It is interesting to note that reserpine sensitivity of NE accumulation (77%) is significantly greater than MgATP sensitivity (60%). These results suggest that some residual electrochemical gradient may be preserved in this medium, and may serve to drive some uptake of NE. It has been demonstrated by other investigators that the internal pH of 5.7 observed in chromaffin granules in the absence of ATP represents the zero-potential energy, equilibrium state of the granule[8]. A similar situation may be operative in the brain storage vesicle, and may serve to drive some ^{3}H-NE accumulation. The non-permeant nature of the anions used in this buffer would preserve any resting gradient of the vesicles, a situation which might not be observed with buffers comprised of phosphate or chloride anions. The obervation that reserpine completely inhibits ATP-dependent ^{3}H-NE accumulation, and inhibits half of the ATP-independent accumulation supports this possibility. A similar situation has been observed previously[3]. Alternatively, some exchange with endogenous NE may be occurring in an isoenergetic fashion. HPLC analysis (electrochemical detection) reveals 17.1 pmoles/mg protein of endogenous NE in the vesicle pellet after isolation (data not shown).

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2 To whom reprint requests should be addressed.
3 Disbrow, J.K., and Ruth, J.A., Life Sci. *29* (1981) 1979.
4 Toll, L., and Howard, B.D., Biochem. J. *17* (1978) 2517.
5 Johnson, R.G., and Scarpa, A., J. biol. Chem. *251* (1976) 2189.
6 Holz, R.W., Proc. natl Acad. Sci, USA *75* (1978) 5190.
7 Seidler, F.J., Kirksey, D.F., Lau, C., Whitmore, W.L., and Slotkin, T.A., Life Sci. *21* (1977) 1075.
8 Holz, R.W., J. biol. Chem. *254* (1979) 6703.

A comparative study of membrane related phenomena in normal and crown gall tissues of red beet (*Beta vulgaris* L.)

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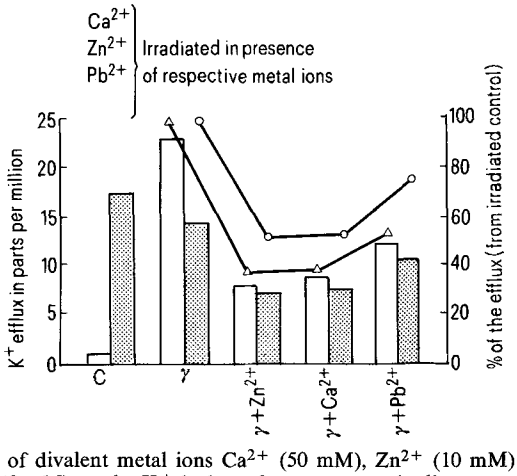
Summary. Divalent metal ions have been found to protect membranes of red beet crown gall tissue more than their adjacent normal regions from thermal or gamma radiation stress, suggesting the possibility of an alteration on the surface charge accompanying tumor formation in plants. Further, tumor tissue has been observed to possess enhanced membrane ATPase activity, a higher tissue sulfhydryl content and increased protein levels, thus suggesting a model of 'source' (normal tissue) and 'sink' (tumor tissue) relationship.

The transformation of a normal cell into a neoplastic cell in plants as well as in animals involves progressive changes in the properties of the membrane systems[3-6]. Altered membrane permeability reported in crown gall tissues (neoplastic tissues in plants formed due to the infection of *Agrobacterium tumefaciens*) by Braun[3] may be due to changes in the membrane lipid composition as has been reported for some crown gall tissues recently[4,5]. Alterations of the membrane surface charge have been frequently encountered with the transformation of cells in animal tissues[6]. There is little evidence to show any such change in the surface charge accompanying tumor formation in plants. In the present study, an attempt has been made to analyze the data obtained on the loss in membrane permeability of normal and crown gall beet root (*Beta vulgaris* L.) tissues. The tissues have been subjected to thermal and/or gamma-radiation stress in the presence of divalent metal ions to show that alteration of the membrane surface charge accompanying tumor formation is also possible in plants.

Besides changes in the membrane permeability, the membrane ($Na^+ + K^+$) ATPase activity and the tissue -SH content which are important factors in the altered function of membrane transport have been studied for both tumors and their adjoining normal regions.

Materials and methods. a) *Tumor induction.* Tumors were induced in beet roots with the help of *Agrobacterium tumefaciens* LBA201 (obtained through the courtesy of Professor S.C. Maheswari, Dept. of Botany, University of Delhi) as had been described earlier[7].

b) *Membrane permeability.* 2-month-old tumors and adjacent normal regions were harvested and cut into slices 4 mm long and 2 mm diameter and washed thoroughly.



Effect of divalent metal ions Ca^{2+} (50 mM), Zn^{2+} (10 mM) and Pb^{2+} (5 mM) on the K^+ leakage from tumor and adjacent normal irradiated tissues. ■, Adjacent normal; □, tumor. ○, %K^+ efflux in presence of metalions in normal tissues; △, %K^+ efflux in presence of metal ions in tumour tissues; C, control; γ, γ-irradiated.

Table 1. Effect of divalent cations on the heat-induced leakage of 260 and 280 nm absorbing materials from tumor and adjoining normal regions (10-12 discs weighing approximately 400 mg). Mean of 3 values. Incubation time - 60 min

Temperature °C	Incubating medium	Tumor		Adjacent normal	
		Optical density (nm)			
		260	280	260	280
20	Buffer (Tricine-NaOH)	0.050	0.070	0.022	0.033
30	-do-	0.069	0.079	0.062	0.077
45	-do-	0.394	0.324	0.090	0.085
45	-do + Ca^{2+} 50 mM	0.086	0.136	0.05	0.052
45	-do + Zn^{2+} 50 mM	0.142	0.155	0.052	0.057
45	-do + Pb^{2+} 5 mM	0.200	0.224	0.05	0.037

Table 2. Sulfhydryl (-SH), protein and membrane bound Mg^+ stimulated ($Na^+ + K^+$) ATPase activity in tumor, adjoining normal and normal uninoculated tissues. Mean values of 3 experimental sets (SE ± 5-18%)

	-SH nM mg^{-1} protein	-SH nm g^{-1} fresh weight of tissue	Protein mg protein g^{-1} fresh weight of tissue	ATPase µmoles of Pi liberated mg^{-1} protein min^{-1}
Tumor tissue	4.7	8.67	2.0869	0.07
Adjacent normal tissue	6.57	6.268	0.954	0.035
Normal tissue (uninoculated)	15.0	18.26	1.217	0.155

10-12 slices weighing approximately 400 mg were taken in 5 ml of buffer (Tricine-NaOH, pH 6.8; 20 mM) or in buffer supplemented with the desired concentrations of metal ions (Ca^{2+}, Zn^{2+} and Pb^{2+}) based upon earlier observations on normal tissues[8], and incubated at different temperatures. The membrane damage was monitored from changes in 260 and 280 nm absorbing materials of the incubating medium as was reported earlier[9].

Similarly, gamma-irradiation of the discs was carried out at a dose rate of 125 rads/sec, estimated by Fricke ferrous sulphate dosimetry[10] (1967) in a 4000 Co^{60}, (5500 Ci) gamma chamber (supplied by BARC, Bombay) for 65 min (total dose, 487.5 kg rads). Post-irradiation incubation of the tissues was carried out at 23 ± 2 °C for 8 h before the K^+ efflux was estimated using an Atomic Absorption Spectrophotometer (AAS 1, Carl Zeiss, Jena) to monitor radiation damage of membranes.

c) *SH estimation.* The sulfhydryl content of the tissue was estimated as described earlier[7] using Ellman's method[11].

d) *Membrane ($Na^+ + K^+$) ATPase.* The membrane pellet was isolated between 2 and 4 °C from both tumor and adjoining normal regions after subjecting the supernatant obtained at 27,000 × g (RC5B sorvall refrigerated centrifuge) to 110,000 × g (Beckman preparative model ultracentrifuge). The Mg^+ stimulated ($Na^+ + K^+$) adenosine triphosphatase (ATPase) activity was estimated in an assay mixture consisting of 50 µl of the enzyme along with the components of the reaction mixture (33 mM Tris - MES buffer, pH 6.0, 3 mM disodium salt of ATP; 25 mM NaCl + KCl and 1.5 mM Mg SO_4) in a total volume of 1.0 ml at 37 °C for 10 min. The pH of the buffer was adjusted to 6.0, as this was found to be optimal for the assay[12] for normal uninoculated tissues. The reaction of the enzyme was terminated by adding 10% ice-cold tricholoroacetic acid. The enzyme activity was measured by the amount of inorganic phosphate liberated mg^{-1} protein min^{-1} (Fiske and Subba Row[13]).

e) Protein was estimated following Vernon and Roberts[14].

Results and discussion. Membrane damage (monitored from the loss in 260 and 280 nm absorbing materials) due to thermal stress was more pronounced in tumor tissues than in their corresponding adjoining tissues (table 1). Similarly, the efflux of K^+ was higher from tumor tissue, due to gamma radiation stress (fig.). Overall, it was observed that when the tissues were subjected to thermal or radiation stress in a medium which was supplemented with metal ions, the protection offered by the latter in terms of the reduced efflux of substances was more pronounced in tumors than in their adjacent tissue.

As has been pointed out earlier by Nagata and Melchers[15], while working with the surface charge of meophyll protoplasts, the zeta potential, a measure of the surface charge, decreased in the presence of Ca^{2+} ions. Further Boss and Mott[16] from their electron spin resonance studies on the carrot protoplast membrane fluidity have proposed that decrease in the fluidity in the presence of Ca^{2+} may be due to calcium binding to phospholipids. Recently, it has been shown that the phospholipid content decreases in completely transformed crown gall tissues[4]. Perhaps the decrease in phospholipid content, as has been shown by Cockerham and Lundeen[4], would reduce the number of anionic sites, thus facilitating the surface charge neutralization in the presence of divalent metal ions. As a result of this, membranes of tumor tissues are stabilized more compared to the corresponding adjoining normal tissues against thermal or radiation stress.

Further protein content and membrane ATPase activity were found to be higher in tumor tissue than in corresponding adjacent normal tissues (table 2), as has been reported for some transformed animal cells[17]. This could be due to the active metabolism and increased membrane permeability reported in crown gall tissues[3,18]. Sufhydryl content g^{-1} fresh weight of tissue was found to be higher in tumors than in the adjoining normal regions; whereas per mg protein, the sulfhydryl content was lower in tumor tissue. The latter may be due to the presence of quinones which are strong oxidants formed during the test[19].

Our data show that tumor tissues are associated with 'increased permeability', enhanced ATPase activity, higher tissue -SH content (g^{-1} fresh weight of tissue) and increased protein levels. This suggests a model of a 'source' (adjacent normal tissue) and 'sink' (tumor tissue) relationship in which the tumor tissues grow by drawing away the necessary nutrients from the adjacent normal regions.

Further work in this direction is in progress to obtain more direct evidence about the membrane surface alterations following tumor induction.

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3 Wood, H.W., and Braun, A.C., Proc. natl Acad. Sci. USA *54* (1965) 1432.
4 Cockerham, L.E., and Lundeen, C.V., Pl. Physiol. *64* (1979) 543.
5 Philips, R., and Butcher, N., Phytochemistry *18* (1979) 695.
6 Nicolson, G.L., Biochem. biophys. Acta *457* (1976) 57.
7 Ramaiah, K.V.A., and Mookerjee, A., Experientia *37* (1981) 132.
8 Ramaiah, K.V.A., and Mookerjee, A., Indian J. exp. Biol. *16* (1978) 857.
9 Ramaiah, K.V.A., and Mookerjee, A., Indian J. exp. Biol. *19* (1981) 561.
10 Fricke, H., and Hart, E.J., in: Radiation dosimetry. Eds H. Attrix and W.G. Rossch. Academic Press 1967.
11 Ellman, G., Archs Biochem. Biophys. *82* (1959) 70.
12 Ramaiah, K.V.A., Ph. D. thesis submitted to the Jawaharlal Nehru University, New Delhi 1980.
13 Fiske, C.H., and Subba Row, Y., J. biol. Chem. *66* (1925) 375.
14 Vernon, F.K., and Robert, W.B., Analyt. Biochem. *82* (1977) 362.
15 Nagata, T., and Melchers, G., Planta *142* (1978) 235.
16 Boss, W.F., and Mott, R.L., Pl. Physiol. *66* (1980) 835.
17 Kasarov, L.B., and Friedman, F., Cancer Res. *34* (1974) 1862.
18 Braun, A.C., Biochem. biophys. Acta *516* (1978) 167.
19 Stonier, T., and Yang, H.Y., Physiologia Pl. *25* (1971) 474.