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.

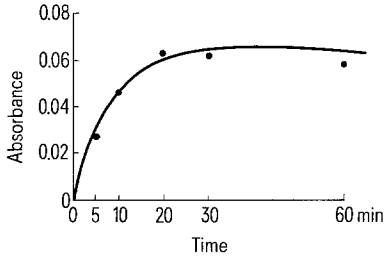
Results and discussion. Esterases were released from the root tips into the surrounding buffer, with the release being proportional to time. The absolute amount of enzymes recovered in the buffer solution, however, was relatively small (average absorbance value for a 30-min immersion period: 0.044). 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

Bhabha Atomic Research Centre, Trombay. From the absorbance characteristics, the concentrations of the transition elements were calculated and have been expressed on dry weight basis (tables 1 and 2).

It can be seen from table 1 that the elements Fe, Zn and Cu are found in fair amounts in all the tissue samples, while Mn, Co and Ni are just above the borderline of detection limits. The level of Fe is found to be increased in the liver compared to the tumour, while the levels of Zn and Cu are almost the same in the liver and the tumour tissues. The same trend is noted in the levels of Mn, Co and Ni in both the tissues where this group is found in relatively low amounts.

The distribution of the elements undergoes a change in the nuclear fractions of the tissues (table 2). The levels of Fe are found to be higher in the NF of liver than the tumour. A reversal of this trend is evident in the levels of Zn and Cu, which are notably higher in the tumour NF than the liver NF. In the case of Mn and Ni, slight but perceptible increase is seen in the tumour NF compared with liver NF.

From tissues to the level of nuclear fractions, the distribution of the transition elements is marked by the occurrence of high levels of Fe in liver, as compared with the MFS tumour. Increase is notable in the levels of Fe, Mn and Ni as well as Zn. A greater recovery is seen in tumour NF of Zn, Mn and Ni than in case of liver NF.

The general trend of increase in the transition metal content from tissues to the nuclear fractions is of interest. In particular, increase in the levels in the nuclear fraction could be attributed to high cellularity and proliferative ability of these tissues. Further Zn has been identified as a constituent of over 70 enzymes inclusive of the polymerases[5]. The relatively higher distribution of Zn in tumour NF confirms the above expections where there is a larger net synthetic activity compared with the normal tissues. The point is borne out in the case of Cu and Mn, which show higher recovery in the tumour NF. In view of the fuller recovery of Cu, Zn and Mn encountered in the tumour, it would be worthwhile to study distribution pattern of these elements in more tumour systems. Srinska et al.[6], in their analysis of transition metals in calf thymus cells and its deoxynucleoprotein (DNP), found that Cu and Fe were concentrated in DNP as compared with the cells, whereas Zn distribution was even in both the fractions. These results are in agreement with our findings too, where we have worked on a larger number of samples. The increased concentration of transition elements in malignant tissues, as well as nuclear fractions, has significance in the pulsed nuclear magnetic resonance (NMR) studies. It is considered that the proton spin-lattice relaxation time values (T_1) of tissues are influenced by the presence of paramagnetic metal ions and their interaction with water present in the cells[7]. In fact, this argument explains the differences observed on the basis of T_1-values in normal and malignant states (higher in latter and lower in former), on the basis that there is a decreased paramagnetic content in malignant state, resulting in enhanced T_1-values in the latter. The present results, therefore, are relevant in this context as they do not support the above, often stated argument. We have observed enhancement in transition metal content both in the malignant tumour as well as nuclear fractions thereof, where the increased T_1-values are associated with elevation in the paramagnetic metal ions. Pulsed NMR-studies of human tissues have been dealt with separately elsewhere[8–10]. The role of paramagnetic impurities in relation to pulsed NMR-studies is and an exploratory study by itself.

Moffitt et al.[11] have shown that alterations in the normal subcellular distribution of transition metals have been produced following acute administrations of carcinogens. In their view, the subcellular metal changes reflected a redistribution of metal stores in the enzyme-responsive tissues. The distribution patterns observed in the present studies could well be explored with other experimental tumours.

It is felt that the current studying of the nuclear fractions has been done to confirm the role of the important ones of the transitional elements as predominantly nucleus associated metals. In view of their recovery and observed enhancement in the nuclear fractions, all 3 elements could be explored further for their potential as markers of malignancy, with other tumour systems.

Table 1. Trace metal analysis of mouse tissues (Swiss mice)

Metal	Amount found Liver (normal)	No. of samples	Amount found Mouse fibrosarcoma	No. of samples
Fe	350±19	24	229±18	23
Zn	120±6	24	117±6	22
Cu	25±2.5	24	21.5±2	24
Mn	4.1±0.44	24	4±1	22
Co	2.3±0.4	23	2.7±0.8	15
Ni	4.1±0.7	23	4.8±0.9	15

* The data are expressed as mean±SE of samples. Amounts of metal in µg/g of dry weight of the tissues.

Table 2. Trace metal analysis of nuclear fractions of tissues (Swiss mice)

Metal	Amount found NF of liver (normal)	No. of samples	Amount found NF of mouse fibrosarcoma	No. of samples
Fe	487±78	21	330±38	24
Zn	68±4	19	155±13	24
Cu	17.9±3.1	21	33.6±5	27
Mn	3.2±0.6	21	9.8±1.8	25
Co	2.7±0.5	15	3.7±0.9	15
Ni	2.8±0.5	15	8.6±1.5	15

* The data are expressed as mean±SE of samples. Amounts of metal in µg/g of dry weight of the nuclear tractions.

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