The Effect of Plant Growth Substances and Natural Products on RNA and DNA Synthesis in Leukocytes

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Summary. The effect of auxins, cytokinins, gibberellins and phenolics on the incorporation of uridine and thymidine into the nucleic acids of human leukocytes was examined. Both the stimulation and inhibition of the incorporation of the precursors was noted. The auxins consistently promoted the incorporation of uridine.

Most plant hormones and associated compounds inhibit or stimulate nucleic acid synthesis in plant systems[1]. Little is known about these compounds in human systems, beyond the work of Rathbone and Hall[2], and it would be of interest in elucidating the mode of action of the plant growth substances to find if they affect nucleic acid synthesis in other systems such as a human leukocyte system.

In this paper we report the effect of certain plant hormones and associated substances on RNA and DNA synthesis in human leukocytes. We utilized the microscreening system developed by Farrow and Van Dyke[3] which is based upon the uptake, phosphorylation and incorporation of thymidine-methyl-^{3}H into DNA of the leukocytes of human whole blood or the uptake, phosphorylation and incorporation of uridine $-5'^3$H into RNA of the leukocytes of human whole blood. Although the screening system was developed to test anti-leukemic drugs it can also test any type of substance that acts at any step in the synthetic pathway of nucleic acids. We have screened the common plant growth substances and several natural products to find if increases or decreases of incorporation of the nucleosides into nucleic acid occurs.

The substances used in this study included gibberellic acid, auxins, cytokinins, and phenolics. 4 doses of each substance were used: 10^{-4} *M*, 10^{-5} *M*, 10^{-6} *M*, and 10^{-7} *M* for each thymidine methyl-^{3}H and uridine-5′-^{3}H set.

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Effect of various substances on uridine or thymidine incorporation

	6-Methoxy-benzoxazolinone (%)	Gibberellic Acid (K^+) (%)	Indole-acetic acid (%)	Indole-butyric acid (%)	α-Naphtalene-acetic acid (%)
Thymidine (*M*)					
10^{-4}	34.8↓	—	—	—	—
10^{-5}	21.9↓	—	—	—	—
10^{-6}	31.2↓	—	—	—	—
10^{-7}	20.7↓	—	—	—	—
Uridine (*M*)					
10^{-4}	61.0↓	33.7↓	40.2↑	53.6↑	49.3↑
10^{-5}	52.7↓	40.7↓	49.5↑	53.8↑	54.9↑
10^{-6}	51.8↓	42.2↓	53.7↑	61.7↑	62.1↑
10^{-7}	62.0↓	54.5↓	61.8↑	57.2↑	67.6↑

	Dihydrozeatin (%)	Zeatin (%)	Kinetin (%)	N-6Benzyl adenine (%)	Kinetin riboside (%)
Thymidine (*M*)					
10^{-4}	—	18.1↓	31.6↓	—	—
10^{-5}	—	—	17.0↑	—	—
10^{-6}	—	12.5↑	49.2↑	—	—
10^{-7}	—	—	—	—	—
Uridine (*M*)					
10^{-4}	66.6↓	22.0↑	—	11.8↓	50.6↓
10^{-5}	55.8↓	15.8↑	—	10.3↓	21.3↑
10^{-6}	60.1↓	36.3↓	43.7↑	—	18.0↑
10^{-7}	65.6↓	64.5↑	—	72.8↑	47.2↑

↑ This arrow indicates percent stimulation of incorporation of tritiated precursor when compared to control without the test substance. ↓ This arrow indicates percent inhibition of incorporation of tritiated precursor when compared to control without the substance. The incorporation values, (percentages indicated above), are based on cpm corrected for efficiency and background. All data presented were significantly different from the control using the Fisher Exact Probability Test, Huntsberger and Leaverton[4]. These dashes indicate no significant differences in incorporation of precursor between test substance and control samples.

The sets were run twice. The method used is reported in detail in FARROW and VAN DYKE[3]. The data were tested statistically with the Fisher Exact Probability Test, HUNTSBERGER and LEAVERTON[4].

Gibberellic acid. In plant systems gibberellins reverse genetic dwarfism in corn and pea. VARNER et al.[5] suggest that the compounds affect de novo protein synthesis.

The potassium salt of gibberellic acid (GA3) was without effect on thymidine incorporation. However, this salt inhibited the incorporation of uridine down to 30 and 50% of the control with no gibberellic acid, in our leukocyte system.

Auxins. The auxins instigate plant growth by cell elongation. Evidence that RNA is directly involved is presented by KEY et al.[6] and NOODEN and THIMANN[7]. EVANS and RAY[8], and EVANS[9] present evidence that points to an effect of auxin on growth via a system other than or in addition to the effect on the nucleic acids.

Indole-acetic acid, indole-butyric acid and α-napthalene-acetic acid did not affect thymidine incorporation at all doses. All concentrations of the auxins stimulated uridine incorporation between 40 and 60% over the control in the leukocyte system.

Cytokinins. The cytokinins are purine derivatives that are required for cell division in many plant systems[10–15]. Cytokinins occur in RNA of many species[15]. ZACHAU et al.[16] showed that in the case of brewers yeast RNA the cytokinin was located adjacent to the 3′ end of the anticodon.

Dihydrozeatin is without effect on thymidine incorporation; however, it decreases uridine incorporation to about 60% of the control at all concentrations in the leukocyte system. At the highest concentration (10^{-4} *M*) zeatin inhibited thymidine incorporation down to about 20% of the control, whereas the lower concentrations were slightly stimulatory. All concentrations of zeatin employed stimulated uridine incorporation.

The synthetic cytokinin N6-benzyladenine was without effect on thymidine corporation; however, at 10^{-4} *M* and 10^{-5} *M* it inhibited uridine incorporation down to about 10% of control. At 10^{-7} *M* a 75% stimulation over the control value was observed.

The synthetic cytokinin, kinetin, inhibited thymidine incorporation at 10^{-4} *M*; whereas a lower concentration (10^{-6} *M*) stimulated thymidine incorporation. Kinetin was either without effect or stimulatory (10^{-6} *M*) to uridine incorporation. Kinetin riboside was without effect on thymidine incorporation; however at 10^{-4} *M* it inhibited uridine incorporation to 50% of the control. Lower concentrations (10^{-5}, 10^{-6} and 10^{-7} *M*) stimulated uridine incorporation.

Phenolics. The phenolics are a diverse group of plant secondary products[17]. It has been suggested that in certain cases these compounds may act as inhibitors. Interestingly enough naringenin and ferulic acid inhibited incorporation of precursors on the order of 50% of the control in certain experiments. 6-Methoxybenzoxazolinone is a breakdown product of 2,4-dihydroxy-7-methoxy-2H-1, 4-benzoxazine-3-(4)-one which occurs in etiolated *Zea mays* seedlings[18]. Inhibition of thymidine incorporation was decreased to about 34% of control while uridine incorporation was decreased to about 60% of the control at the 10^{-4} *M*. All other doses also produced an inhibition of incorporation of the nucleoside precursor in the leukocyte system.

The compounds studied may affect nucleic acid metabolism in plants. No specific mechanisms have been established. Probably, in order to inhibit or stimulate the incorporation of thymidine-methyl-^{3}H or uridine-5′-^{3}H into DNA or RNA of the leukocyte, the compound must have acted at some point in pathway leading to synthesis of the nucleic acids. The possibility of nonspecific inhibition of the uptake of nucleosides as reported in HILL and WINGO[19] seems unlikely, because both stimulation and inhibition were observed[20, 21].

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[21] The substance 6-methoxy 2–3 benzoxazolinone was obtained through Dr. SMISSMAN, University of Kansas.

Structural Changes of Deoxyribonucleoprotein Fibres Following γ-Irradiation under Aerobic and Hypoxic Conditions

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Summary. The DNP fibres γ-irradiated under aerobic condition showed a reduction of their diameter, while no remarkable changes were observed in the DNP fibres irradiated under hypoxic condition by scanning electron microscopy.

In general, the radiosensitivity of macromolecules and biological systems irradiated in the presence of oxygen is higher than when they are irradiated in the absence of oxygen, as reviewed by DERTINGER and JUNG[2]. There are a considerable number of physicochemical and biological studies on this phenomenon. As far as the authors are aware, however, no literature of electron microscopic investigations pertaining to this subject is accessible.

The present study has been undertaken with the hope of elucidating the structural changes of deoxyribonucleo-