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Attempts at DNP isolation from callus and tumour tissues of *Nicotiana tabacum*

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Abstract:

This paper deals with a preliminary investigation of DNP originating from tumour and callus tissues of *Nicotiana tabacum*.
DNP preparations from tumour tissue contained on the average: 54,8% of protein, 38,4% of DNA and 2,9% of RNA. The N/P ratio value fluctuated within the limits 4,8—5,8. The respective mean values for DNP preparations from callus tissue were: 57,3; 21,6 and 12,1%. The N/P ratio was 4,7—6,6.

INTRODUCTION

Physiological and biochemical studies on callus and tumour tissues of *Nicotiana tabacum* var. White Burley, have been carried on in this Laboratory for many years (R e n n e r t 1965, 1968). Data concerning the deoxyribonucleoprotein complex (DNP) — an essential component in the transformation of genetic information are lacking in the biochemical characteristic of both these kinds of tissue.

The present paper describes a preliminary attempt to isolate DNP from callus and plant tumour tissues.

MATERIAL

Crown-gall tumour tissue and normal (callus) tissue of *Nicotiana tabacum* var. White Burley isolated in 1961 in the Department of Physiology and cultured on the modified White and Burley medium supplemented with growth substances (R e n n e r t 1965) were used for DNP isolation. The optimum age of cultures (4—5 weeks) for DNP isolation from

both tissue types was established on the basis of preliminary fractionation of phosphorus compounds according to Schmidt and Thanhauser (1945) with delipidation after Niemierko (1953). Tissue cultures 2, 4, 6 and 8 weeks old were tested in these experiments. In 2- and 4-week-old cultures DNAP content in callus tissue was about 12 mg% and in tumour tissue — about 30 mg%. In 6-week-old tissue cultures the DNAP content considerably decreased: 8-weeks-aged cultures, however, hardly showed any DNAP. (Maybe in this material DNA underwent degradation). The use of 2-week tissue cultures was inconvenient because of their small weight (about 0,2 g).

ANALYTICAL METHODS

a) The mineralization of substances intended for N and P determinations was performed by means of H_2SO_4 and hydrogen peroxide or by the selenium reagent according to Filipowicz et al. (1962).

b) Nitrogen was determined in a Kjeldahl apparatus modified by Skarżyński (1963). A solution containing 1 ml of a mixture prepared from 1% methylene blue (2,5 ml) and saturated H_3BO_3 was used as indicator.

c) Phosphorus was determined by the method of Bartlett (1959) modified by Filipowicz and co-workers (1960).

d) RNA content was determined by the orcinol method (Kerr, Seraidarian 1945) in samples prepared according to Kłyszejko and co-workers (1965), DNA content — by the diphenylamine method (Dische 1930) in a definite volume of the solution obtained by way of threefold namely 15-, 10-, and 5-minutes lasting extraction with hot (90° C) 5% $HClO_4$ of the precipitate remaining after washing RNA hydrolysed in alkaline medium.

e) Protein content was determined by the method of Lowry and co-workers (1951) modified by Fiszer (1964).

Isolation of DNP from callus and tumour tissues

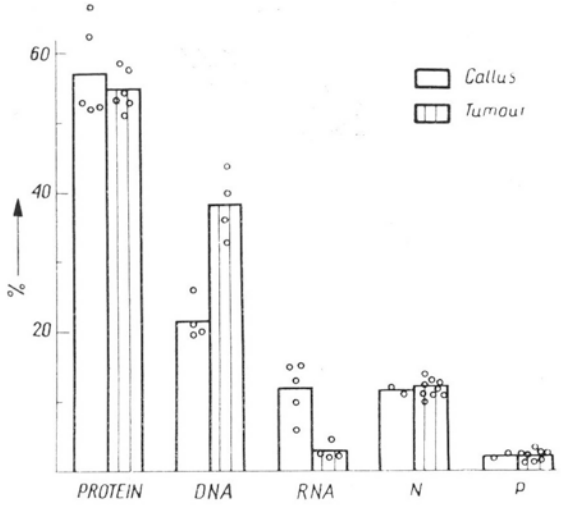
DNP was isolated immediately from the RNP-free tissue. All the manipulations were performed in a cold room with the use of an ice bath and cooled centrifuge.

a) RNP extraction. 1 g of a tissue was ground in a porcelain mortar with 0,14 M NaCl (with 0,02 M trisodium citrate added) for 10 minutes. The extract was centrifuged at 1800 r.p.m. for 10 minutes (Janetzky centrifuge K-50). This extraction was repeated three times more.

b) DNP extraction. To a residue remaining after RNP extraction from 12 successive 1-gram portions 1,5 M NaCl and 0,02 M trisodium ci-

trate was added and several hours lasting extraction with the use of a low-speed glass stirrer was performed. After centrifuging the extract (Janetzky centrifuge K-50, rotor „star", conical tubes, 3000 r.p.m. for 25 min.), DNP was precipitated from the supernatant by 9-fold diluting with ice-cold water (NaCl was diluted to 0,14 M). DNP of callus origin was precipitated in granular form, DNP from tumours, however, in the form of fibers could easily be wound around a glass rod. After threefold washing with cooled acetone, the precipitates were dried in a dessicator over $CaCl_2$ and used for chemical analysis.

Chemical analysis of DNP preparations



The results of chemical analysis of DNP preparations from callus and tumour tissues of *Nicotiana tabacum*, protein DNA, RNA, nitrogen and phosphorus contents are summarized in the diagram.

RESULTS AND DISCUSSION

The great difficulties connected with obtaining of DNP from tumour and callus tissues of *Nicotiana tabacum* should be emphasized. Our numerous preliminary preparations were unsuccessful.

We established in a series of experiments the following conditions for DNP isolation from both tissue types: 1) optimum age of tissue culture is 4—5 weeks; 2) the material should be taken from the culture room immediately before preparation; 3) the entire preparation procedure

should be carried out at approximately 0° C; 4) mild homogenization in a porcelain mortar as well as in a teflo-homogenizer; 5) the time since material taking from the tissue culture to starting of DNP extraction from 12 g of tissue should not exceed 90 minutes.

The yield of DNP preparation was low and fluctuated from 4,9 to 9,6 mg (tumour) and from 3,6 to 7,6 mg (callus) as related to 12 g of fresh tissue. These values concern seven preparations of DNP from tumorous of both DNP kinds in 0,14 M NaCl solutions should be mentioned: the DNP sediment from tumours had the form of long fibers, which were never isolated from callus (granular DNP precipitate).

Protein, DNA, RNA, nitrogen and phosphorus contents in DNP preparations were determined. The results of chemical analysis seem to confirm some findings in animal material. Recently Borchsenius and co-workers (1969) found in calf thymus 58,1% of protein, 40,0% of DNA and 1,9% of RNA. The protein content, however, in DNP of both kinds of plant tissue, was about 55%, but DNP preparations from tumour tissue contained much more DNA (approximately 38%) than those of callus origin (about 20%). The RNA percentage in DNP from plant tumours was almost in agreement with Borchsenius's data (1969), but the preparations from callus tissues contained four times more RNA. This was probably connected with the fact hat DNP of callus origin was precipitated in granular form, which has to be centrifuged. Perhaps during this procedure more contaminations were transferred to DNP of callus origin, than in the case of tumour DNP, where centrifuging was not needed.

If we take into consideration Zbarskij and Jermołajeva's (1960) data for the N/P ratio in DNP obtained from various animal tissues, being 4,1—4,3, the corresponding values obtained in this study are too high, particulary in the case of DNP preparations from callus tissue.

It should be mentioned, that we undertook some attempts to isolate DNP from the nuclei of tumour and callus cells. We tried to isolate nuclei from plant cells applying several methods worked for animal (Hogeboom, Schneider 1948; Neelin, Butler 1959; Sripati) or plant (Leish 1963; Rozijn, Tonino 1964; Vasiljev, Govsztein 1964) tissues. The technique described by Vasiljev, Govsztein (1964) seems to be the most useful for this purpose, but 0,02 M citrate buffer must be used instead of 0,01 M phosphate buffer. The yield of DNP preparations obtained in this way was, however, very low. In the case of callus tissue, DNP preparations precipitated always in granular form. The nitrogen/phosphorus ratio in these preparations fluctuated from 4,0 to 5,9. They, moreover, showed the presence of RNA. Further investigations are in progress.

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Próby izolowania DNP z tkanki kalusowej i tumorowej Nicotiana tabacum

Streszczenie

W ramach wstępnych doświadczeń otrzymano po 7 preparatów DNP pochodzących z tkanki tumorowej i kalusowej *Nicotiana tabacum*.

Preparaty DNP z tkanki tumorowej zawierały średnio 54,8% białka, 38,4% DNA oraz 2,9% RNA, wartości stosunku N/P wahały się w granicach od 4,8—5,8. W preparatach DNP pochodzenia kalusowego odpowiednie wartości średnie były następujące: 57,3; 21,6; 12,1% oraz 4,7 do 6,6 dla stosunku N/P.