ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVIII, nr 2: 131—145
1979

Nucleic acids synthesis in normal and tumorous tobacco calluses treated with hydroxyurea *in vitro*

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(Received: March 10, 1979)

Abstract

In normal and tumorous tobacco calluses treated with hydroxyurea RNA synthesis was investigated with the use of $NaH_2{}^{32}PO_4$ as precursor and the method of fractionation on MAK columns. Low inhibitor concentrations show a specific action, and the influence of this compound on the RNA of the two tissues is different. In tumour tissue the population of the rapidly labelled RNA localized in region IV of the elution profiles exhibits remarkable resistance to hydroxyurea (HU). Moreover, disturbances of equilibrium between synthesis and degregation of heavier RNA, indicating inhibition of its degradation, are observed.

INTRODUCTION

Previously the susceptibility of the tissue of a bacterial tobacco tumour and of homologous normal tissue to the action of HU had been tested. Although these tissues reacted to different ranges of inhibitor concentration, the foremost effect of its action in both systems was the depression of the DNA level and of growth rate. In the callus tissue a proportional fall of the protein level and inhibition of their synthesis measured by ^{14}C-leucine incorporation was noted. On the other hand, in the tumour HU accelerated proteins biosynthesis with simultaneous inhibition of the process of their degradation. This led to a considerable accumulation of proteins in this tissue, which could be observed in a rather wide range of HU concentrations (Rennert, 1977a). Similar results were obtained for tumorous and normal calluses of sunflower (Rennert, 1977b). It is believed that the different reaction of tissue of crown-gall type to HU is the result of a specific modification of the course of the processes of protein and RNA biosynthesis.

According to the data of Manse (1974) concerning the action of cycloheximide on *Vinca rosea* cells, the state of transformation of crown-gall is, at least partly, the result of a change in the system of cytoplasmic proteins synthesis. The results of Börner (1969, 1971) and Srivastava (1968) indirectly also point to a modification of the processes of cytoplasmic ribosome synthesis and their RNA in crown-gall tumour tissues. The above named authors found changes in the nucleotide composition of the ribosomal RNA fraction in the transformed cells as compared with normal ones in *Vicia faba* and *Nicotiana tabacum*.

Earlier investigations (Rennert, 1977a) suggested that protein synthesis induced by HU in tumorous tobacco tissue occurs with a decreasing RNA amount, this indicating that it is independent of the synthesis of the latter.

The present study was undertaken to compare the HU action on the course of RNA synthesis in tumorous and normal tobacco calluses. Considering the characteristic influence of this inhibitor on protein synthesis in tumour tissue particular attention was paid to ribosomal RNA.

MATERIAL AND METHODS

Tissue cultures. Strains of normal and of tumorous calluses of *Nicotiana tabacum* L. cultivar White Burley of many years culture were used. The culture conditions on standard media and media supplemented with HU have been described in an earlier paper (Rennert, 1977a).

^{32}P-orthophosphate incorporation into RNA. After a definite time of culture 6-10-g portions of tissues freed remains of the medium were placed in 20 cm^3 of sterile basic medium from which agar and KH_2PO_4 were excluded. This medium contained 10 μCi/cm^3 of sterile $NaH_2{}^{32}PO_4$. Incubation lasted 2 h with continuous shaking under illumination at 26°C. Then the tissues were washed repeatedly with cold water and sterile basic medium, dried on filter paper and frozen on dry ice.

RNA extraction. Nucleic acids were extracted by the detergent-phenol method used by Cherry et al. (1965). The final pellet was dissolved in 10 cm^3 SSC (0.15 M NaCl + 0.015 M Na citrate), then 15 cm^3 of 0.05 M phosphate buffer containing 0.4 M NaCl, pH 6.8 were added. The extracts thus prepared were used immediately for chromatography on MAK columns. The particular extracts of control tissues contained 2—3.0 mg RNA, in dependence on the type of tissue. This constituted

about 70 per cent of the RNA obtained from the same tissues by the modified method of Schneider (Rennert, 1977). The OD_{280}/OD_{260} ratio of the preparations obtained was about 0.4.

MAK Chromatography. The columns (1.8 × 25 cm) were prepared by a simplified method (Nonoyama, Ikeda, 1964) with the use of Celit 545. The columns were washed with 150 cm^3 of phosphate buffer (pH 6.8) with 0.2 M NaCl and the RNA extracts were placed on them. The unbound components were removed with 150 cm^3 of buffered 0.2 M NaCl solution. RNA was eluted at room temperature (25—27°C) with the application of a discontinuous NaCl concentration gradient for more complete separation of the particular RNA fractions (Srivastava, 1967). 50 cm^3 portions of the following solutions: 0.4, 0.6, 0.8, 0.9, 1.0, 1.2, 1.6 M NaCl were placed on the columns. Seventy fractions of 5 cm^3 were collected by an automatic fraction collecter and a drop counter. After 18—20 h, TB-RNA was eluted from the column with 100 cm^3 of 0.5 M NaOH and 20 fractions of 3.5 cm^3 were collected.

Absorption (A_{260nm}) of each fraction was measured spectrophotometrically. For radioactivity measurements 1 cm^3 of the eluate was taken from each sample, placed on an aluminium plate, dried (60°C) and the number of impulses was read on an NZO-615 counter.

The results were well repeatable as regards separation. The numerical data of the particular experiments are means from 2—3 series of determinations.

In experiments with the isotope fraction I exhibited a very high radioactivity which could only partly be removed by dialysis. This may have been due to the presence in this fraction of nonspecific components which are found in tissue cultures from agar media. These contaminations consist of polysaccharide material (Srivastava, 1967) and also inositol hexaphosphate which becomes strongly labelled when ^{32}P is used and interferes with interpretation of the elution profile from MAK columns (Beisenherz, 1972). Therefore, in the present paper the radioactivity peak representing fraction I was disregarded.

RESULTS

RNA separation on MAK columns

The profiles of optic density (OD) of eluates from the MAK columns under the conditions here described are shown in diagrams (Fig. 1, 2).

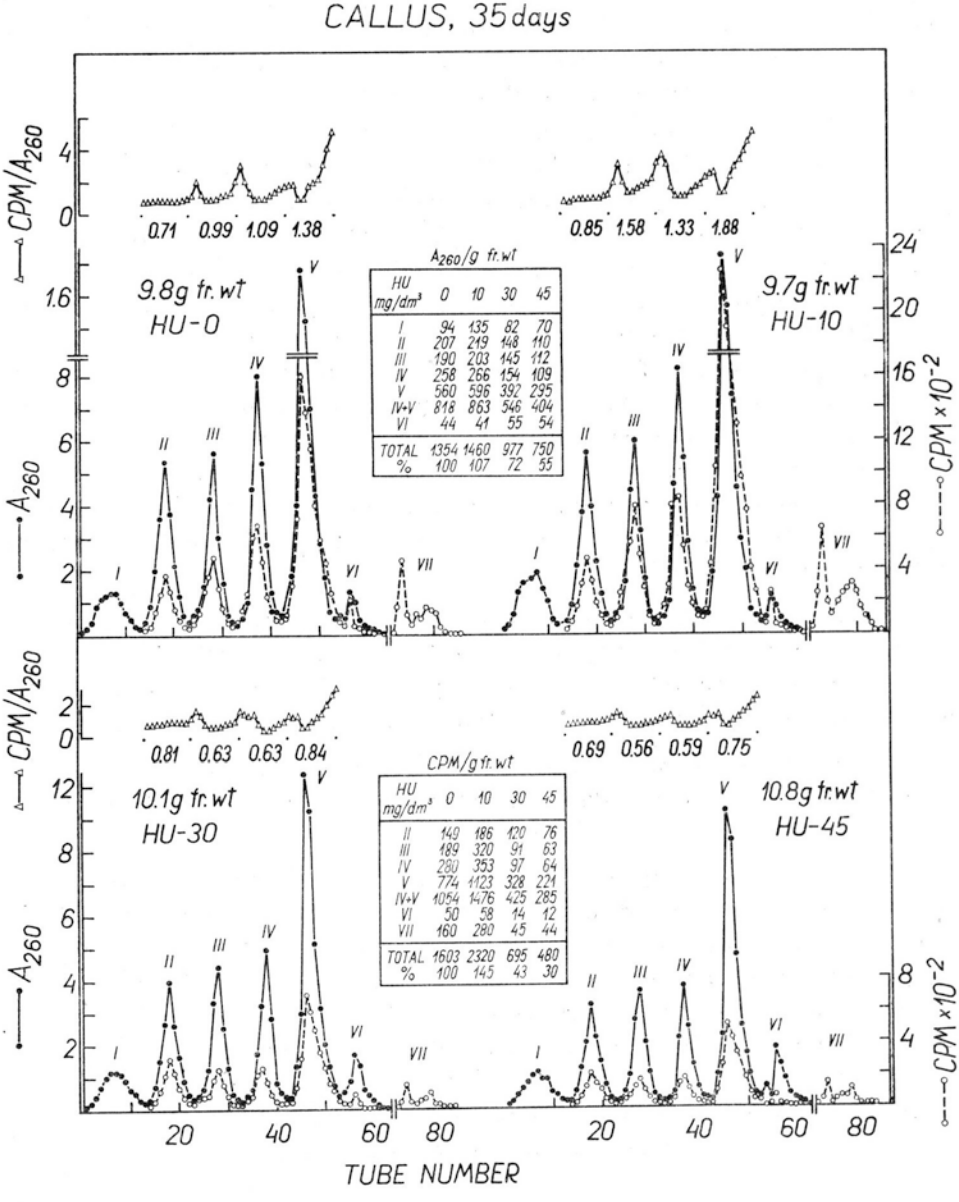

Fig. 1. MAK chromatograms of total RNA of *Nicotiana tabacum* (cv. White Burley) tissue cultures

1 — normal tissue, *2* — tumour, after 2 h incubation in 10 μCi ^{32}P-orthophosphate/cm^3. RNA extracted by the detergent-phenol method and eluted from column by successive NaCl portions (0.4, 0.6, 0.8, 0.9, 1.0, 1.2, and 1.6 M) in phosphate buffer (0.05 M, pH 6.8), 5-ml fractions were collected. At each main profile (A_{260} and CPM) the amount of tissue mass taken for analysis and the HU concentration in mg/dm^3 in the growth medium are given. Above the main profiles the specific activity curves (CPM/A_{260}) of RNA are shown. The figures under them represent mean values of specific activity of whole RNA populations: 5S, DNA-RNA, 18S and 25S. The figures in the small tables denote the OD_{260} sum or the sum of impulses/min/ml of the succesive RNA fractions converted to tissue mass unit. The remaining details in the text (Methods).

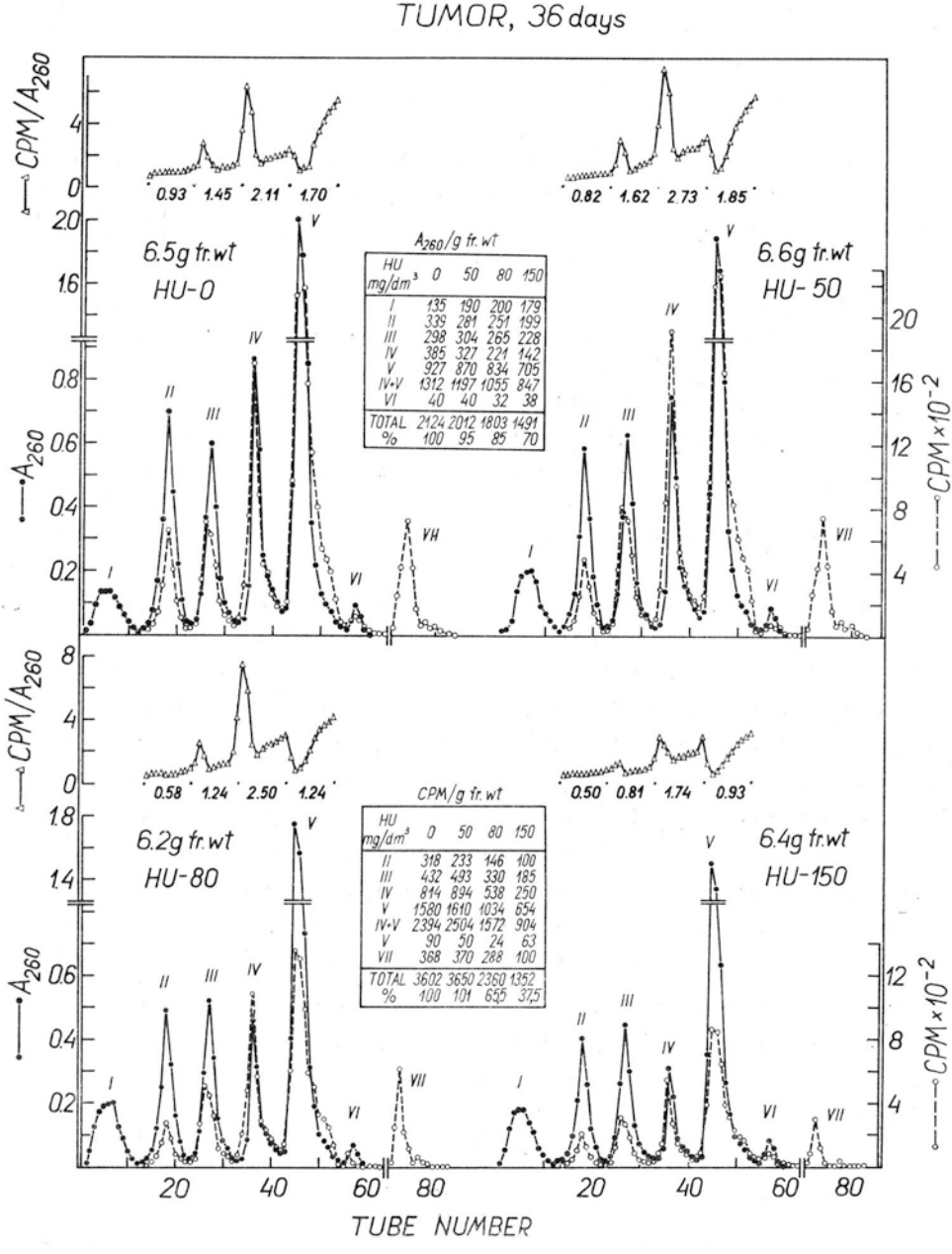

Fig. 2. MAK chromatograms of total RNA of *Nicotiana tabacum* (cv. White Burley) tissue cultures.

(Explanations as in Fig. 1)

Among the fractions eluted with salt, six well separated absorption peaks were obtained: five typical identified in the plant material as 4S and 5S RNA (I, II), the DNA-RNA complex (III) and lighter and

heavier ribosomal RNA (18S, 25S, IV, V). The small fraction VI eluted after rRNA is some closer not identified RNA. A similar one was obtained by Srivastava (1967) when using a discontinuous NaCl concentration gradient for separation of RNA from tobacco pith tissue and barley leaves. This RNA constituted in this material several per cent of the total amount. In the present study this fraction amounted to 2—3 per cent and is of no major importance in the interpretation of the results for the remaining fractions.

In the profiles of RNA synthesised during 2-h incubation with ^{32}P-orthophosphate the radioactivity peaks coincide with those of absorption. In this type of gradient it is difficult to distinguish the fraction defined as mRNA or D-RNA occurring usually directly after rRNA. In the presented profiles a slight shoulder on the right side of the heavier rRNA radioactivity peak can, however, bee seen. Calculations point to a distinct increase in specific radioactivity in this part of the fraction (figs 1 and 2, curves CPM/A_{260}). Increase in specific radioactivity is also observed in the region of fraction III. This is connected with the presence of the rapidly labelled DNA-RNA complex frequently occurring in plant material (Cherry, 1964; Julien, Guitton, 1972). The increased specific radioactivity in the light rRNA region indicates that this fraction is not homogeneous and contains a specific class of rapidly-labelling RNA. The latter which starts elution immediately preceding ribosomal RNA from tobacco tissue cultures has been described by Sittert et al. (1975). The last fraction (VII) consists of RNA thightly bound to the column (TB-RNA) eluted with NaOH. Two subfractions can be distinguished in it denoted as α and β (Sittert et al., 1975). In the tumorous tissue RNA the β subfraction is hardly noticeable while subfraction α is more pronounced.

The rather low per cent of TB-RNA radioactivity is due to the kind of precursor applied. Because of the high AMP content, this fraction is labelled more strongly when the label is derived from ^{3}H-adenosine, and weakly when ^{3}H-uridine or ^{32}P-orthophosphate is the precursor (Sittert et al., 1975).

Influence of HU on the optical density and radioactivity (CPM) of the RNA fractions

Callus. At low HU concentration (10 mg/dm^3) the OD of the sum of fractions is slightly higher than the control value (Table in Fig. 1). This is mainly the result of the increase in the amount of soluble RNA in fraction I (44%). At this HU concentration ^{32}P incorporation into all fractions is stimulated. TB-RNA and DNA-RNA activity

increases most (75% and 70%, respectively), while ribosomal RNA-s jointly increases by about 40 per cent. Higher HU concentrations (30 and 45 mg/dm^3) produce in normal tissue a considerable progressive depression of the radioactivity peaks, and in a somewhat smaller degree of the absorption peaks. The amount of ribosomal RNA decreases relatively most, whereas ^{32}P-incorporation inhibition in the particular RNA populations is enhanced with the increase of the NaCl ionic strength at which they are eluted (Table in Fig. 1).

T u m o u r. At low HU concentration (50 mg/dm^3) absorption of the sum of fractions is very similar to the control value, however, the amount of soluble RNA (fraction I) increases (40%) and ribosomal RNA decreases (ca. 10%). The total amount of the label incorporated into the RNA is the same as in the control. On the other hand, within the fraction, labelling was more pronounced in the DNA-RNA and lighter rRNA fraction and weaker in 5S RNA and fraction VI of RNA.

The total decrease of absorption in the UV of RNA preparations from tumourous tissue observed at higher HU concentrations (80 and 150 mg/dm^3) is not very drastic (15 and 30%). The OD peaks of low molecular weight RNA of fraction I remain the whole time at a level higher than that of the control, whereas the amount of ribosomal RNA-s diminishes, particularly of the light one and somewhat less of DNA-RNA. These changes are accompanied by an increased reduction of radioactive precursor incorporation into the RNA of all fractions. At an intermediate HU concentration (80 mg/dm^3) TB-RNA and DNA--RNA radioactivity diminished much less than in the remaining RNA fractions.

Percentual share of OD and radioactivity of various fractions in total RNA

The influence of HU on nucleic acids of tobacco tissues is manifested by changes in the quantitative proportions between the particular species of RNA (Tables 1 and 2). For both tissues and all inhibitor concentrations applied an increased share of sRNA I and DNA-RNA OD and a decrease of the share of rRNA OD in total RNA OD is characteristic. In callus tissue this is associated with changes in ^{32}P distribution running along the same lines (Table 1). In the tumour on the other hand, the contribution of the label incorporated into rRNA is higher or equal to that in the control (Table 2). Moreover, in tumour tissue a disturbance of the quantitative equilibrium is observed between ribosomal light and heavy RNA. The decrease of the percentual

share of rRNA OD concerns exclusively the lighter fraction. It is this fraction of the tumour that, at low HU concentration (50 mg/dm³) the amount of incorporated precursor increases and the share of its radioactivity also increases.

Table 1

Share of particular fractions in total RNA of normal tobacco callus at various hydroxyurea concentrations

HU mg/dm³ / Fraction	0	10	30	45	0	10	30	45
	Optical density				Radioactivity			
I	7.0	9.2	8.4	9.3	—	—	—	—
II	15.3	15.0	15.2	14.7	9.3	8.0	17.3	15.8
III	14.0	13.9	14.8	14.9	11.8	13.8	13.1	13.1
IV	19.0	18.2	15.9	14.5	17.5	15.2	13.9	13.3
V	41.4	40.8	40.1	39.3	48.3	48.4	47.2	46.0
IV+V	60.4	59.0	55.9	53.9	65.8	63.6	61.1	59.3
VI	3.3	2.8	5.6	7.2	3.1	2.5	2.0	2.5
VII	—	—	—	—	10.0	12.1	6.5	9.2
Sum of fractions	100	100	100	100	100	100	100	100
Total RNA	1354	1460	977	750	1603	2320	695	480

Table 2

Share of particular fractions in total RNA of tumorous tobacco callus at various hydroxyurea concentrations

HU, mg/dm³ / Fraction	0	50	80	150	0	50	80	150
	Optical density				Radioactivity			
I	6.4	9.4	11.1	12.0	—	—	—	—
II	16.0	14.0	13.9	13.3	8.8	6.4	6.2	7.4
III	14.0	15.1	14.8	15.3	12.0	13.5	14.0	13.7
IV	18.1	16.2	12.2	9.5	22.6	24.5	22.8	18.5
V	43.6	43.3	46.2	47.2	43.8	44.1	43.8	48.3
IV+V	61.7	59.5	58.5	56.8	66.4	68.6	66.6	66.8
VI	1.9	2.0	1.8	2.6	2.5	1.4	1.0	4.6
VII	—	—	—	—	10.2	10.1	12.2	7.4
Sum of fractions	100	100	100	100	100	100	100	100
Total RNA	2123	2012	1803	1491	3602	3650	2360	1352

Influence of HU on specific radioactivity of the RNA fractions

Numerical data representing the specific radioactivity of the total fractions (successively: 5S, DNA-RNA, light rRNA, heavy rRNA) lie below the curves of specific activity (CPM/A_{260}) (Figs. 1 and 2). If we compare both control tobacco tissues (HU O, Figs. 1 and 2), it is seen

that the highest specific radioactivity appears in the region of heavier rRNA of callus and the lighter rRNA of the tumour. This relation persists also in the experiments with HU. Low concentration of this inhibitor (10 mg/dm^3) evokes in normal tissue rise of the specific activity of all four RNA fractions: the stimulation is strongest in DNA--RNA and heavier rRNA. At higher concentrations (30—45 mg/dm^3) HU strongly inhibits the activity of all RNA fractions in this tissue: the characteristic peaks on the specific radioactivity curves disappear gradually. In tumour tissue at low HU concentrations (50 mg/dm^3) specific DNA-RNA and heavy rRNA activity increases slightly, the increase is larger in the lighter rRNA. Stimulation of activity associated with the region of lighter rRNA persists at higher HU concentration (80 mg/dm^3), at which specific radioactivity of the remaining fractions diminishes considerably. Synthesis of this particular RNA fraction begins to decline only when HU concentration reaches 150 mg/dm^3, and specific radioactivity of the remaining regions is about two times lower as compared with that in the control.

RECAPITULATION AND DISCUSSION

HU in higher concentrations (above 10 and 50 mg/dm^3 respectively, for callus and tumour) inhibits biosynthesis of all RNA fractions (II—VII) of both the studied tissues; simultaneously the level of these macromolecules diminishes. Inhibition deepens with the increase of inhibitor concentration. This is in agreement with the results of chemical analysis of the RNA level (Rennert, 1977a).

Moreover, in both tissues an increase in the percentual share of radioactivity incorporated into the DNA-RNA fraction is observed under the influence of HU, but independently of its concentration. This may be the reflection of a tendency to maintain such a metabolic activity of the cells which is not directly associated with DNA replication (Barlow, 1969; Rennert, Knypl, 1972). The realization of this tendency and degree of the intensity of metabolic activity seem to be dependent on the activity of HU and the metabolic characteristics of the given tissue. In lower concentrations (10 and 50 mg/dm^3, respectively for callus and tumour) the influence of HU on RNA synthesis in the two types of tissue is different. In normal tissue a certain accumulation of low molecular weight RNA is observed together with an enhancement of synthesis mainly of TB-RNA and DNA-RNA as well as of the fraction localized in the region of heavy rRNA. These changes seem to indicate the mechanism of growth stimulation evoked by subthreshold HU concentration in "normal" tissue cultures (Rennert, 1977a and b).

The occurrence of rapidly labelled DNA-RNA complexes has been observed many times in plant tissues. According to Julien and Guitton (1972), synthesis of this hybrid occurs independently of that of the bulk of cellular DNA and is not coupled with cell division. This stresses the metabolic character of the DNA of the complex and its role in cell enlargement.

It is difficult to establish which part of the newly formed ^{32}P-RNA in region V represents ribosomal RNA alone. On the basis of the generally accepted view concerning the slow turnover rate of this species of RNA, it may be supposed that simulation of synthesis of this fraction, evoked by low HU concentration, involves also other rapidly-labelling RNA population.

In the total population of rapidly labelled, rich in AMP RNA-s (D-RNA), the RNA eluted from the MAK column with salt and the RNA bound to the column and non-removable with salt solutions (TB-RNA) can be distinguished. Both these classes of RNA seem to be polydispersional and differ by their half-life (Key, 1972). The radioactive component localized in the rRNA region and immediately beyond it may contain mRNA (Wollgiehn and Munsche, 1970; Munsche and Wollgiehn, 1970; Key 1972) and so may the high molecular weight rRNA precursor (Beisenherz, 1972; Seitz and Seitz, 1972). On the basis of investigations of RNA from *N. tabacum* callus cultures Sittert et al. (1975) suggest, however, that the D-RNA eluted with salt is heterogeneous nuclear RNA, but not mRNA. According to these authors, the molecules which might represent mRNA are present in TB-RNA. The radioactivity of this fraction in the callus at low HU concentration is stimulated most (Table in Fig. 1; HU 10 mg/dm^3). It seems, therefore, that the stimulating effect of low HU concentration on normal callus starts by intensified mRNA transcription processes and an increase in the sRNA pool, leading to stimulation of ribosomal RNA synthesis. This not very high but harmonious metabolic activation of "normal" cells may involve their enlargement with normally occurring divisions or their slight enhancement. It is believed that this is the natural defence reaction of the system and that it represents the phenomenon repeatedly observed when minimal concentrations of various inhibitors were used (Ivanova, 1966). When, however, HU concentration exceeds the threshold value, the DNA level and the tissue mass increase start to fall, then synthesis of all RNA populations also declines, beginning with mRNA. Thus, in normal tabacco callus, the course of RNA (and protein — Rennert, 1977a) synthesis seems rather strictly correlated with DNA synthesis and the tissue growth rate.

In tumorous tissue treated with a low HU concentration (50 mg/dm^3) total absorption and radioactivity of RNA show no changes; on the

other hand, the quantitative proportions between the particular fractions undergo certain changes. The increase of specific activity and depression of the ribosomal RNA level are evidence of a certain activation of the metabolism of cytoplasmic ribosome RNA, both as regards synthesis and degradation. This coincides with the earlier observed stimulation of protein biosynthesis (Rennert, 1977a). It is possible, however, that this may occur independently of synthesis of new RNA, since at a 80 mg/dm^3 HU concentration the radioactivity of all RNA fractions decreases. Both HU concentrations (50 and 80 mg/dm^3) at which protein biosynthesis stimulation occurred, inhibit the tissue mass increment and the DNA level also decreases in this tissue. This situation may be compared to the phenomenon of unbalanced growth evoked by HU in HeLa cells. Owing to the inhibited DNA synthesis, these cells completely ceased to divide, their volume, however, increased considerably; the amount of RNA and proteins also increased proportionally to the volume. Puromycin and actinomycin D application demonstrated that the unbalanced growth of HeLa cells depends on protein synthesis and may occur after RNA synthesis inhibition by actinomycin D (Cohen, Studziński, 1976).

It is, therefore, probable that the activation of ribosome RNA metabolism observed in tumour callus at the lowest HU concentration (50 mg/dm^3) is connected with the stimulation of cell enlargement, occurring in spite of the inhibition of their proliferation. This is also indicated by a certain stimulation of specific activity of the DNA-RNA complex. Synthesis of the DNA of the complex may occur in the absence of cell division, and the complex itself could play a role in the initiation of RNA synthesis for the eventual increase of the cell size (Julien, Guitton, 1972).

Considering the differences in the action of HU on normal and and tumorous tissues, two important details should be stressed.

[1] An increase of HU concentration causes in both tissues a marked inhibition of ribosomal RNA synthesis. Its accumulation also decreases at a somewhat slower rate. In both tissues the optical density of light rRNA diminishes more than that of the heavy one. In normal callus this disproportion is, however, less pronounced, whereas in tumour tissue the bulk of heavy rRNA is reduced barely to 76 per cent, while that of light one to as little as 37 per cent of the control value (Table in Fig. 2, HU 150 mg/dm^3). This indicates an inhibition of degradation processes of the heavy fraction of ribosomal RNA, and indirectly a disturbance of degradation of heavier ribosome units under the influence of HU.

If this is true, the considerable accumulation of proteins occurring in the tumour tissues at HU concentrations which already inhibit their

synthesis (Rennert, 1977a and b) may partly involve the structural proteins of ribosomes.

Comparative studies of *Partenocissus tricuspidata* cell suspensions prove that tumour cells exhibit a higher content of ribosomes bound to membranes and may produce much more membrane proteins than do normal cells (Grienenberger et al., 1976). It can be that the protein accumulation observed in tumorous callus of tobacco involves as well the proteins of the membraneous cell structures. There are grounds to suppose that the system of protein synthesis connected with the endoplasmic reticulum is more stable that the system of free polysomes (Steveninck, Steveninck, 1971). This would explain why the protein synthesis evoked by HU in the tumour may occur in the case of inhibited RNA synthesis.

[2] In tumour tissue the rapidly-labelled RNA fraction localized in region IV of the elution profiles shows a remarkable resistance to the action of HU. Its synthesis at first is enhanced (50 mg/dm^3 HU) and the high specific activity persists when the inhibitor concentration is increased (Fig. 2. CPM/A_{260} curve, HU 80 mg/dm^3). This RNA population shows the highest specific radioactivity characteristic for the investigated tumour tissue.

Table 3

Data on the action of hydroxyurea (HU) on growth, RNA and protein in tobacco tissue cultures. Results expressed as per cent of control

Tissue	Callus			Tumour		
HU, mg/dm^3	10	30	45	50	80	150
Fresh mass	112	64	40	77	65	44
Protein level *	100	80	70	115	125	133
RNA level *	100	80	68	104	86	72
OD of sum of fractions	105	72	55	95	85	70
Spec. act. of sum of RNA fractions	135	60	54	106	77	52
Spec. act. of protein *	—	75	67	122	130	90
Spec. act. of 1V RNA fraction	123	58	55	129	115	83

* — data from earlier work (Rennert, 1977a).

The degree of disturbance of the metabolic activity in tumourous and normal tobacco calluses at various inhibitor concentrations is shown in Table 3. It is difficult to explain the mechanism of induction by HU of synthesis stimulation in the particular fraction of rapidly-labelled RNA in region IV of the elution profiles. This is the only tumour RNA fraction which coincides in the rate of synthesis with the non-

typical (unphysiological) stimulation of protein synthesis, under the effect of HU in this tissue (Table 3, 50 and 80 mg/dm^3 HU). This fraction most probably represents the lighter component of the polydispersive D-RNA which among the fractions eluted with salt starts to be eluted immediately before rRNA; according to Sittert et al. (1975), D-RNA of *N. tabacum* cv. White Burley tissue culture consists of heterogeneous nuclear RNA. According to Britten and Davidson's model (1969), RNA corresponding to heterogeneous nuclear RNA mediates the processes of integration of the protein production systems controlled by repeated DNA sequences (redundant DNA).

This research was partly supported by the Section of Agricultural and Forestry Sciences of the Polish Academy of Sciences within the project MR II/7, 1.2.3.

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Przebieg syntezy kwasów nukleinowych w kalusach normalnych i tumorowych tytoniu, traktowanych hydroksymocznikiem w hodowli in vitro

Streszczenie

W normalnych i tumorowych kalusach tytoniu, traktowanych hydroksymocznikiem (HU), badano syntezę RNA stosując jako prekursor $NaH_2{}^{32}PO_4$, oraz metodę frakcjonowania RNA na kolumnach MAK. Wysokie stężenia inhibitora silnie hamują biosyntezę wszystkich frakcji RNA u obu badanych tkanek. W stężeniach niskich wpływ HU na RNA dwu typów tkanek jest zróżnicowany. W tkance kalusowej obserwuje się akumulację RNA niskocząsteczkowego oraz ogólne wzmożenie aktywności właściwej wszystkich frakcji RNA. W tkance tumorowej populacja szybko znakowanego RNA, zlokalizowana w regionie IV

profilów elucji, wykazuje znaczną oporność na działanie HU. Jej synteza początkowo wzmaga się (50 mg/dm^3), a wysoka aktywność właściwa utrzymuje się również przy zwiększeniu stężenia inhibitora. Ponadto w tkance tumorowej występuje pod wpływem HU zaburzenie równowagi między syntezą a degradacją RNA rybosomalnego ciężkiego, świadczące o przypuszczalnym zahamowaniu rozpadu cięższych podjednostek rybosomów w tej tkance.

Wyniki te rzucają światło na mechanizm odmiennej reakcji tkanki tumorowej na działanie HU, w porównaniu z tkanką prawidłową.