7,1, après lavages répétés avec NaCl 9‰. Elles sont broyées au Potter de Thomas pendant 2 min en présence de Triton X100 1‰, ce qui permet de recueillir la totalité de l'activité enzymatique dans le surnageant à 2000 g.
La proline et l'hydroxyproline cellulaires sont séparées et mesurées d'après la méthode chromatographique précédemment décrite[5]. Les protéines sont dosées selon la méthode de Lowry.
Résultats et discussion. La figure 1 montre que les cellules KB contiennent des protéines ayant le même caractère antigénique que le collagène de type I et non la fraction C q du complément à laquelle il aurait été possible d'attribuer partie ou totalité de l'hydroxyproline présente dans ces cellules.
La figure 2 indique que les cellules KB possèdent une activité spécifique prolyl hydroxylase similaire à celle des fibroblastes quand ces activités sont mesurées avec des lots semblables de substrat procollagénique. En revanche, le rapport Hyp/Pro, qui mesure la concentration du matériel intracellulaire contenant l'hydroxyproline, est bien en accord avec le rapport $\Delta C/\Delta P$ qui distingue ces 2 types de cellules et manifeste la prépondérance de la synthèse du collagène dans les fibroblastes[3].
Cette activité prolyl hydroxylase relativement élevée chez les cellules KB, dont la concentration en matériel collagénique est faible, est à rapprocher des résultats de Golberg et Green[6] ainsi que de Langness et Udenfriend[7]. Alors que l'enzyme intracellulaire est abondante, son substrat pourrait être soit synthétisé en faible quantité, soit inaccessible du fait de la compartimentation cellulaire, ou bien les conditions de milieu à l'intérieur des cellules d'origine tumorale pourraient être défavorables à la réaction enzymatique.
Le tableau complète ce caractère de disparité entre activité de la prolyl hydroxylase et synthèse du collagène dans les cellules KB en montrant qu'aux différents temps d'une subculture notés sur la figure 2, la concentration du matériel collagénique intracellulaire est correlée avec l'activité prolyl hydroxylase chez les fibroblastes mais pas chez les cellules KB. 4 paramètres pourraient rendre compte de cette différence: la perméabilité de la membrane cellulaire à la proline, extracellulaire, l'activité prolyl hydroxylase, la concentration du procollagène, la vitesse de sécrétion du matériel collagénique hydroxylé.
Conclusion. Les cellules épidermoïdes KB synthétisent du collagène bien qu'en faible quantité. L'activité de la prolyl hydroxylase, enzyme marqueur essentiel au collagène, ne semble pas ici le facteur limitant dans la synthèse du collagène. L'observation du comportement particulier de ces cellules met l'accent sur la complexité des mécanismes régulateurs de la synthèse du collagène dont l'expression quantitative est une des caractéristiques cellulaires.

Calcul d'une corrélation linéaire entre la prolyl hydroxylase et le rapport Hyp/Pro

Cellules	r	t calculé	t 0,05
Fibroblastes de peau humaine (17° P)	0,8618	3,399	2,776
Cellules KB	0,1861	0,328	3,182

La corrélation est établie pour différents temps d'une même subculture.

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Release of nonspecific esterases from the roots of *Vicia faba* L.

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Summary. Roots of *Vicia faba* L. immersed in a buffer solution were found to release esterases into the environment. The substrate α-naphthylacetate was taken up by the roots and degraded, the hydrolysis products being released back into the surrounding medium.

In a previous study, the activity of non-specific esterases in various parts of young, developing seedlings of *Vicia faba* was investigated[1]. Since a pronounced maximum of enzyme activity was found in root tips, the question arose whether the unknown natural substrates are localized within the plant itself, or, at least in part, in its soil environment. The present study was conducted to investigate some of these aspects.
Materials and methods. Seedlings of *Vicia faba* L. were grown as previously described[1]. Only 8-day-old plants were used for the experiments, since the activity of esterases in root tips was highest at this age.
Release of esterases: The roots of 3 intact plants were immersed to a depth of 1 cm in small test tubes containing 4 ml of Sörensen phosphate buffer (pH 7.2). After various time intervals, the plants were removed, and 0.1 ml of an ethanolic solution of α-naphthylacetate was added to the buffer (final concentration of α-naphthylacetate: 2.5×10^{-4} M). The mixture was then incubated at 32 °C for 10 min, and finally mixed with 0.5 ml of reagent solution (40–50 mg Diazoblue B in 5 ml of distilled water and 10 ml

Influence of substrate on esterase release from the roots

Substrate concentration	Absorbance	Replicates
0	0.0233	6
10^{-5} M	0.0316	3
$2 \cdot 10^{-5}$ M	0.025	3
10^{-4} M	0.0566	3
$2 \cdot 10^{-4}$ M	0.1266	3
10^{-3} M	0.436	3

of a 5% aqueous solution of sodium laurylsulfate). After 4 min, the absorbance of the colored solution was determined photometrically at 579 nm.

In order to measure the influence of an ester upon enzyme release, α-naphthylacetate was added to the buffer before immersion of the root tips (final concentrations of α-naphthylacetate, see table). Enzyme activities were determined after an immersion period of 10 min.

Release of hydrolysis products: 2 plants were immersed in 4 ml of buffer solution (pH 7.2) containing the substrate (final concentration: 2.5×10^{-4} M) for 10 min as described above.

The roots were then carefully washed with distilled water, and re-immersed in a fresh buffer solution without the ester. The amount of α-naphthol released was determined photometrically after various time intervals.

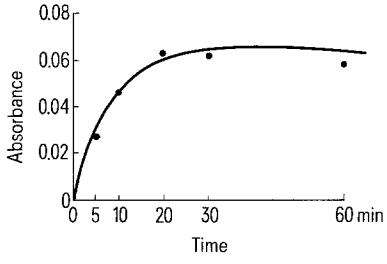
Results and discussion. Esterases were released from the root tips into the surrounding buffer, with the release being proportional to time. The absolute amount of enzymes recovered in the buffer solution, however, was relatively small (average absorbance value for a 30-min immersion period: 0.044). When the buffer solution was boiled for a few min after immersion time and then subjected to the enzyme assay, no color formation occurred. This indicates that the absorbance measured was indeed the result of an enzymatic reaction.

Another proof for the release of esterases was obtained from tests in which buffer subsamples were mixed with the substrate for increasing periods of time, which clearly demonstrated a linear relationship between absorbance, i.e. amount of ester hydrolyzed, and incubation time.

The presence of α-naphthylacetate in the buffer solution appears to stimulate esterase release (table). The 2 lowest concentrations tested did not exert any influence, but concentrations above 10^{-4} M did. The limited solubility of α-naphthylacetate in water prevented test concentrations above 10^{-3} M.

The product release experiments showed that α-naphthylacetate is taken up by roots of *Vicia faba,* and that the hydrolysis product α-naphthol is excreted back into the surrounding buffer solution. After 20 min, however, an equilibrium was reached which indicates either completed product release after this period of time, or a balance between product intake and release (figure).

The physiological function of esterases is unknown. Many reports on high esteratic activities associated with cell walls[2-6] and the phloem[7,8], however, point to a possible function of these enzymes in the transportation of substances through cell walls. As our results show, this transportation need not only occur between cells inside the plant, but also between a root and its soil environment.



α-Naphthol release from roots after various time intervals. Each value represents the average of 3 replicates.

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Transition metals in an experimental tumour system[1]

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Summary. In this report we present an analysis by atomic absorption spectrometry of some of the transition metals Fe, Zn, Cu, Mn, Co, and Ni in tissues and nuclear fractions in an experimental tumour system.

Although, the occurrence of transition metal ions in biological tissues has been known for long[2], at present there is notable interest in their cellular distribution and physiological role. There is increased identification of their intrinsic association with organelles or macromolecules leading to a stage where their distribution pattern itself may be employed as a parameter of measuring the normal or abnormal state, such as cancer. Secondly, the presence of transition metal ions is considered to influence the magnetic resonance properties of cells/tissues[3]. In view of this, we have undertaken the estimation of these elements in normal and malignant tissues of experimental animals and nuclear fractions isolated from them. The relative differences between the distribution of trace elements from tissues to nuclear fractions were determined to pinpoint their association and focus attention on the nuclear associated reaction.

In this work, an experimental tumour system in Swiss mice was employed. The tumour, mouse fibrosarcoma (MFS), originally induced by chemical carcinogen in this institute and maintained by serial transplantation, was used. In the present studies, 2-week-old tumours were employed.

The nuclear fractions (NF) were prepared using the procedure of Chauveau et al[4]. The tissues were chopped, minced and homogenized with a glass teflon homogenizer in sucrose-EDTA solution (sucrose 0.25 M, ethylene-diaminotetracetic-acid 0.001 M) till the cells were completely dissociated. The homogenate was filtered through cheese-cloth and centrifuged at 1000 rpm for 10 min. The nuclear pellet was washed a few times until it was free from debris to ensure the intactness of the nuclei with microscopic examination. All the steps were carried out in the cold (4 °C). The nuclear pellets were lyophilized. The transition elements were determined from the lyophilized samples as follows. The dry samples were dissolved in (1:2) a mixture of perchloric and nitric acid, and diluted. The diluted solutions were analyzed on an atomic absorption spectrophotometer in the laboratory of Health Physics Division of the