Metabolic aspects of growth in HU-treated crown-gall tissue cultures

I. *Nicotiana tabacum*

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Abstract

An influence of hydroxyurea (HU) on the growth, DNA and RNA contents and protein synthesis in the tobacco tumour tissue culture was studied in comparison with a homologous callus tissue. In conformity with expectations considerable decrease of DNA level in both tissues is a primary effect of HU activity. This results in the growth inhibition and in the secondary metabolic effects; these effects depend not only on the concentration of inhibitor but also on the age of tissue. In spite of some common features the character of these changes shows a distinct differentiation depending on the tissue type. This points to specific modifications of the biochemical regulation of growth in a tumour.

INTRODUCTION

HU strongly inhibits DNA synthesis in bacteria (Gale et al., 1964; Rosenkranz, Levy, 1965), mammalian organisms (Schwartz et al., 1965; Yarbo et al., 1965 b), animal cells cultured *in vitro* (Young, Hodas, 1964, Yarbro et al., 1965 a; Adams et al., 1967), *Acetabularia mediterranea* (Heilporn-Pohl, Limbosh-Rolin, 1969), *Euglena gracilis* (Buetow, Mego, 1967) and in the root tips of *Vicia faba* (Odmark, Kihlman, 1965; Kihlman et al., 1966). A reversibility of induced inhibition (Rosenkranz et al., 1966; Buetow, Mego, 1967; Truhant et al., 1967; Phillips et al., 1967), a certain selectivity as related to DNA type (Heilporn-Pohl, Lombosh-Rolin, 1969; Becker, Asher, 1975; Grüneisen et al., 1975) and the fact that protein synthesis is

indispensable for the biological activity of HU manifesting (Rosenkranz, Carr, 1966, Rosenkranz et al., 1968) are the most characteristic attributes of this compound effects.

This extremely specific mode of HU action-dissimilar to that of any known inhibitor of DNA synthesis — makes it very suitable to researches on the role of DNA in various macromolecular processes. A great majority of investigations with HU applied was performed with animal and bacterial materials.

The aim of our previous work was to test (1) the effectiveness of HU action on the fundamental physiological processes in higher plants (Rennert, Knypl, 1971, 1972) and (2) a possibility of this compound application in the control of tumorous growth (Maciejewska-Potapczykowa et al., 1970). However a different reaction of pathological tissue from that of a "normal" one as response to the same compound should be a ground for realization of such a possibility. Our preliminary investigations were performed with tobacco bacterial tumour tissue cultures in vitro and homologous callus tissue being a control system (Maciejewska-Potapczykowa et al., 1970). The results of this research allowed to state that the effects of HU activity in both tissue types are differential. The growth of tumour tissue was less sensitive to HU than that of callus. However tumour tissue, in contrast with the callus one, responded with considerable changes in the metabolic indices under examination. An increase in the protein nitrogen content — induced by HU — at simultaneous lack of changes in nucleic acids N content (callus) or even their decrease (tumour) is surprising. On account however of a kind of analytical method applied and also a slow growth rate of tissues under examination (White's medium) these results have only an approximate value.

This investigation deals with a detailed analysis of growth, RNA and DNA contents and protein synthesis in *Nicotiana* callus and tumour tissues treated with HU. Recently Bilecka (1975 a, b) using autoradiographic methods and the same slowly growing tissue strains performed similar investigations. In the present research tissue cultures showing more intensive growth were used; this ensured an appreciable level of changes induced by HU providing facilities for a more precise — as it seems — estimation of the values.

MATERIAL AND METHODS

Tissue strains and growth. The tissue cultures of *Nicotiana* callus and bacterial tumour (*Nicotiana tabacum* variety White Burley) isolated in 1961 were grown in a number of years on a White's

medium (Rennert, 1965). They showed a good rate of growth only in the presence of natural complex growth stimulators (Rennert, 1968). The strains actively growing on a sythetic medium of Murashige and Skoog (1962) modified by Linsmaier and Skoog (1965) derived from these tissues. Calluses were grown in the presence of IAA and kinetin (2 and 0.2 mg/l respectively), tumourous tissues however without these substances. Varying quantities of N-hydroxyurea (Schuchardt, München) in the form of freshly prepared solutions were added to these standard media before their sterilization in an autoclave. Initial tissue fragments weighed approximately 200 mg. They were grown in tubes (25×180 mm) on a slightly skewed agar surface (20 ml). Tubes were allowed to stand in an incubator provided with a continuous fluorescent light (500 lux) and constant temperature 26°C. Analysis of growth in tissue cultures was based on fresh and dry weights measurements made at the end of each experiment. Tissues were dried initially at 100°C for 40 minutes and then at 75° up to a constant weight. Vertical sections of tissues were cut off and used for biochemical analyses. All parts of colonies were taken into consideration in the replicates.

Protein determination. Approximately 150 mg tissue fragments were homogenized in a cold (+5°C) TCA and centrifuged. The precipitate was washed successively with a cold 5% TCA, 80% ethanol, 95% ethanol; ethanol-ether mixture (3:1). Then the precipitate was treated with 5 ml of 1N NaOH at 100°C for 10 minutes to dissolve protein. After cooling the extract was filtered and protein determined in this extract by the method of Lowry et al. (1951). Each experimental variant contained 4 equivalent samples and each series of determinations was repeated three times.

Radioisotope techniques. Three tissue fragments, each deriving from a separate colony of one variant (total weight approximately 600 mg of callus and 300 mg of tumour) were placed in a standard liquid medium containing U-¹⁴C-L-leucine (1 μ Ci/ml; spec. act. 90 mCi/mM). Tissues were incubated in a shaker at temperature 26°C for 4 hours, then washed with a diluted solution of unlabelled precursor and put into a boiling solution of leucine (0.001 M) in 80% ethanol for 1 minute. The plant material prepared in this way was homogenized with 80% ethanol and centrifuged. The precipitate was washed successively with 80% ethanol, cold 5% TCA, 95% ethanol (twice), ethanol-ether mixture (3:1), ether. The proteins present in the precipitate were dissolved in 1N NaOH as described above. Protein precipitate was cooled and centrifuged. A repeated precipitation of proteins and determinations of the radioactivity of protein fraction and of ethanol extract were made as previously (Rennert,

Knypl, 1971). The radioisotope experiments were repeated 2-3 times, each combination contained 3 identical samples.

Extraction of nucleic acids. 1 g of tissue was homogenized in 5 ml of a cold (5°C) methanol. The centrifuged precipitate (+5°C) was again washed with a cold methanol, then with a cold 0.2N HClO_4 , 95% ethanol and finally ethanol-ether mixture (2:1) for 30 minutes at 50°C. 5 ml of 0.5N HClO_4 were added to the tissue homogenate and the extraction of nucleic acids was made at temperature 80° for 10 minutes, or for comparison at 70° for 20 minutes (Schneider, 1945). The cool extract was centrifuged and the precipitate washed again with 0.5N HClO_4 . The acid extract were pooled and used for RNA and DNA determinations.

Quantitative determinations of nucleic acids. Total contents of both nucleic acids were established spectrophotometrically measuring the absorption of respectively diluted extracts in UV at 260 and 290 nm (Cherry, 1962). Yeast RNA hydrolysed in the same way was used as a standard. RNA contents were determined by means of the reaction with deoxyribose according to Burton (1956). Standard solutions of sodium salt of the highly polymerised calf thymus DNA (Koch-Light Lab. LTB) were treated analogously as tissue extracts.

All experiments were made in three parallel tissue samples and the respective experimental series were repeated 3 times.

RESULTS

Growth

A range of HU concentrations being examined in the present work contains these values at which the growth inhibition in both tobacco tissues increases up to nearly 70% (Fig. 1).

This range for the callus tissue is 20—60 mg/l. HU concentrations needed to obtain the same degree of inhibition in tumour tissue are several times higher and they are included within 25—200 mg/l. It is interesting that in the case of callus tissue low concentrations of HU induce slight stimulation symptoms, particularly in younger tissues. Therefore the run of curves representing the growth inhibition depending on HU concentration is different in both tissue types (Fig. 2). This attests — similarly as previously (Maciejewska-Potapczykowa, Rennert, Bilecka, 1970) — to a higher sensitivity to HU of growth processes in a callus tissue than in a tumour one.

Observations of the tissues of different age show that HU — induced growth inhibition increases in time; a difference between continuously growing control tissue and that treated with HU increases.

Fig. 1. Effect of various hydroxyurea (HU) concentrations on the course of growth of tobacco (*N. tabacum* variety White Burley) tissues: callus and tumor

Fig. 2. Comparison of two tobacco tissue types sensitivity to HU

The tissue colonies grown *in vitro* pass several successive stages of growth: after several days lasting latency stage they enter the period of intensive growth; its maximum falls on a period of

about 5 weeks, then the senescent phase arises. During this phase the processes of cellular syntheses undergo inhibition, though an increase in fresh matter is continued (Douce, 1963, 1964; Maroti, 1966). The tissues grown on a medium containing HU do not attain any phase of maximum growth and they do not enter a senescent phase which is typical for control tissues.

An influence of HU on the dry matter content in tissues under examination is rather insignificant; it increases at higher concentrations of inhibitor and a little more in tumour tissue (Fig. 3).

Fig. 3. Dry matter of tobacco tissues cultured in the presence of HU

Metabolic changes

Callus. The tissues growing on a medium containing HU (22.5—60 mg/l) are disguised by a lower protein content as related to 1 g of fresh matter. This decline rises with an increase in the concentration of the compound and also with age of the tissue. However it is insignificant and even in the oldest tissues (40 days), at a high HU concentration (52.5 mg/l) it does not attain 30% (Fig. 4). At lower HU concentrations, particularly in younger tissues, the changes in protein level show the same trend and of the same order as changes in the growth intensity. However, at high concentrations of inhibitor and in older tissues, the growth inhibition exceeds a degree of protein content lowering. Such a regularity maintains also if the results are calculated in relation to dry matter, but in this case the influence of HU on protein content is stronger (Fig. 5).

Fig. 4. Protein content in fresh matter of tobacco tissues at various HU concentrations and various culture duration

A considerable reduction of the incorporation of labelled leucine to protein fraction influenced by HU was observed too. In tissues treated with HU a specific activity of protein decreases and the inhibition percentage is approximate to a decrease of protein content determined under the same experimental conditions. It amounts nearly 35% (52.5 mg/l HU). HU does not influence any uptake of compounds from the medium by a tissue. A radioactivity of the fraction soluble in 80% ethanol, which is similar for all HU concentrations applied speaks for that.

At the lowest HU concentration (7.5. mg/l) producing a slight stimulation of the growth and protein level, no changes in nucleic acid contents were found (Fig. 7), at higher ones however these contents, were lower as related to both: fresh and dry matters. At the highest HU concentration (60 mg/l) RNA content in younger callus

Fig. 5. Protein content in dry matter of tobacco tissues at various HU concentrations and various culture duration

Fig. 6. HU-induced stimulation of ^{14}C -leucine incorporation to protein fraction and protein accumulation in tumorous tobacco tissue

tissues is lower as related to control by 25%, in older ones however by 35%. An inhibitory effect of HU on DNA content is the greatest one, it amounts approximately 45% (Fig. 9).

Tumour. HU activity results in an increase in the protein level in tumorous tobacco tissue (Fig. 4). With an increase in the concentration of the compound protein content rises in the tissues up to

Table 1
Incorporation of L-¹⁴C-leucine to proteins in callus tobacco tissue at various HU concentrations; 38 day old tissue

CALLUS											
HU, mg/l	0	7.5	15	22.5	30	37.5	45	52.5	60		
Subst. sol. in 80% ethanol; imp. /min/ 100 mg fr. mass	12780	12420	13000	13040	14250	13850	14720	13680	13670		
Protein, imp./min/100 mg fr. mass	7110	6750	5820	4850	4150	3720	3210	3010	2680		
%	100	94.9	81.8	68.2	58.4	52.3	45.1	42.3	37.7		
Sum of imp./min/ 100 mg fr. mass	19890	19170	18820	17890	18400	17570	17930	16690	16350		
%	100	96.4	94.6	89.9	92.5	88.3	90.1	84	82.2		
Protein spec. act., imp. /min/mg	26044	25424	22707	21546	19600	18600	17540	17054	16047		
%	100	97.6	87.1	82.7	75.2	71.4	67.3	65.5	61.6		
Protein, µg/g fr. mass	2730	2655	2475	2251	2105	2000	1830	1765	1670		
%	100	97.2	90.7	82.4	77.1	73.3	67.0	64.6	61.2

Fig. 7. Effect of HU on RNA content in tobacco callus tissue at various culture stages
RNA in fresh matter (A) and in dry matter (B)

some level, then it decreases forming a curve with maximum peak. In 30 days tissues the protein peak occurs at 175 mg/l of HU giving a value higher than that of control by nearly 45%; in older tissues this peak is lower and it shifts towards a lower HU concentration. Any decrease below a control value of protein level has not been found in the experimental variants containing HU. It deals with the results related to both: fresh and dry matter, though in the latter case HU-induced stimulation of protein is insignificant (Fig. 5). The experiments with ^{14}C -leucine have shown that its incorporation to protein fraction influenced by HU increases considerably (Table 2). A specific radioactivity of tumourous tissue protein is stimulated at 50, 75 and 100 mg/l of HU by nearly 25% as related to control, but at higher concentrations of HU (150–175 mg/l) an inhibition can be observed. Under the same conditions protein level increases in the tissues but this increase maintains even then as the specific radioactivity falls rapidly (Fig. 6). HU does not really influence any uptake of compounds from a medium by the tissue. A radioactivity of the fractions soluble in 80% ethanol is similar to that of control. It slightly decreases only at high HU concentrations (Table 2).

An influence of HU on nucleic acids in tumourous tissue is revealed by their content decrease (Fig. 8). However in younger tissues low

Table 2
Incorporation of L-¹⁴C-leucine to proteins in tumourous tobacco tissue at various HU concentrations; 38 day old tissue

TUMOUR									
HU, mg/l	0	50	75	100	125	150	175		
Subst. sol. in 80% ethanol; imp./min/100 mg fr. mass	25830	25100	25663	22270	22600	22250	22860		
Protein, imp./min/100 mg fr. mass %	7204 100	10346 143.6	11894 165.1	12324 171.0	10597 147.1	8308 115.1	6442 89.4		
Sum of imp./min/100 mg fr. mass %	33034 100	35446 107.3	37557 113.7	34594 104.7	33197 100.5	30558 92.5	29302 88.7		
Protein spec. act., imp./min/mg %	15674 100	19088 121.8	20366 130.0	19782 126.1	17302 110.3	14177 90.4	11586 73.9		
Protein, µg/g fr. mass %	4596 100	5420 120.1	5839 127.0	6231 135.6	6125 133.3	5860 127.5	5786 121.0

Fig. 8. Effect of HU on RNA content in tobacco tumour tissue at various culture stages

Explanations as in fig. 7

concentrations of HU (25 and 50 mg/l) induce some increase in RNA content (several per cent) which declines with the culture ageing. Under the same conditions DNA contents are approximate to control (Fig. 9). Maximum RNA decrease as related to 1 g of fresh tissue weight is 33%; in relation to dry weight it is a little higher (Fig. 8B). DNA decrease is the most distinct; at the highest HU concentration (200 mg/l) it attains 33–46% depending on the age of tissue (Fig. 9).

HU activity effects depending on the tissue type

Comparing the effects of HU activity on both types of tobacco tissue: tumorous and "normal" ones the following statements can be made.

Low concentration of HU (7.5 mg/l) produces in the callus tissue a slight stimulation of growth and protein level, and the lack of appreciable changes in RNA and DNA contents. This effect is perceptible in the earlier phase of culture but it declines with time. In tumour tissue (25 and 50 mg HU) any stimulation of growth does not occur. On the contrary the growth of tissue is inhibited but protein biosynthesis is advanced, RNA content also increases.

Fig. 9. DNA content in callus and tumorous tobacco tissues at various HU concentrations and various culture duration

Higher concentrations of HU markedly inhibit the growth of both tissues. At a comparable inhibition degree of their growth HU induces a parallel decrease of protein and nucleic acids in the callus tissue (Fig. 10). In the case of DNA and successively RNA and protein a degree of inhibition is the highest one. Therefore it could be supposed that a decrease of RNA and protein contents and the growth inhibition are secondary effects of DNA level decrease.

In tumorous tissue however at a reduced level of nucleic acids an increase in the level of protein is observed (Fig. 11). This fact could be an effect of both: increased protein synthesis and their degradation inhibition. The results presented show that both events occur here. An increase in the specific activity of protein labelled with a radioactive leucine (Table 2) which, at the concentration of HU 50 mg/l, coincides with some increase in RNA level attests to an increased synthesis (Fig. 8). A high protein level maintaining at decreased protein synthesis points to the inhibition of its degradation (Fig. 5).

Fig. 10. Convergence of changes in DNA, RNA and protein levels induced by HU in tobacco callus tissue

Fig. 11. Disproportionate changes in DNA, RNA and protein levels induced by HU in tumorous tobacco tissue

DISCUSSION

Our previous investigation has shown that HU induces in a tumorous tobacco tissue a great increase in the protein nitrogen content at simultaneous decrease of the nucleic acids fraction nitrogen (Maciejewska-Potapczykowa et al., 1970). In the light of fun-

damental rules of molecular biology these results could be doubtful. However Bilecka (1971) basing on autoradiographic examination of the same tissue has found a considerable, HU-stimulated, ^3H -uridine incorporation to nuclei at an equally strong inhibition of tritiated thymidine incorporation. An increase in the intensity of RNA synthesis found in this way could justify HU-induced increase in protein level, but simultaneous decrease of nitrogen content in the nucleic acids fraction still remains unexplained.

Therefore on purpose to verify these facts other analytical methods and other tobacco tissues, differing from those previously used in the rate of metabolic processes and much more intensive growth, were applied in the present work. This resulted in the necessity of verifying the range of HU concentrations needed to obtain a definite degree of inhibition.

Observations of everlasting influence of HU on the growth allowed to estimate a range of tissues' tolerance of this compound and to seize a picture of changes rising gradually in successive phases of the growth of colonies. Taking into consideration the dynamics of metabolic transformations in tissues during a whole passage duration and a specific character of HU activity, it seems that the analysis performed in only one term of growth gives incomplete results leading to erroneous conclusions.

If 39 days tissues will be assumed as a basis of HU activity estimation and following the results presented here it can be stated, that HU (proportionally to its concentration) causes an inhibition of growth, RNA and DNA contents lowering in both tissues. In the case of DNA this decrease is more clear. The only difference between both tissue types deals with proteins. In the callus tissue HU-induced changes in nucleic acids contents are followed by changes in the level and biosynthesis of protein of the same direction but at a little slower rate.

HU acted similarly on isolated cucumber cotyledons strongly inhibiting the incorporation of ^{14}C -leucine to proteins (Rennert, Knypl, 1971). Also in HU-treated *Euglena* an intensity of DNA synthesis lowered first of all and then that of RNA and protein (Buetow, Mego 1967). In tumourous tissue however, at nearly all HU concentrations the protein content is higher than in control though the contents of both nucleic acids decrease. This confirms entirely the results of our previous work (Maciejewska-Potapczykowa et al. 1970). Such a situation could be in part explained by HU-blocking processes of protein degradation (see graphs 3 and 5), such a conclusion is drawn also by Bilecka (1975b); it is however more difficult to give reasons for the stimulation of protein biosynthesis.

But if younger tissues (28—34 days) will be assumed as a basis of HU activity estimation, then any disagreement in trends of changes

in RNA and protein levels does not occur. An increase in protein content is compensated — at least in part — by an increase in RNA content (25 and 50 mg HU/l — Fig. 8)). This is in accordance with the results of Bilecka (1971, 1975 a) who found that HU (10 and 100 mg/l) produces an increase in the number of nuclei incorporating ^3H -uridine in the tobacco tumour tissue.

Thus it seems that if HU — as was found — stimulates protein biosynthesis then at the lowest HU concentrations this synthesis is conditioned on the earlier initiated increase in RNA synthesis. However this stimulation is insignificant (Fig. 8) and it declines in older tissues because an extension of time of HU acting exerts the same influence as an increase in its concentration. This can be clearly seen in graph 4: the longer is a time of exposition to HU the lower concentration is sufficient to evoke the same effect. Therefore the symptoms of HU-induced stimulation disappear with time; on the other hand the inhibition increases. Presumably for this reason Bilecka (1975 b), examining exclusively the older tissues, did not find any HU-stimulated incorporation of ^{14}C -leucine to nuclei and cytoplasm of tumourous tissue, but she has shown a deep stimulation of the synthesis of arginine-rich proteins.

However at two higher successive HU-concentrations: 100 and 125 mg/l an increased activity of protein biosynthesis still remains unchanged though RNA level markedly decreases. The causes of such a situation could be various.

1. This could be a result of the retarded processes of protein synthesis as related to RNA synthesis. Our several years lasting observations of tobacco and sunflower tissue cultures really show that at an early phase of the tissue growth, RNA synthesis increases faster than that of protein, at the later growth stages however protein synthesis prevails RNA synthesis. This is also consistent with the data of another authors (Douce 1963, 1964; Maróti 1966, 1971). Thus if the influence of HU on protein synthesis is only a contributory effects so a decrease of RNA level should involve an inhibition of protein synthesis but with some delay or — in practice — at a higher HU concentration. In tumourous tissue this delay is rather significant because during an initial phase of its activity HU promoted protein biosynthesis in this tissue. A distinct inhibition appeared only at HU concentration of 175 mg/l (Table 2, Fig. 6). Similarly in the experiments with *E. coli* (Rosenkranz, Levy, 1965; Rosenkranz et al., 1966) and *Euglena gracilis* (Buetow, Mego, 1967), an inhibition of protein synthesis required higher HU concentrations than that of RNA.

2. Maybe under the influence of HU in tumourous tobacco tissue the synthesis of a total RNA pool decreases but m-RNA synthesis is continued. Its quantity is however too slight to be proved by the

way of UV absorption measurements. In the *E. coli* cells treated with HU and then incubated with ^{14}C -uracil for 30 seconds Rosenkranz et al., (1966) observed even some stimulation of m-RNA synthesis. HU induced also an increase in β -galactosidase amount depending on the synthesis of specific m-RNA.

3. It is also possible that the protein synthesised in the presence of HU is controlled by a longer-lived m-RNA. In this situation HU could provide for a change of properties of m-RNA produced by the cell or the cells possess some pool of "stable" m-RNA which reveals its activity in the presence of HU. There are some data that the conformation and lifetime of bacterial m-RNA could be changed under the influence of various factors (Schumacher, Ehrling, 1975; Blum, 1974). In higher organisms however: animals and plants, whose m-RNA is characterized by a longer lifetime than that of bacterial m-RNA, the presence of mechanisms stabilizing templates for protein synthesis was found. Due to accumulation of these templates the processes of translation in the embryos and differentiating cells can be separated in time from the moment of transcription. Apart from that it is known that definite types of cells can contain m-RNA molecules with various degrees of stability (Clement, Ursprung, 1974).

Thus, in conformity with expectations, a great decrease of DNA level in both: callus and tumour is the foreground and significant effect of HU activity on tobacco tissues. This result in a considerable growth inhibition and appearance of some secondary metabolic effects. They depend on both: the concentration of inhibitor and the age of tissue. In spite of some common features the character of these changes is clearly differentiated depending on the type of tissue. The results of Bilecka's autoradiographic examination (1975b) concerning the synthesis of proteins containing arginine and lysine also attest to that.

It would point to the presence of a different mechanism of the growth processes regulation in tumourous tissue, maybe at the translation level. It is known at present that the process of a normal, healthy cell transformation to a tumourous crown-gall cell involves the modification of genetic information (Guillé, Grisvard, 1971; Schilperoort, 1972, Schilperoort, et al., 1973). As was recently shown by Novak and Galston (1975), the nucleic acids isolated from a bean hypocotyl crown-gall tumour, infiltrated to a normal receptor tissue induced a stimulation and modification of the course of protein metabolism.

If however different effects of HU activity on the tobacco tumourous tissue are conditioned upon a specificity of the metabolism of crown-gall type, then the crown-gall tissues of other plant species should respond similarly. The experiments made with sunflower tissues con-

firm this assumption (in press). An unexpected stimulation of growth occurring in the callus tissue at low HU concentrations is surprising. This could attest to: (1) natural defensive response tending towards the elimination of slight metabolic defects in the way of total metabolic tissue activity increasing or (2) this tissue holds additional control systems involving the synthesis of DNA or its precursors.

A stimulative effect of low concentrations of some metabolic inhibitors and antibiotics on the growth of various plant tissues has already been observed (Tso, Burk, 1962; Woodstock, Brown, 1963; Schaeffer, Sorokin, 1966; Solovei, 1974). However the growth stimulation of tobacco callus found in the present work is insignificant and a supposition arises that this stimulation deals exclusively with cells enlargement but not with their mitotic activity. This possibility could be examined by cytological methods.

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Metaboliczne aspekty wzrostu tkanki tumora crown-gall, traktowanej hydroksymocznikiem w hodowli in vitro I. Nicotiana tabacum

Streszczenie

Badano wpływ HU na wzrost, zawartość DNA, RNA i syntezę białek w kulturze tkanki tumora bakteryjnego tytoniu. Układ odniesienia stanowiła homologiczna tkanka kalusowa. Efekty działania HU zależą wyraźnie od trzech czynników: stężenia inhibitora, wieku tkanki oraz typu tkanki. Przy niskich stężeniach HU (7,5 mg/l) obserwowano w młodych tkankach kalusowych pewną stymulację wzrostu i poziomu białek oraz brak uchwytynych zmian w poziomie kwasów nukleinowych. W tkance tumorowej natomiast (25 i 50 mg/l HU), brak jest stymulacji wzrostu, przeciwnie, tkanka rośnie wolniej, lecz biosynteza białek ulega przyspieszeniu, a w tkankach młodych wzrasta również poziom RNA. Wyższe stężenia HU silnie hamują wzrost obu badanych tkanek. W kalusie inhibicja wzrostu przebiega z równoległym obniżeniem się poziomu DNA, RNA i syntezy białek. W tkance tumorowej natomiast ilość kwasów nukleinowych spada, lecz zawartość białek jest zwiększona.

Wyniki wskazują, że w tkance tumora HU powoduje z jednej strony wzmocnienie procesów syntezy, z drugiej zahamowanie degradacji związków białkowych.

Zmiany indukowane przez HU w tkance tumora crown-gall mogą odzwierciedlać specyficzny mechanizm regulacji procesów wzrostu u tej tkanki.