o b e y and G r i s s m a n, 1972).

The above seems to apply rather well to results of the present study. Namely, onion root meristematic cells with DNA synthetized in the presence of hydroxyurea were not capable to divide unless incubated in the absence of hydroxyurea, since the first labelled mitotic figures appeared only 12 hrs after transfer of roots to water (Fig. 4b). This indicates that DNA synthesis performed in the presence of hydroxyurea

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PLATE I

Photo 1. Incorporation of ³H-uridine (5 μCi/ml) to onion root meristematic cells during 12 hrs contact in the water medium

Photo 2. Incorporation of ³H-uridine (5 μCi/ml) to onion root meristematic cells during 12 hrs contact in the presence of 2.6 mM hydroxyurea after 24 hrs preincubation in a solution of hydroxyurea alone

Photos 3 and 5. Incorporation of ³H-thymidine (5 μCi/ml) to onion root meristematic cell nuclei during 6 hrs contact in the absence of hydroxyurea, in bulbs not pretreated with hydroxyurea

Photos 4 and 6. Incorporation of ³H-thymidine (5 μCi/ml) to meristematic cell nuclei of onion roots pretreated for 24 hrs with 2.6 mM hydroxyurea, and subsequently incubated with ³H-thymidine for 6 hrs in the presence of hydroxyurea

The cells were stained with aceto-orceine. Exposition time — 3 weeks. Photos 1—4, ×800.

Photos 5 and 6, ×1500

PLATE I
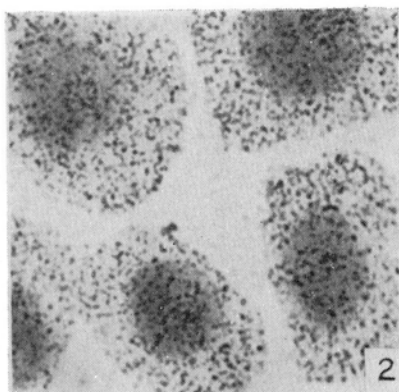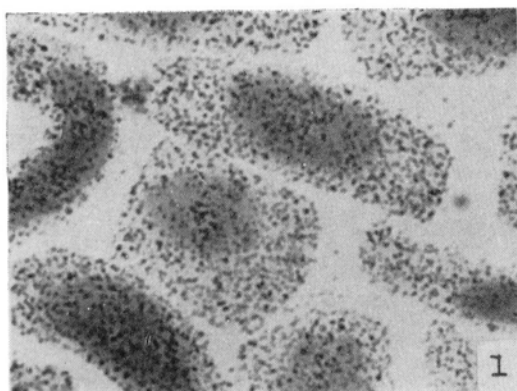
PLATE II

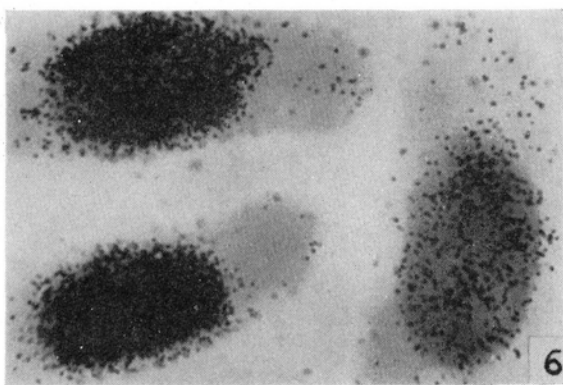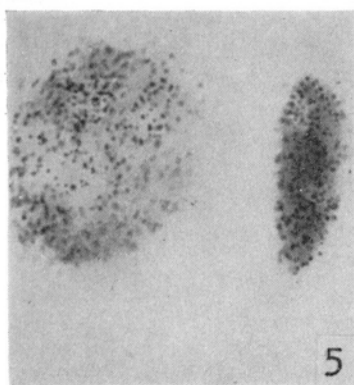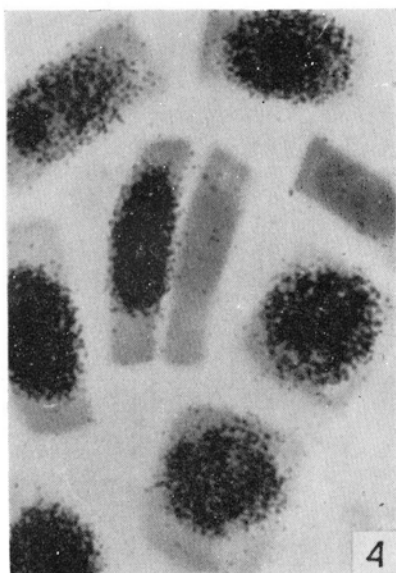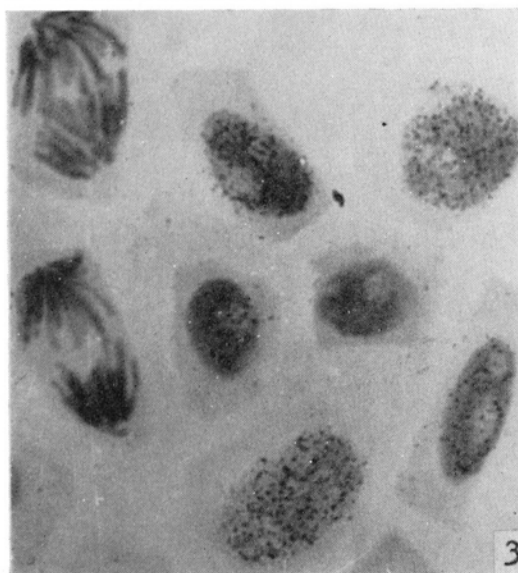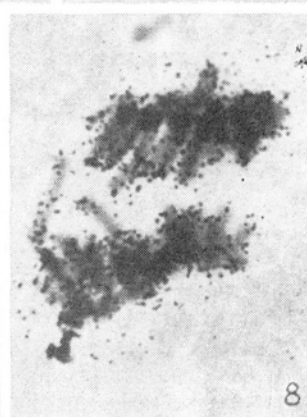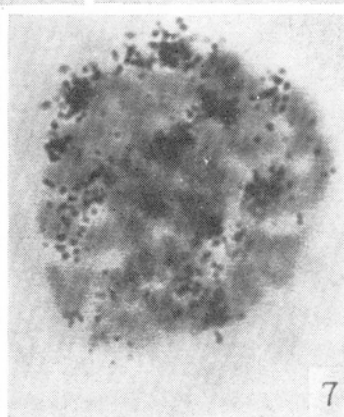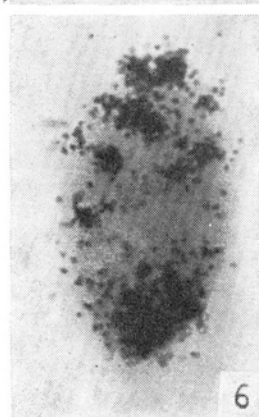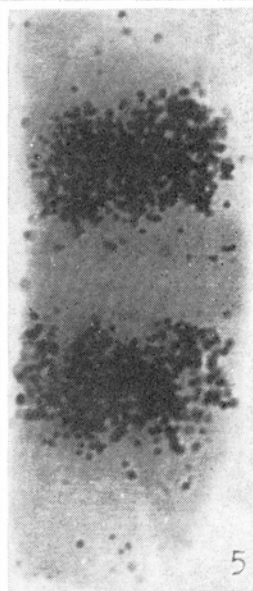had to be completed in its absence. Furthermore, autoradiography has shown discontinous labelling of some mitotic figures and irregular distribution of the label in some nuclei (Plate II, photos 6—8), what might relate to synthesis of short fragments of DNA.

High enhancement of ^{3}H-thymidine incorporation to onion root meristematic cell nuclei, found in the presence of hydroxyurea, has to be emphasized (Plate I, photos 4, 6). Some potentiation of ^{3}H-thymidine incorporation by hydroxyurea treatment was also noted in mouse fibroblast cell cultures (A d a m s and L i n d 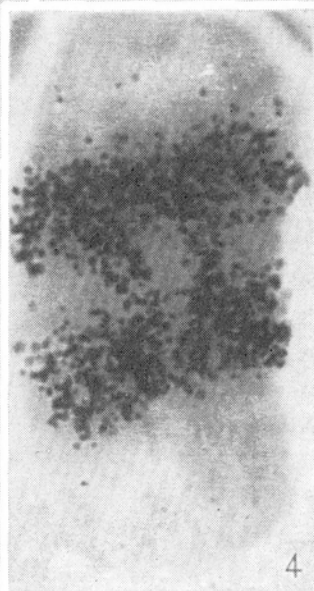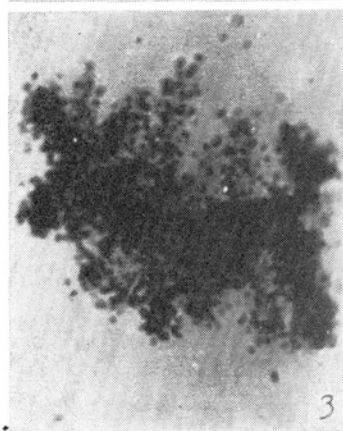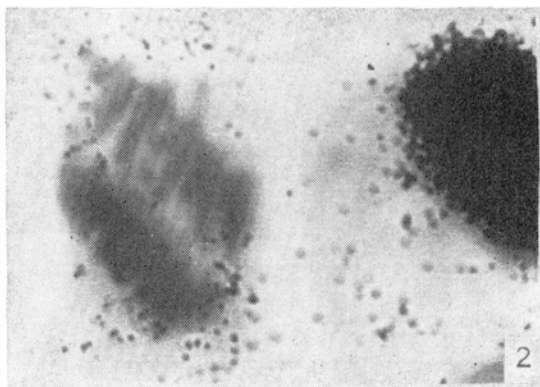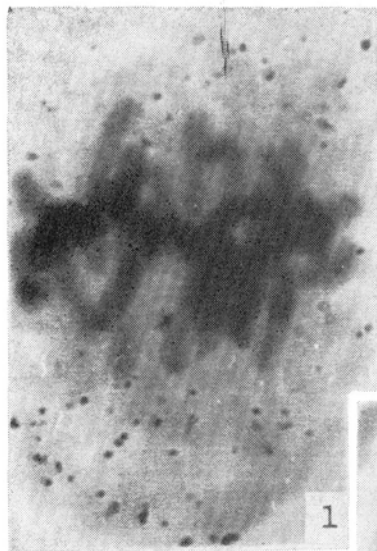s a y, 1967) and in cultures of *Crithidia* sp., *Haemoflagellata* (I n j e y a n et al., 1974). Moreover, enhancement of ^{3}H-thymidine incorporation following specific arrest of thymidylate synthesis with 5-fluorodeoxyuridine or methotrexate (R u b i n i, 1966; R o b e r t s and W a d i n s k y, 1968; B o g e n h a g e n and C l a y t o n, 1976) has to be taken into account, since it clearly shows that shortage of endogenous thymidylate reflects in increased incorporation of exogenous thymidine.

At this place, it has to be mentioned that thymidine kinase is not inhibited by hydroxyurea (A d a m s and L i n d s a y, 1967).

In view of the above data, high potentiation of incorporation of ^{3}H-thymidine, noted in onion root meristems in the presence of hydroxyurea, may be considered to reflect a selective arrest of deoxyuridylate (dUMP) formation, the precursor of thymidylate (TMP). However, the question arises on source of the remaining three deoxyribonucleotides, since their formation in other biological objects seems to be generally inhibited, except for some microorganisms (e.g. *Lactobacillus leichmanni*) reducing ribonucleotides at the triphosphate level with the cobamide dependent reductase (E l f o r d, 1968).

Induction of unlabelled divisions in the presence of hydroxyurea was another effect of exogenous thymidine (Fig. 2, 3, 4a). Absence of the label in mitotic figures indicates that the cells had passed the S phase before the contact with ^{3}H-thymidine. Thus, they were arrested by hydroxyurea in the G_2 phase. This effect of hydroxyurea was found to

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PLATE II

Photos 1 and 2. Non-labelled mitotic figures induced in the presence of 2.6 mM hydroxyurea by 6 hrs incubation with 0.25 μM ^{3}H-thymidine (5 μCl/ml) after previous 24 hrs treatment with hydroxyurea

Photos 3—5. Labelled mitotic figures in meristems of onion roots pretreated for 24 hrs with 2.6 mM hydroxyurea, incubated subsequently with ^{3}H-thymidine (5 μCi/ml) in the presence of hydroxyurea for 6 hrs, and finally transferred to water for 12 hrs

Photos 6—8. Irregular distribution of the label in nuclei (Photo 6) and discontinous labelling of mitotic figures (Photos 7 and 8) in meristems of onion roots treated for 24 hrs with 2.6 mM hydroxyurea, incubated subsequently with ^{3}H-thymidine (5 μCi/ml) in the presence of hydroxyurea, and finally transferred to water

The cells were stained with aceto-orceine. Exposition time — 3 weeks. Magnification ×1500

be released by minute amounts of exogenous thymidine, in spite of continued treatment of onion roots with hydroxyurea. The latter finding indicates that some residual DNA synthesis, not revealed by autoradiography, occurs in meristematic cells during the G_2 phase.

In view of the presented results, it may be concluded that hydroxyurea arrests onion root meristematic cells both in the S phase and the G_2 phase by inducing a particular shortage of thymidylate, released by addition of thymidine to the incubation medium.

Acknowledgments

REFERENCES

A d a m s R. L. P., L i n d s a y J. G., 1967. Hydroxyurea: Reversal of inhibition and use as a cell-synchronizing agent. J. Biol. Chem. 242: 1314—1317.

B iᵗl e c k a A., 1975a. Influence of hydroxyurea on nucleic acid content and ^{3}H-uridine incorporation in callus and tumorous tobacco tissues cultured *in vitro*. Acta Soc. Bot. Pol. 44: 393—405.

B i l e c k a A., 1975b. Effect of hydroxyurea on the protein content and synthesis in normal and tumorous tobacco tissues cultured *in vitro*. Folia Histochem. Cytochem. 13: 139—150.

B o'g e n h a g e n D., C l a y t o n D., 1976. Thymidylate nucleotide supply for mitochondrial DNA synthesis in mouse L-cells. Effect of 5-fluorodeoxyuridine and methotrexate in thymidine kinase plus and thymidine kinase minus cells. J. Biol. Chem. 251: 2938—2944.

B r o w n J. S., W h e a t l e y D. N., 1974. DNA synthesis in the presence of hydroxyurea. Brit. J. Cancer 30: 173. (Abstract).

B r u l f e r t A., D e y s s o n G., 1973. Synchronisation des divisions méristématique d'*Allium sativum* L. par l'hydroxyurée. Conditions d'application à l'analyse cinétique des effects cytoxiques. Annales pharmaceutiques franc. 31: 19—27.

B u e t o w D. E., M e g o J. L., 1967. Hydroxyurea inhibition of greening, macromolecule synthesis and cell division in *Euglena gracilis*. Biochim. Biophys. Acta 134: 395—401.

C o y l e M. B., S t r a u s s B., 1970. Cell killing and the accumulation of breaks in the DNA of HE-2 cells incubated in the presence of hydroxyurea. Cancer Res. 30: 2314—2319.

E l f o r d H. L., 1968. Effect of hydroxyurea on ribonucleotide reductase. Biochem. Biophys. Res. Commun. 33: 129—135.

F u j i w a r a Y., 1975. Postreplication repair of ultraviolet damage to DNA, DNA--chain elongation, and effects of metabolic inhibitors in mouse L-cells. Biophys. J. 15: 403—415.

G i l l e t t e E. L., W i t h e r s H. R., T a n n o c k I. F., 1970. The age sensitivity of epithelial cells of mouse small intestine. Radiology 96: 639—643.

H a l l E. J., B r o w n J. M., C a v a n a g h J. J., 1968. Radiosensitivity and the oxygen effect measured at different phases of the mitotic cycle using synchronously dividing cells of the root meristem of *Vicia faba*. Rad. Res. 35: 622—634.

Heilporn-Pohl V., Limbosh-Rolin S., 1969. Effects de l'hydroxyurée sur *Acetabularia mediterrenea*. Biochim. Biophys. Acta 174: 220—229.

Injeyan H. S., Meerovitch E., Mc'Laughlin J., 1974. Anomalous enhancing effect of hydroxyurea on DNA synthesis. Life Sciences 14: 1687—1693.

Jyssum K., 1973. Cell division after inhibition of DNA and protein synthesis in *Neisseria meningitidis*. Acta pat. microbiol. scand. Sect. B 81: 127—137.

Kihlman S. A., Eriksson T., Odmark G., 1966. Effects of hydroxyurea on chromosomes, cell division and nucleic acid synthesis in *Vicia faba*. Hereditas 55: 386—397.

Krakoff I. H., Brown N. C., Reichard P., 1968. Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. Cancer Res. 28: 1559—1565.

Maciejewska-Potapczykowa W., Rennert A., Bilecka A., 1970. Influence of hydroxyurea on growth and nitrogen fraction content in callus and tumorous tissues of *Nicotiana tabacum* cultured *in vitro*. Zesz. Nauk. UMK N. Mat.-Przyr. Biol. 13: 257—262.

Magnusson G., 1973. Hydroxyurea-induced accumulation of short fragments during polyoma DNA replication. I. Characterization of fragments. II. Behavior during incubation of isolated nuclei. J. Virol. 12: 600—608, 609—615.

Manso-Martinez R., Frank W., 1972. Die Wirkung von Hydroxyharnstoff auf die DNA-Synthese embryonaler Rattenzellen in Kultur. Zeit. Naturforsch. (B) 27b: 1500—1507.

Martin R. F., Radford I., Pardee M., 1976. Accumulation of short DNA fragments in hydroxyurea treated mouse L-cells. Biochem. Biophys. Res. Commun. 74: 9—15.

Miyamoto T., Watanabe M., Takabe Y., Terasima T., 1976. Synchrony of Burkitt lymphoma cells induced by hydroxyurea. Cell Structure and Function 1: 177—185.

Mohler W. C., 1964. Cytotoxicity of hydroxyurea (NCS-32065) reversible by pyrimidine deoxyribosides in a mammalian cell line grown in vitro. Cancer Chemoth. Rep. 34: 1—6.

Moore E. C., 1969. The effects of ferrous ion and dithioerythritol on inhibition by hydroxyurea of ribonucleotide reductase. Cancer Res. 29: 291—295.

Necas E., Neuwirt J., 1976. Control of haemopoietic stem cell proliferation by cells in DNA synthesis. Brit. J. Haemat. 33: 395—400.

Odmark G., 1971. Effects of hydroxyurea on the synthesis of deoxycytidine phosphate and the uptake of nucleosides in excised root tips of *Vicia faba*. Physiol. Plant. 25: 158—163.

Rabes H. M., Iseler G., Tuczek H. V., Kerler R., 1974. Increased synchrony of DNA synthesis in regenerating rat liver after continous infusion of hydroxyurea. Experientia 30: 1116—1117.

Rajewsky M. F., Hülser D. F., Fabricius E., 1971a. Untersuchungen zur Synchronisation in vivo: Temporare Inhibition der DNA-Synthese durch Hydroxyharnstoff in normalen und malignen Säugerzellsystemen. Z. Krebsforsch. 76: 266—292.

Rajewsky M. F., Fabricius E., Hulser D. F., 1971b. Synchronisation in vivo: Temporary inhibition of DNA synthesis in the rat embryo with hydroxyurea. Exp. Cell Res. 66: 489—492.

Rennert A., 1977a. Metabolic aspects of growth in HU-treated crown-gall tissue cultures. I. *Nicotiana tabacum*. Acta Soc. Bot. Pol. 46: 79—99.

Rennert A., 1977b. Metabolic aspects of growth in HU-treated crown-gall tissue cultures. II. *Helianthus annuus*. Acta Soc. Bot. Pol. 46: 101—118.

Rennert A., Knypl J. S., 1971. Hamowanie syntezy chlorofilu i RNA w izolowanych liścieniach ogórka przez N-hydroksymocznik. Acta Soc. Bot. Pol. 40: 669—679.

Rennert A., Knypl J. S., 1972. Wpływ N-hydroksymocznika na kiełkowanie nasion, wzrost izolowanych liści ogórka i innych biotestów. Zesz. Nauk. UŁ. N. Mat.-Przyr. (s. II) 40: 101—114.

Roberts D., Wodinsky I., 1968. On the poor correlation between the inhibition by methotrexate dihydrofolate reductase and deoxynucleoside incorporation into DNA. Cancer Res. 28: 1955—1962.

Rosenkranz H. S., Garro A. J., Levy J. A., Carr H. S., 1966. Studies with hydroxyurea. I. The reversible inhibition of bacterial DNA synthesis and the effect of hydroxyurea on the bactericidal action of streptomycin. Biochim. Biophys. Acta 114: 501—515.

Rosenkranz H. S., Gutter B., Becker Y., 1973. Studies on the developmental cycle of *Chlamydia trachomatis:* Selective inhibition by hydroxyurea. J. Bact. 115: 682—690.

Rubini J. R., 1966. In vitro DNA labeling of bone marrow and leukemic blood leucocytes with tritiated thymidine. II. ^{3}H-Thymidine biochemistry in vitro. J. Lab. Clin. Med. 68: 566—576.

Schwartz H. S., Garofalo M., Sternberg S. S., Philips F. S., 1965. Hydroxyurea: Inhibition of deoxyribonucleic acid synthesis in regenerating liver of rats. Cancer Res. 25: 1867—1870.

Sinclair W. K., 1965. Hydroxyurea: Differential lethal effects on cultured mammalian cells during the cell cycle. Science 150: 1729—1731.

Sinclair W. K., 1967. Hydroxyurea: Effects on Chinese hamster cells grown in culture. Cancer Res. 27: 297—308.

Sinha N. K., Snustad D. P., 1972. Mechanism of inhibition of deoxyribonucleic acid synthesis in *Escherichia coli* by hydroxyurea. J. Bact. 112: 1321—1333.

Slater M. L., 1973. Effect of reversible inhibition of deoxyribonucleic acid synthesis on the yeast cycle. J. Bact. 113: 263—270.

Theiss J. C., Fisher G. A., 1976. Inhibition of intracellular pyrimidine ribonucleotide reduction by deoxycytidine, arabinosylcytosine and hydroxyurea. Biochem. Pharmacol. 25: 73—79.

Tobey R. A., Crissman H. A., 1972. Preparation of large quantities of synchronized mammalian cells in late G_1 in the pre-DNA replicative phase of the cell cycle. Exp. Cell Res. 75: 460—464.

Walters R. A., Tobey R. A., Hildebrand C. E., 1976. Hydroxyurea does not prevent synchronized G_1 Chinese hamster cells from entering the DNA synthetic period. Biochem. Biophys. Res. Comm. 69: 212—217.

Wanka F., Moors J., Krijzer F. N. C. M., 1972. Dissociation of nucleolar DNA replication from concomitant protein synthesis in synchronous cultures of Chlorella. Biochim. Biophys. Acta 269: 153—161.

Young C. W., Schochetman G., Karnofsky D. A., 1967. Hydroxyurea-induced inhibition of deoxyribonucleotide synthesis: Studies in intact cells. Cancer Res. 27: 526—534.

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Wbudowywanie tymidyny do jąder komórek merystematycznych korzeni cebuli w obecności hydroksymocznika i jej rola w odnowie aktywności mitotycznej

Streszczenie

Stwierdzono, że blok mitotyczny powodowany w merystemach korzeni cebuli działaniem hydroksymocznika jest znoszony nie tylko przez przeniesienie cebul do wody, lecz także przez dodanie do roztworów hydroksymocznika ^{3}H-tymidyny lub zimnej tymidyny. W obecności hydroksymocznika zaobserwowano bardzo intensywne wbudowywanie ^{3}H-tymidyny do jąder komórkowych, dające indeks znakowania 40—70%. Mimo to wszystkie figury mitotyczne pojawiające się w obecności hydroksymocznika nie zawierały znacznika. Natomiast znakowane mitozy wystąpiły po przeniesieniu do wody korzeni preinkubowanych z ^{3}H-tymidyną w obecności hydroksymocznika. Otrzymane wyniki wskazują, że hydroksymocznik zatrzymuje komórki merystematyczne korzeni cebuli zarówno wfazie S, jak i w fazie G_2. Wzmożone wbudowywanie się ^{3}H-tymidyny do jąder komórkowych w obecności hydroksymocznika wskazuje ponadto, że hydroksymocznik wybiórczo wywołuje w merystemach korzeni cebuli niedobór tymidylanu.

Wbudowywanie ^{3}H-urydyny do komórek merystematycznych nie zmieniło się pod wpływem hydroksymocznika.