Zusammenfassung. Nach Bebrütung von «Erythrocyten» mit DHEA oder DHEA Sulfatid konnte eine Abnahme des intracellulären c-AMP's festgestellt werden. Eine angenommene Aktivierung der Phosphodiesterase liess sich durch die verstärkte Hydrolyse von c-AMP zu 5'-AMP in Gegenwart von DHEA bestätigen. Auf der anderen Seite bewirkte DHEA über die Hemmung der G-6-PDH auch eine indirekte Hemmung der Phosphodiesterase, die auf einem geringeren Verbrauch inhibierenden G-6-P's beruht. Unter physiologischen Bedingungen scheint letztere Wirkung vorzuherrschen.

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Presence of Adenosine Triphosphate in Tobacco (*Nicotiana tobacum*) Tissue Grown in Nutrient Medium Containing Various Concentrations of Kinetin

Kinetin has been known to affect cell division, differentiation, and organ development[1]. Kinetin (cytokinin) has also been reported to be an inhibiting agent for the production of glycolytic kinases, hexokinase, and pyruvic kinase[2]. Furthermore, it is known that cytokinins induce RNA synthesis and consequently affect protein synthesis[1]. While logic would make one assume that ATP contents of tissues would vary according to the kinetin concentration present, this paper sets down the computed ATP contents of tissues grown in vitro with various concentrations of kinetin.

Materials and methods. Cylinders of fresh tissue were cut through the stem of *Nicotiana tobacum* var. Wisconsin 38 with a cork borer (6 mm ⌀). 5-mm segments were then cut from the cylinder. Each piece of tissue weighed 0.31 ± 0.02 g. 3 pieces of such tissue were grown in a 125 ml flask containing 50 ml nutrient agar medium. The medium contained the basic substances previously used[3]. Plant hormones in the medium included 2 mg/l of indole acetic acid and varied concentrations of kinetin. All of the flasks were placed in a growth chamber with a temperature of 27 °C and approximately 100 Lux of light.

The liquid culture was identical to the solid medium except for the agar. Flasks containing pith tissue in nutrient solution were placed on a shaker in a room kept at a constant temperature of 24 °C and approx. 350 Lux of light.

Adenosine triphosphate was extracted from the pith tissue (2 g) with 5 ml of boiling water for a period of 10 min. The extract was cooled and the ATP content was analyzed by the luciferin-luciferase method[4] and an Aminco Chem-Glow Photometer. The luciferin-luciferase was prepared from the commercial firefly lantern extract (Sigma Chemical Co., St. Louis, Mo.). The ATP standard and the N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid/magnesium buffer were also from the same source.

Percentage of increases of ATP contents or fresh weights shown by Tables I and II was calculated as:

$$\text{Percent increase} = \frac{\text{Tissue grown in vitro} - \text{Fresh pith tissue}}{\text{Fresh pith tissue}} \times 100$$

Table I. Changes of ATP content and fresh weight of tobacco pith tissue grown in vitro comparing to fresh pith

Kinetin in medium (mg/l)	Increase of fresh weight (%)	Increase of ATP content in tissue (%)
0	174	300
0.02	266	150
0.20	550	720
2.00	145	658

Each number (for either fresh weight or ATP content of tissue) was calculated from 3 pieces of pith tissue in 1 flask comparing same number of fresh pith of tobacco stem.

Table II. Changes of ATP content in tobacco pith tissue grown in liquid nutrient medium containing various concentration of kinetin

Kinetin in medium (mg/l)	Increase of ATP in pith tissue (%)	
	8 days[a]	14 days[a]
0.02	33	120
0.20	337	820
1.00	718	929

ATP content was calculated based on 1 g of tissue comparing to the fresh pith (0 day growth in medium). [a] Growth period in medium.

Results and discussion. The Figure shows the changes of ATP contents of tobacco pith tissue grown in nutrient agar. Different experiments utilized different concentrations of kinetin in the nutrient. Tissue grown in the medium containing 0.2 or 2 mg/l of kinetin produced more ATP than tissue grown on the medium without kinetin (control). Tissue grown for 6 days in medium containing 0.02 mg/l of kinetin contained much less ATP per g of fresh weight of tissue than the control. The longer growth period (14 days) made the difference even more dramatic.

Table I shows a comparison of the fresh weight and ATP contents of the tissue grown in vitro with that of pith tissue freshly cut from tobacco stem. After 14 days of growth, it can be seen that kinetin in the medium enhanced the growth, both by weight and overall size. High concentrations of kinetin (2 mg/l) retarded the growth when compared to tissue grown in the absence of kinetin. The greatest ATP content was found in tissue grown in such medium (2 mg/l).

Similar experiments were performed using liquid media. The overall growth rates of tissue in any of these media were faster than those grown on agar, probably due to

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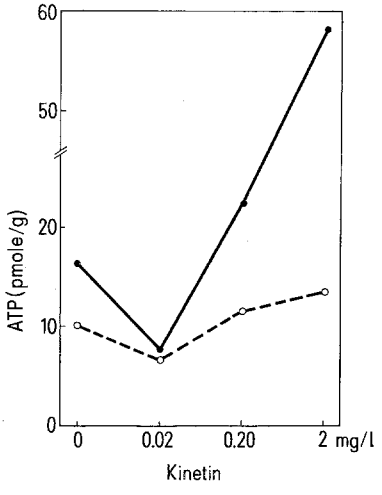
increased availability of nutrients to the tissue grown under such conditions. As can be seen in Table II, the amount of ATP content per gram of tissue increased with the increase of growth period as well as to the amount of kinetin in the liquid nutrient.

It has been reported[5,6] that various amounts of cytokinin, in combination with auxin, may affect cell division and morphogenesis of tissue grown in vitro. In our work, it was apparent that tissue produced in the 0.02 mg/l of kinetin was soft with large cells; callus produced on medium containing 2 mg/l of kinetin was compact in form and was composed of small cells.



ATP content of tobacco pith tissue grown in medium containing various concentrations of kinetin for 6 days (---) and for 14 days (—).

Different concentrations of kinetin apparently affect the rate of cell division and enlargement. Tissue on a medium containing 2 mg/l kinetin grew very little (Table I). However, as shown in both Tables I and II, the amount of ATP in this tissue was far greater than in the tissue grown on other media. A possible explanation is that ATP consumption was not high, due to lowered growth activity but that the supply of sugar was great enough for the formation of ATP itself.

It is suggested that the amount of ATP in the pith tissue grown in vitro very likely relates to the amount of kinetin in the nutrient medium and might also relate to the growth and or cell differentiation that could be influenced by the exogenous plant hormones.

Résumé. On a fait croître du tissue de la moelle du tabac (*Nicotiana tobacum*) dans l'agar nourrissant ou dans un liquide contenant des minéraux basique et des hormones végétales. La croissance du tissu médullaire se montra directement dépendante de la kinétine contenue dans le milieu. La teneur en ATP du tissu a varié. On estime que celle de la moelle dépend de la croissance et de la division cellulaire affectées par la kinétine dans le milieu nutritif.

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Glyoxalase II Activity in Tumours

The glyoxalase enzyme system, discovered independently by DAKIN and DUDLEY[1] and by NEUBERG[2] in 1913, was later shown to be a compound system of two enzymes (RACKER[3]) which catalyzes the following reaction:

$$\text{methylglyoxal} + \text{glutathione} \xrightarrow[\text{E.C 4.4.1.5}]{\text{glyoxalase I}} \text{S-lactoyl-}$$

$$\text{-glutathione} \xrightarrow[\text{E.C 3.1.2.6}]{\text{glyoxalase II}} \text{D-lactate} + \text{glutathione}$$

The role of the glyoxalases in tissues has not as yet been elucidated. Particularly noteworthy is the hypothesis formulated by SZENT-GYÖRGYI et al.[4–6] which postulates that the glyoxalases substrate, methylglyoxal, and the glyoxalases play a significant part in the mechanism of cell division. It should be stressed that methylglyoxal has also been shown to have tumour-inhibiting properties[7,8]. During investigations of the distribution of the glyoxalases in normal and cancerous tissues, when determining the activity of the whole glyoxalase system, we have observed that this is in general lower in cancerous than in normal tissues[9,10]. Earlier work on this problem showed values either lower or higher than those for normal tissues[11,12]. When employing more precise spectrophotometric methods, which allow each enzyme to be determined separately, it was found that as a rule glyoxalase II was absent in cancerous tissues or cells, while glyoxalase I activity was either reduced or was similar to that in normal tissues[9]. Absence of glyoxalase II was observed by us in several animal tumours and also in operation sections from human tumours. This fact

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