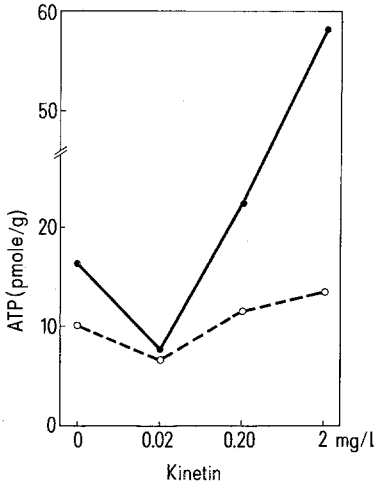
increased availability of nutrients to the tissue grown under such conditions. As can be seen in Table II, the amount of ATP content per gram of tissue increased with the increase of growth period as well as to the amount of kinetin in the liquid nutrient.

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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would appear to be of potential significance in view of the postulated role of the glyoxalase system in cell division and in cancer genesis.

*Methods.* Glyoxalase II were determined in the supernatants of homogenates prepared from normal and cancerous tissues, using the methods described in earlier works[13,14]. Normal and cancerous tissues were homogenized in 0.15 *M* KCl (1 + 5); leukaemia, sarcoma Yoshida and Ehrlich tumour cells were homogenized as described in[15]. Protein in the supernatants of the cell homogenates was determined by the spectrophotometric method[16]. Glyoxalase I was purchased from Boehringer. Glyoxalase II was obtained by molecular filtration as described earlier[13]. Methylglyoxal was obtained from glyceraldehyde[17], and S-lactoyl-glutathione (substrate for glyoxalase II) was obtained from methylglyoxal and glutathione (Merck) by the method described by WIELAND[18]. Other reagents were of analytical purity.

*Results and discussion.* Results of measurements are set out in the Table. Together with the activity of the glyoxalases in cancerous tissues and cells, several examples of values for normal tissues are given. It is worth noting that accurate tests with spectrophotometric methods on the distribution of glyoxalase II in normal tissues showed activity of this enzyme for various animals and in all types of tissue investigated, i.e. liver, brain, kidneys, spleen, heart and muscle tissue, with the exception of muscles of certain mammals, e.g. rats, where the glyoxalase II content was very small[9,13]. This universal distribution of glyoxalase is confirmed by earlier research, before the compound nature of the glyoxalase system had been demonstrated, when the reaction of the whole system was studied, that is the conversion of methylglyoxal to D-lactate. Glyoxalase activity has been ascertained throughout the whole living world[19].

From the evidence of both previous and current investigations showing high activity of glyoxalase enzymes in normal tissues, a hypothesis may be put forward that absence of glyoxalase II activity in cancerous tissues is a characteristic feature distinguishing such tissues from normal tissues. Based on a reasonably large number of our own observations, we venture to postulate that in neoplastic tissues the enzyme synthesis mechanism is impaired. It may be taken that this is not due to the presence of some enzyme inhibitor, since none of our many experiments with extracts from human and animal cancerous tissues (Fibroadenoma mammae, Carcinoma infiltrans mammae, Yoshida sarcoma, Ehrlich carcinoma, Guerin tumour, Melanoma malignum amelanoticum AL) added to glyoxalase II preparations showed any evidence that these extracts inhibit enzyme activity. The absence of methylglyoxal (substrate for glyoxalases) in neoplastic cells as reported in other papers[4], plus the absence of glyoxalase II as shown by us, provides a basis for assuming that a fundamental factor in the cancer process is a 'dislocation' of the regulating system (or one of the regulating systems ?) as envisaged in the hypothesis of SZENT-GYÖRGYI[4], EGYÜD et al.[5,6]. Our observations require confirmation on a broder range of tumours.

Activity of glyoxalase II in some tumour tissues or cells and in some normal tissues in units[a] per mg of protein

| Samples | Glyoxalase II activity[b] |
|---|---|
| Tumours: | |
| Morris hepatoma 5123 D (rat)[c] | 27 ± 4 |
| Morris hepatoma 7777 (rat)[c] | 0 |
| Kirkman-Robbins hepatoma (hamster)[d] | 12 ± 3 |
| Sarcoma 180 (mouse)[d] | 25 ± 6 |
| Sarcoma TW (hamster)[e] | 9 ± 2 |
| Guerin tumour (rat)[c] | 0 |
| Melanoma malignum (hamster)[f] | 0 |
| Melanoma malignum amelanoticum AL (hamster)[f] | 0 |
| Ehrlich carcinoma cells (mouse)[f] | 26 ± 5 |
| Yoshida sarcoma cells (rat)[d] | 0 |
| Leukemia 1210 cells (mouse)[c] | 0 |
| Carcinoma infiltrans mammae (human)[g] | 0 |
| Carcinoma hepatocellulare (human)[g] | 0 |
| Carcinoma papillare ovarii (human)[g] | 0 |
| Glioblastoma multiforme (human)[g] | 16 ± 2 |
| Fibroadenoma mammae (human)[g] | 8 ± 2 |
| Normals: | |
| Liver (human)[g] | 108 ± 4 |
| Liver (hamster) | 246 ± 21 |
| Liver (rat) | 167 ± 32 |
| Brain (human)[g] | 245 ± 47 |
| Muscle (human)[g] | 108 ± 21 |

[a] Enzyme unit: enzyme activity is expressed as 1 nmol of substrate transformed/min at 25 °C. [b] The results are given as mean ± SE of 3–5 measurements of different samples. Zero (0) means values not detectable in the experimental conditions. Sources of tumours: donor animals with tumours were kindly supplied by: [c] Institute of Oncology in Gliwice. [d] Dept. of Pathological Anatomy Medical Academy in Wrocław. [e] Dept. of Pathological Anatomy Medical Academy in Krakow. [f] Dept. Biology Silesian Medical Academy in Katowice. [g] Human tumour and normal samples were supplied (from operation sections) by Dept. of Pathomorphology Silesian Medical Academy in Katowice.

*Résumé.* Les auteurs ont constaté que l'activité de la glyoxalase II, très intense dans les tissus normaux, est au contraire très faible dans les cellules et tissus cancéreux, où elle peut même manquer complètement.

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