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THE INFLUENCE OF ORGANOPHOSPHORUS COMPOUNDS ON RNA SYNTHESIS IN ISOLATED CELL NUCLEI[1]

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Summary. The influence of malathion (O:O-dimethylo-1/2 dicarboetoxy-ethylo--dithiophosphorate) and IPO-63 (O,O-dimethylo-O-1/2, 4-dichlorophenylo-2-bromovinylophosphate) on the level of H^3UTP incorporation during transcription in cell nuclei isolated from calf gland was studied.

It was found that in the presence of organophosphorus insecticides the level of RNA synthesis decreased depending on the applied dose and the time of action with these compounds.

In the case of both applied compounds the course of inhibition of the radioactive precursor incorporation in RNA in nuclei was similar.

Earlier studies (Walter, Czajkowska, Lipecka 1980; Czajkowska, Walter 1980) showed that malathion — an organophosphorus pesticide inhibits nucleic acid synthesis in human lymphocytes stimulated by PHA to a degree depending on the dose and time of malathion introduction in culture. Thus, it seemed interesting to study the influence of malathion and IPO-63 on RNA synthesis in isolated, transcriptionally active cell nuclei. The investigation of transcription process on active cell nuclei looked reasonable since that model may be treated as an intermediate between RNA transcription in intact cells and the process studied in vitro by using isolated RNA polymerases. The advantage of that system is that transcription in cell nuclei proceeds linearly during a long period. There are little literature data concerning the influence of malathion on metabolism of nucleic acids in eukaryotic cells.

MATERIAL

Material for the studies was a fresh calf gland transported in ice bath from the Meat Plant in Łódź.

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METHODS

1. Isolation of cell nuclei: Cell nuclei were isolated from calf gland by modified Allfrey and Mirsky's method (1967). In sucrose solutions used by us, $CaCl_2$ was substituted by $MgCl_2$ without the addition of KH_2PO_4. 12.5 g fresh crumbled tissue was homogenized for 0.5 min. with the addition of 0.5 M sucrose+0.003 M $MgCl_2$ in "Laurdes" homogenizer and then 100 ml of 0.25 M sucrose+0.003 M $MgCl_2$ were added and again homogenized for 5 min. at 25 - 30% of maximum circulations. The obtained homogenate was filtered through 4 layers of gauze and centrifuged for 7 min. at 1000 g. The sediment of nuclei was washed three times with 0.25 M sucrose+ 0.003 M $MgCl_2$, always centrifuging nuclei suspension for 7 min. at 1000 g. The sediment of nuclei washed in that way was suspended in Tris-HCl buffer, pH 7.8, containing: 12% glycerole, 5 mM $MgCl_2$, 50 mM Tris, 0.1 mM EDTA, 2.5 mM dithiotreitol and dialized at 0°C to that buffer for 24 hours. The purity of nuclei was examined in a contrast-phase microscope (Carl Zeiss Jena).

2. RNA synthesis in isolated cell nuclei: Conditions of synthesis were established on the basis of papers by: Marzluff et al. (1973), Land, Schafer (1977), Hoflack et al. (1980) and Hayashi, Mikami (1981). Incubation mixture$_1$ (0.25 ml) contained: 12% glycerole, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.8, 0.1 mM EDTA, 0.1 mM dithiotreitol, 0.1 M $(NH_4)_2SO_4$, 0.5 mM: ATP, GTP, CTP, 0.1 mM UTP and 4 uCi H^3UTP. Transcription reactions were started by adding 100 µl of nuclei suspension.

Samples were incubated at 25°C for 30 min., — the reaction being interrupted, when adding 2 ml of cold 10% TCA, and cooled down on ice. Acid-insoluble fractions were precipitated on Millipore HA 0.45 µm filter, then washed with 100 ml of 3% TCA and 10 ml of 99% ethanol. After drying up, the filters were placed in phials containing 5 ml of Scintol 3 and radioactivity was determined in a scintilation Beckman counter.

3. RNA synthesis in nuclei in the presence of α-amanitin: An attempt has been made to identify RNA polymerases, which are responsible for active incorporation of H^3UTP into synthesized RNA α-amanitin in cell nuclei isolated from the calf gland. For that purpose, α-amanitin was added the incubation mixture to until the final concentration of 1 µg/ml or 200 µg/ml and transcription was made as in point 2.

4. Conditions of incubation with organophosphorus compounds: Cell nuclei at the concentration of 10^6-10^7 nuclei/ml were suspended in Tris-HCl buffer, pH 7.8, and incubated in the presence of 10, 40, 70 µg/ml malathion or IPO-63 for 1 and 2 hours at 37°C. Transcription was made as in point 2.

RESULTS AND DISCUSSION

To study RNA synthesis in vitro functionally active cell nuclei isolated from the calf gland, the system, in which transcription occurs linearly for a long period, was used. Optimal conditions of RNA synthesis in cell nuclei were established by performing studies on the dependence of its productivity on temperature, incubation

time and concentration of nuclei. Temperature was 25°C, the time of transcription reaction 30 min. and the concentration of nuclei 10^6 and 10^7 nuclei/ml (Fig. 1). Our conditions of RNA synthesis were adequate to those of other authors (Marzluff et

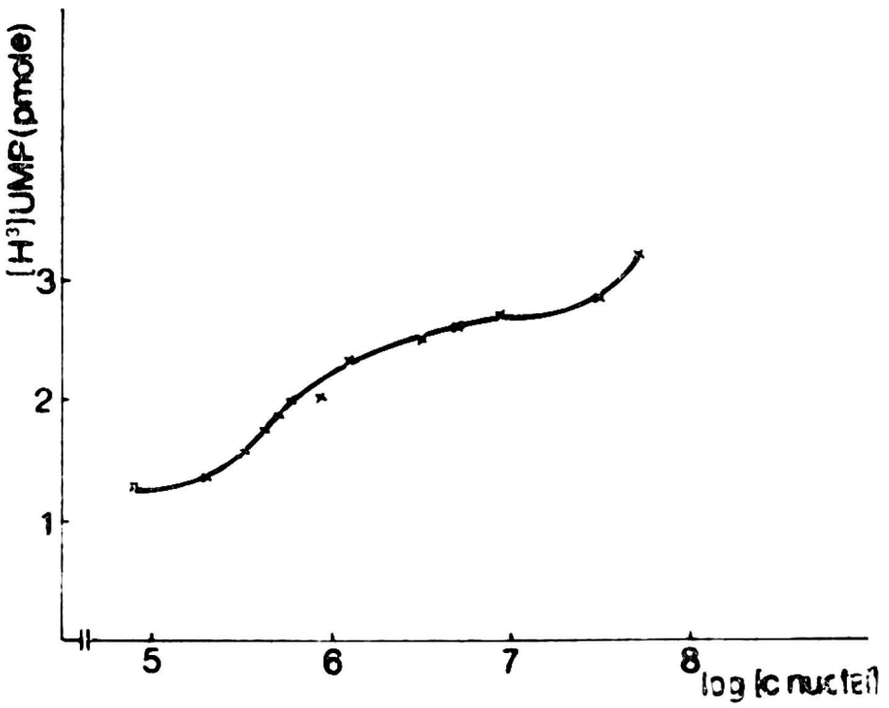

Fig. 1. The influence of cell nucleus concentration in transcription mixture on the level of RNA synthesis

al. 1973, Land, Schäffer 1977, Hoflack et al. 1980, Hayashi, Mikami 1981, Wojcierowski et al. 1981). An attempt has been made to classify, which polymerases are responsible for an active incorporation of H^3UTP into RNA synthesized by us in the studied cell nuclei of the calf gland. Results of that experiment are presented in Fig. 2. They clearly show the presence of eukaryotic RNA polymerases A, B and C in the nuclei of all the classes. Our results are supported by the literature data. Hoflack et al. (1980) found the occurence of three classes of RNA polymerases dependent on DNA in the nuclei isolated from KB cells. Ueno et al. (1981), using diffe-

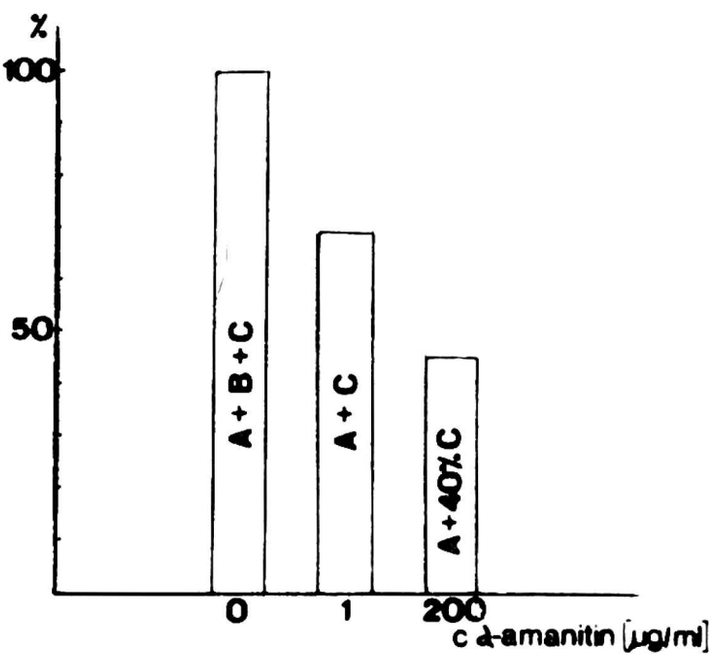

Fig. 2. Distribution of the activity of DNA-dependent RNA polymerases in cell nuclei depending on α-amanitin concentration

rent concentrations of α-amanitin, an inhibitor of RNA polymerases, displayed the presence of polymerases I, II and III in the nuclei of Ehrlich's cancer cells and lien cells of anemic mice.

In view of a wide application of organophosphorus insecticides in plant protection and their probable harmful influence on metabolism of live organisms an attempt has been made to elucidate the mechanism of the action of these compounds on the genetic material of live cells. In the present studies, two compounds from this group, malathion and IPO-63, were applied for the experiment. The influence of these in-

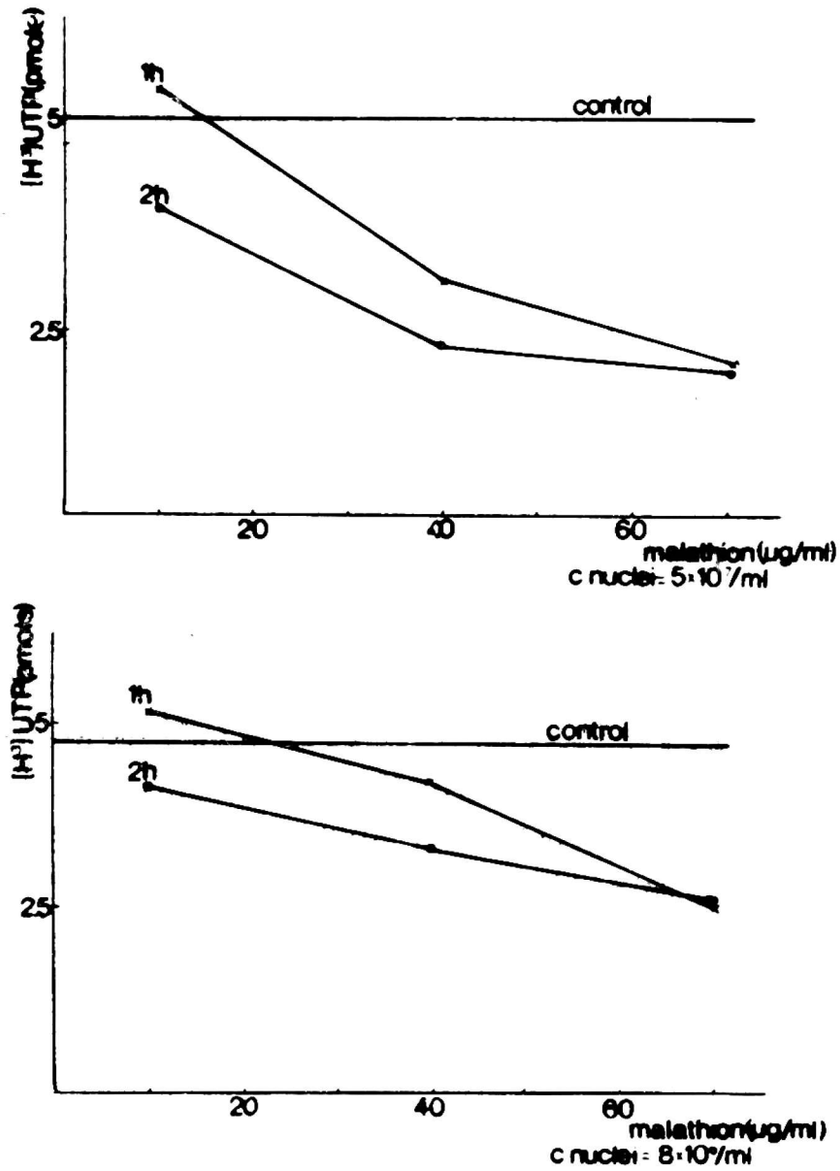

Fig. 3. The influence of different malathion concentrations on RNA synthesis in isolated cell nuclei

secticides on the rate of RNA synthesis was determined depending on their concentration and the time of action on cell nuclei. Figs. 3 and 4 clearly illustrate the inhibiting effect of malathion and IPO-63 on the rapidity of H^3UTP incorporation into synthesized RNA in the cell nuclei from calf gland. The action of both applied compounds depends first of all on the concentration and to lesser extent — on the time of action on the genetic material under study. In the case of low concentrations of both insecticides — 10 µg/ml and 40 µg/ml (malathion) and 10 µg/ml of IPO-63 the level of RNA synthesis markedly decreased depending on the time of reaction with an inhibi-

tor (1 and 2 hours). At the highest concentrations of both compounds (70 μg/ml) the inhibition degree of the transcription reaction did not depend on the time of their action. Fig. 5 shows a comparison of the action mode of the two insecticides and illu-

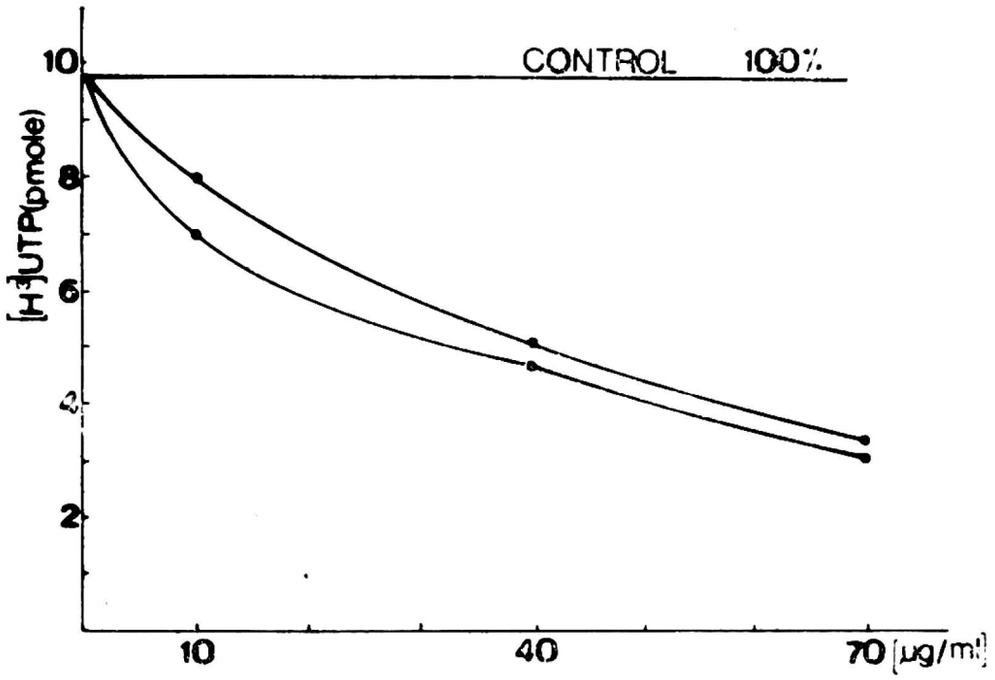

Fig. 4. The influence of different IPO-63 concentrations of RNA synthesis in isolated cell nuclei

strates a similar course of RNA synthesis inactivation in nuclei for both IPO-63 and malathion. Malathion and IPO-63 are considered to be factors, which modify cell metabolism in a non-specific way. There is very little literature data concerning this problem. Myhr (1973) studied the influence of malathion on RNA synthesis in HeLa cells and that factor decreased the rate of incorporation of labelled uridine into synthesized RNA. Walter et al. (1980), Czajkowska and Walter (1980) showed that malathion at the concentrations of 30-70 μg/ml of culture caused a decrease in

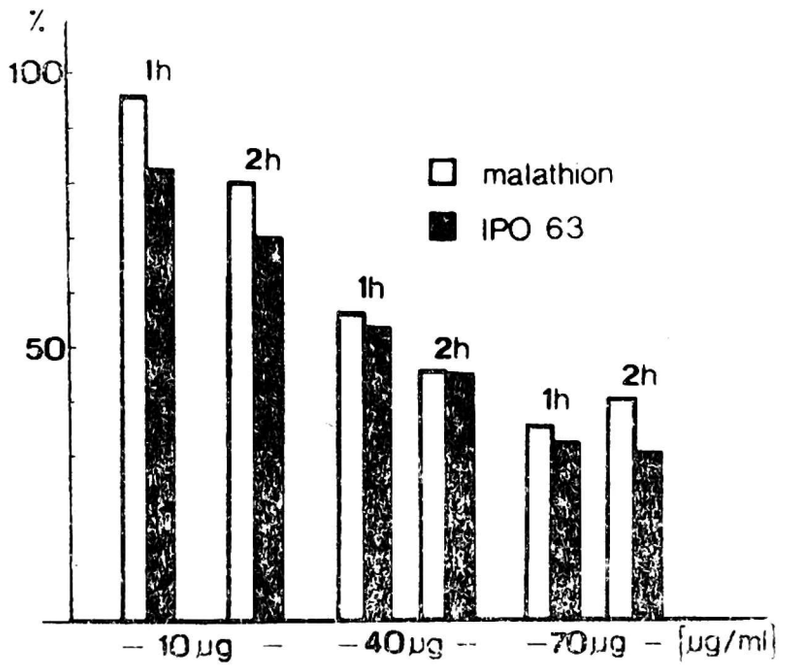

Fig. 5. A comparison of the action of malathion and IPO-63 on RNA synthesis in cell nuclei

nucleic acid content, inhibited the rate of their synthesis and induced chromosome aberration in PHA-stimulated human lymphocytes.

Our results are in agreement with the literature data concerning transcription performed on isolated cell nuclei. A decrease in the incorporation rate, a labelled RNA precursor (H^3UTP) may be a result of two mechanisms: a) modification of DNA molecule, a matrix in the transcription process in cell nuclei, b) disturbance in the action of enzymes participating in RNA synthesis.

Studies in vitro carried out on isolated DNA by other authors showed that organophosphorus compounds induce structural changes in this molecule. The observed changes in physico-chemical properties of DNA are caused to a considerable extent by its methylation, the main product of which is 7-methyloguanine (Oliński et al. 1980). Sites modified under the influence of these compounds are more labile what can lead to the degradation of nuclei acid molecule. On the other hand, it is known that DNA in the cell nuclei, occurs in a chromatin complex. Studies of Wild (1975) showed that DDVP, acting on the entire cells in culture, causes a 30-fold larger methylation of proteins than DNA.

Therefore, on the basis of the literature data and our results, none of the previously proposed mechanisms can be either excluded or accepted. Disturbances in the rate of RNA synthesis in nuclei under the influence of insecticides are likely to be the effect of these two phenomena simultaneously.

CONCLUSIONS

1. Using various concentrations of α-amanitin, an inhibitor of RNA polymerases, the cell nuclei from the calf gland were found to have the activity of all three classes of RNA polymerases — A, B, and C.

2. In the presence of both applied organophosphorus insecticides (malathion and IPO-63) the level of RNA synthesis in the nuclei decreases depending on the dose. The decrease was the largest for the lowest doses of the inhibitor (10, 40 μg/ml).

3. The incubation time for small doses of insecticides (10, 40 μg/ml) significantly influenced the level of transcription activity. The level of RNA synthesis decreased by 10-15% of the extended incubation time up to 2 hours.

4. In case of application of organophosphorus compounds the course of RNA synthesis in cell nuclei was similar.

5. Malathion and IPO-63 are considered to be factors modifying cell metabolism in a non-specific way. It may be suggested that they inhibit RNA synthesis by disturbing the action of enzymes involved in that process or modifying DNA molecule.

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WPŁYW ZWIĄZKÓW FOSFOROORGANICZNYCH NA SYNTEZĘ RNA W IZOLOWANYCH JĄDRACH KOMÓRKOWYCH

Streszczenie

Zbadano wpływ malationu (O:O-dwumetylo-1/2dwukarbo-etoksy-etylo-dwutiofosforan) i IPO-63 (O,O-dwumetylo-O-1/2, 4-dwuchlorofenylo-2-bromowinylofosforan) na poziom włączania H^3UTP podczas reakcji transkrypcji zachodzącej w jądrach komórkowych wyizolowanych z grasicy cielęcia.

Stwierdzono, że w obecności zastosowanych insektycydów fosforoorganicznych poziom syntezy RNA obniżał się w zależności od stosowanej dawki i czasu oddziaływania z tymi związkami. W przypadku obu zastosowanych związków przebieg hamowania włączania radioaktywnego prekursora do RNA w jądrach był podobny.

ВЛИЯНИЕ ФОСФОРООРГАНИЧЕСКИХ СОЕДИНЕНИЙ НА СИНТЕЗ RNA В ИЗОЛИРОВАННЫХ КЛЕТОЧНЫХ ЯДРАХ

Резюме

Исследовалось влияние малатиона (0 : 0 - диметило-1/2 дикарбо-этокси-этило-дитиофосфат) и IPO-63 (0,0-диметило-0-1/2, 4-дихлорофенило-2-бромовинилофосфат) на уровень включения H^3UTP во время реакции транскрипции, происходящей в клеточных ядрах, изолированных из телячей железы.

Обнаружено, что в присутствии применяемых фосфороорганических инсектицидов уровень синтеза RNA снижался в зависимости от дозы и времени воздействия этих соединений. У обоих применяемых соединений процесс торможения включения радиоактивного прекурсора до RNA в ядрах был подобный.