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DNA SYNTHESIS AND EFFECTS OF HYDROXYUREA ON REGENERATING HYDRA

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Abstract Regenerating Hydra littoralis incorporate tritiated thymidine into DNA. The synthesis of DNA begins at least within six to eight hours following transection, and continues up to 24 hours. There is a decline in synthesis at 45 hours following transection, indicating possible metabolic turnover of DNA. Hydroxyurea (10^{-3}M) inhibits the synthesis of DNA by regenerating hydra, but inhibition is statistically significant only at the eight-hour period following transection. At eight hours there is an approximately 50% inhibition of DNA synthesis compared to 30% inhibition at 24 hours. At 45 hours inhibition is negligible, suggesting a possible breakdown of hydroxyurea or a completion of DNA synthesis at that time.

INTRODUCTION

The discovery that regenerating hydra incorporate tritiated thymidine into their cells (Burnett, Baird, and Diehl, 1962) provides a means by which the localization and extent of aspects of metabolic activity may be ascertained. The sequential pattern for cell renewal in hydra is as yet not fully known, but there is little doubt that a wide range of factors, both physiological and environmental, come into play. There is the indication of an overall decrease in catabolic activity during regeneration, and a lack of specific information by the regenerating hydra as to its anatomical completeness (Lentz and Barrnett, 1962).

In association with the decrease in metabolic activity is the synthesis of nucleic acids or precursors during regeneration. In the synthesis of nucleic acids necessary for cell replacement, hydra must either possess a reservoir of available constituents for this replacement or, upon transection or trauma, must form these constituents at a fairly rapid rate. Once the regenerative process is "triggered", there is an obvious need for utilizable organic substances from which the cells assemble the components necessary for the process.

The present paper deals only with the sequential synthesis of DNA with time in regenerating hydra, and with the effects of hydroxyurea on this activity.

* Part of this work was initiated in the Department of Physiology, Chicago College of Osteopathic Medicine, Chicago, Illinois.

MATERIALS AND METHODS

Hydra littoralis were cultured in tap water containing $CaCl_2$, Na_2EDTA, $CaCl_2$, and TRIS buffer. Stock culture medium was renewed at least every ten days and kept at laboratory temperature of 22°C ± 2°C at pH 7.0. Hydra were fed Artemia nauplii daily and, at selected times, every other day for a 30- to 60-minute period. All animals used for experimentation were nonbudding, asexual organisms starved at least 48 hours prior to transection.

Deoxyribonucleic acid (DNA) extractions were made, utilizing fifty hydra transected through the lower stomach region and incubated for periods of 8, 24 and 45 hours in 9 ml of culture medium containing 1.0 ml tritiated thymidine (20 microcuries/ml). Identical procedure was followed for the inhibition experiments, except that a 10^{-3} M (0.076 mg/ml) solution of hydroxyurea in culture medium was used in place of culture medium alone.

In addition, groups of ten hydra were incubated for periods of 2, 4, 6, 8, 16, 24 and 45 hours in 1.8 ml of culture medium containing 0.2 ml tritiated thymidine, and the DNA extracted.

Following the incubation periods, the hydra were centrifuged and the medium decanted. Animals were washed three times, suspended in 10% trichloroacetic acid (TCA), and homogenized in the cold with a glass-teflon homogenizer. The homogenate was centrifuged for five minutes, and the pellet was treated with 1.0 ml of 0.6N KOH followed by an incubation period of 18 to 24 hours in a 37°C water bath. At the end of this time, 0.5 ml of 6N HCl and 1.0 ml of 10% TCA were added in the cold.

After precipitate formation, the homogenate was centrifuged for five minutes. The resulting pellet was washed and centrifuged three times in 2.0 ml of 5% TCA, resuspended in 2.0 ml of 10% TCA, and incubated in a 90°C water bath for 20 minutes to solubilize the DNA. The sample was then chilled in ice and centrifuged for five minutes, yielding the DNA supernatant fraction. This fraction was then counted by liquid scintillation for tritiated thymidine incorporation.

All samples — 0.2 ml in 10 ml of dioxane-based scintillant — were counted for ten minutes in a CPM-100 Liquid Scintillation Counter. Stock scintillant medium contained 5 grams of 2, 5-diphenyloxazole (PPO) and 100 grams of naphtalene made to 1,000 ml with dioxane.

RESULTS

Regenerating hydra, transected through the lower stomach region, display a definite pattern regarding the incorporation of tritiated thymidine into DNA. There is a relatively limited degree of incorporation during the first six hours, followed by a notable increase at the eight-hour period. There is continued increase in incorporation at 16 hours up until the apparent maximum is reached at about 24 hours. At this time, the organism must have at least the minimum amount of DNA required to further propagate the regenerative processes.

The decline in incorporation noted at the 45-hour period may indicate leakage or metabolism of the precursor as the regenerative processes have all but ceased, a loss of cells by the organism, or a metabolic turnover of DNA (Fig. 1).

FIG. 1. Incorporation of Tritiated Thymidine Into DNA of Regenerating Hydra (With Standard error)

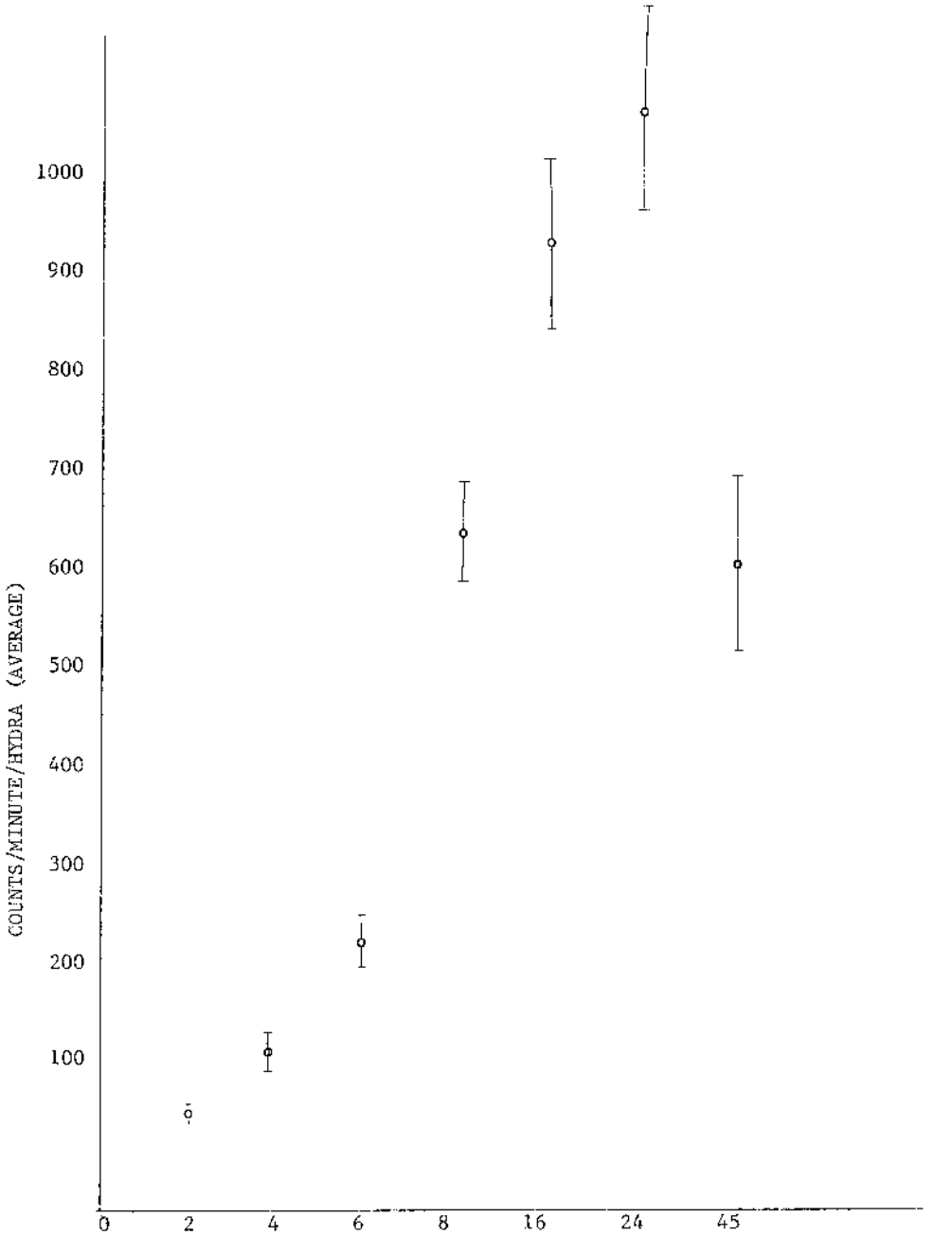
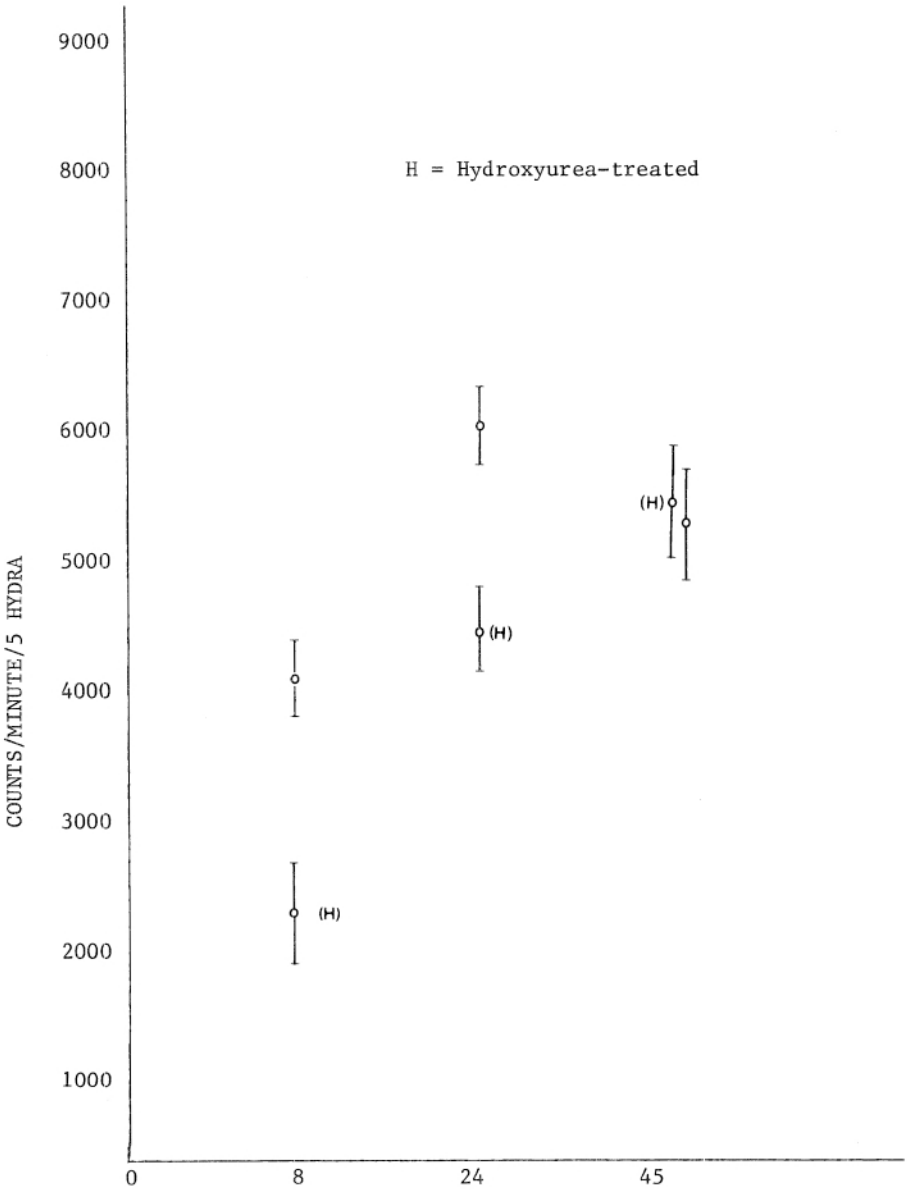


Fig. 2. Incorporation of Tritiated Thymidine into DNA of Hydroxyurea-Treated and Nontreated Regenerating Hydra at 8 hours, 24 hours, and 45 hours (with standard error)



The incorporation of tritiated thymidine into DNA of hydroxyurea-treated and nontreated regenerating hydra is shown in Figure 2. Counts per minute (CPM) at the eight-hour period show a range of from 3,900 to 4,800 for untreated organisms, and a range of 1,500 to 3,700 for hydroxyurea-treated organisms. At 24 hours the range is from 4,000 to 9,200 CPM for untreated animals, and from 1,700 to 8,100 CPM for treated animals. There is little detectable inhibition at 45 hours as the range of CPM is from 2,500 to 8,500 for untreated hydra, and from 2,400 to 8,400 for hydroxyurea-treated hydra. At each time period, however, the CPM for hydroxyurea-treated organisms are less than that for the corresponding untreated controls. There is thus blockage of isotope incorporation at the eight-hour period and 24-hour period. Results from the "t" test show that the inhibitory effect of hydroxyurea is statistically significant (.05 level) for the eight-hour period only.

There is an approximately 50% inhibition of synthesis at the eight-hour period compared with 30% inhibition at 24 hours. There is no determinable percentage of inhibition at the 45-hour period.

All data presented represent isotope incorporation into both proximal and distal sections taken together.

DISCUSSION

The incorporation of tritiated thymidine with time into regenerating hydra represents the relative progression of the synthesis of nucleic acids or precursors by the organism. This is predicated on the supposition that transection of hydra initiates the onset of synthesis of these materials, necessary for tissue renewal.

According to Ham and Eakin (1958), there is an eight-hour period following transection during which no visible differentiation can be observed, with the actual physical rebuilding of lost structures beginning at about the twelfth hour (Fig. 1) and the subsequent increase in synthesis corroborate these data and lend credence to the idea that DNA synthesis does increase during regeneration.

Work by Clarkson (1969) demonstrated that DNA synthesis was inhibited in hypostomes of hydra by approximately 60% during the first hour of distal regeneration, after a 20-hour pretreatment with hydroxyurea at a concentration of 1 mg/ml.

The significant inhibitory effect of hydroxyurea, noted at the eight-hour period (Fig. 2), may indicate that this is the period during which relatively larger amounts of DNA are being synthesized. That the relative percentages of inhibition by hydroxyurea between 8 hours and 45 hours are decreasing could indicate that the hydroxyurea is being metabolized or that there is completion of DNA synthesis. The major metabolic effect of this drug is reported to be a diminution in oxidative phosphorylation and a subsequent diminished total concentration of adenosine triphosphate (ATP) (Fishbein and Carbone, 1963).

From this base, it may be considered that available energy sources for hydra during regeneration are diminished by the action of hydroxyurea on the anabolic mechanisms. Evidence for this hypothesis is seen in the increased time required for hydroxyurea-treated hydra to complete fully the replacement of lost parts over that of nontreated organisms.

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