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THERMAL AND RADIATION EFFECTS ON THE PERMEABILITY OF BEET ROOT MEMBRANES

Dissertation Submitted to the Jawaharlal Nehru University in partial fulfilment for the degree of MASTER OF PHILOSOPHY

by

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PREFACE

The research work embodied in this dissertation has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full for any other degree or diploma of any University.

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CONTENTS

CHAPTER - I

INTRODUCTION

Temperature and radiation are some of the important environmental factors that affect the functioning of living organisms. Today, each of these factors by itself has become a full fledged branch in biology viz., Thermobiology and Radiobiology.

The internal cellular environment is constantly modified and influenced by external surroundings. External physical forces like temperature, radiation, wind, humidity and pressure etc., regulate the rate of cellular reaction and determine if they proceed normally and optimally.

To understand the thermal stress or radiation effect or for that matter any parameter affecting the functioning of cell membranes, the past history and the present status of the various components of cell membrane or plasma membrane and their packing arrangements need a brief discussion. The cell membrane has been envisaged by Wallach (1972) as "an assembly of numerous diverse, but specifically and genetically regulated functional systems arrayed at the cell periphery each encompassing defined receptors and playing biological roles far more intricate than their well studied participation in permeability and transport". Thus plasma membrane has been viewed as a living and dynamic entity surrounding the cell.

Inside the cell also there are various membrane systems like thylakoid membranes of chloroplasts, mitochondrial cristae endoplasmic reticulum and also membranes are found covering cell organelles in the eukaryotic cells. These membranes provide sites for photosynthesis, electron transport, protein-synthesis and provide regions for organisation of energy transduction mechanisms such as photoreceptors. Beside the above functions, the membranes are found to be highly selective and regulate the flux of various organic and inorganic substances from inside to outside the cell and vice-versa. Thus, cell membranes play a very crucial role in the active and passive transport of substances of the cell.

Essentially, the plasma membrane consists of proteins, lipids and small amount of carbohydrates. Many models have appeared to explain the structure (Fox 1972; Singer and Nicolson 1972; Capaldi 1974; Gitler 1972) and organisation of membrane and its components (Davson and Danielli 1952; Robertson 1964; Benson 1966; Leonard and Singer 1966; Malhotra 1970; Singer and Nicolson 1972). But out of all those models, the Fluid Mosaic model proposed by Singer and Nicolson has been widely accepted because it is found consistent with the restrictions imposed by thermodynamics and is also supported by many experimental evidences and physical techniques

(Nicolson *et al* 1971; Frye and Eddin 1970; Portes *et al* 1970; Engelmann 1971; Melchoir *et al* 1971).

The fluid mosaic model is analogous to a two-dimensional oriented solution of integrated proteins (or lipo-proteins) in the viscous phospholipid bilayer solvent. The integral proteins are a set of heterogenous globular molecules and they are amphipathic. Proteins are embedded partially in the bulk of the phospholipid matrix which is a discontinous fluid bilayer (Nicolson and Singer 1972). The components of the biological membranes are asymmetrically distributed between the membrane surfaces and the asymmetry is maintained because of lack of trans-membrane diffusion (Rothman and Leonard 1977).

A spin label study of biological membranes for specific lipids is being carried out (Shun-Ichi Ohnishi 1976) for the study of individual roles. Biological membranes contain many kinds of lipids. In phospholipids there are a variety of classes differing in the polar head groups and fatty acid composition. Each phospholipid appears to have its own role in biological membranes. The spin label detects conformational change through change in the local environment of the labelled site. Ca^{++} binding lipid molecules have been investigated through this method.

Isolated membranes are found to have markedly different properties compared to the intact membranes insitu. It is a challenge to the biophysicist to search for those procedures that will describe the intimate mechanisms associated with membrane functions. It is likely such procedures have to include methods which affect the structure in a transitory reversible manner including temperature, pressure, electric, magnetic fields, and radiation effects coupled with those techniques that might detect transients in the range of microseconds-milliseconds (Gitler 1972).

In the present study, the effect of temperature, γ-irradiation and the effect of some metallic cations like calcium, lead, zinc and magnesium have been tested to see the permeability changes and the protective action offered by the above mentioned metallic cations on the beet root tissue, Beta vulgaris L. The involvement of beet root membranes in the tissue's response to temperature fluctuation has been recognised by several workers quite some time ago (Siegel 1969; Yochevad Toprover and Glinka 1976).

The system is convenient to handle. The pigment betacyanin is present in vacuoles of the cells. It is released out when the cell membrane is damaged. So by spectrophotometric analysis of the eluted betacyanin from the tissue and hence the integrity of the membrane can be determined. Membrane properties are quite sensitive to environmental influences. Generally mammalian cells can buffer effectively against the environmental fluctuations by elaborate regulatory devices but the mechanisms may not be the same in lower organisms and plants.

In nature, sometimes the temperature of plants or some of their organs will be more than their atmospheric temperature because these plants may not be in a position to transfer all the excess of heat to their environment. Sometimes the rise of temperature in the plant compared to its surroundings may be as high as 11 - 12°C (Vrolik and de Vrise 1839). Eg., in the case of fleshy organs of Colacasia odora and thus temperature may exceed to 45 - 55°C range which is usually accepted as a normal temperature limit for most plants, and animals die at those temperatures (Huber 1935; Rouschal 1938b). Huber (1932) recorded that fruits of Arum, pine tree cambium and opuntia reach in nature to the highest temperatures of 50°C, 55°C and 60°C respectively. Lundegardh (1949) noted that soil exposed to insolation reached to temperatures as high as 55 - 75°C. There are many reports that seedlings die at the soil level (Baker 1929; Munch 1913 and Franco 1961) because of the fatal temperatures recorded at the soil level. So, it was of interest to investigate the changes in membrane permeability with increase in temperature and also to see calcium ions repairing the damage caused due to the increased temperature. Similar experiments have been also carried out with gamma irradiation of the tissue either singly or in conjunction with thermal exposures. This type of task acquires importance in cosmic plant growing and in raising plants under conditions of high irradiation levels (Grodzinskii 1976).

In conducting such studies, it will be possible to understand the possible dynamic alterations in the normal biochemical reactions due to the increased temperature or even radiation levels and the intimate mechanisms associated with the membrane functions. Such studies may also be useful in understanding the nature of membrane components and their packing arrangements which determine the functioning of membranes. Moreover, interest can also be focussed on the stabilising and destabilising properties of membranes.

C H A P T E R - II

MATERIALS AND METHODS

Commercially available red beet roots (*Beta vulgaris L.*) were purchased from the local market and stored in the refrigerator. Cylinders of tissue were taken out from roots by using stainless steel cord borer. Tissues were cut into slices of 2 mm thickness and 0.7 cms in diameter by using a tissue slice cutter which has been designed and fabricated in the Central Work-shop, Jawaharlal Nehru University.

After cutting the tissues into slices, the latter were washed under the running tap water for about six to ten hours so that the pigment betacyanin that leaked out from the tissue in the process of cutting into slices should be thoroughly washed out. Afterwards, the slices were washed twice or thrice with double distilled water and finally with Tris ($C_4H_{11}NO_3$-Tris-hydroxymethyl aminomethane - (Merck) - HCl buffer maintaining a concentration of 10mM and pH 6.8.

Later, those tissue slices were transferred to aerated buffer (10 mM, pH 6.8) and kept at 8 - 10°C overnight. Next morning (i.e. after 12 - 14 hours) slices were brought to the room temperature maintaining below 27°C. 10 slices weighing approximately 2.1 gms were put in 50 ml conical flasks so that piling of tissue slices one over the other was avoided. 10 ml of Tris buffer was put in each 50 ml conical flask containing 10 slices.

Now the slices in the conical flasks were subjected to a temperature stress of 25°C, 27°C, 30°C, 45°C, 55°C and 60°C in the temperature controlled shaker water-bath with an accuracy of ± 1°C. After treating the tissue at various temperatures (25° - 60°C), the efflux of betacyanin was measured for 2 - 3 hours for every 30 minutes interval at 535 nm by using the spectronic-20. The set which was kept at 45°C has been transferred after 30, 60, 90, 120 and 150 minutes of interval to a temperature, 25 ± 2°C, maintained by using cold water bath.

Chemicals used in the experiments are $CaCl_2.2H_2O$; $MgCl_2.4H_2O$; HNO_3; $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ (All are of BDH analar quality). Besides the above chemicals, Tris buffer (Merck) and Tricine buffer (Sigma Chemicals) have been used.

Effect of Calcium:

Effect of calcium ions have been tested by using solutions of $CaCl_2.2H_2O$ of various concentrations viz., 200 mM; 100 mM; 50 mM; 25 mM and 12.5 mM.

Effect of Zinc and Magnesium:

Effect of zinc and magnesium ions on the efflux of betacyanin from beet roots have also been tested by using various(50, 100 and 200 mM) ionic strengths of those respective salt solutions, $ZnSO_4 \cdot 7H_2O$ and $MgCl_2 \cdot 4H_2O$.

Effect of Lead:

Lead ions have been tested to see whether lead being an atmospheric pollutant can cause any damage to membranes. In order to make $Pb(NO_3)_2$ solution of various concentrations, various buffers have been tried but formation of precipitate while adjusting the pH(6.8) became a problem. Tricine (From Sigma Chemicals) buffer was used after many trials because recently Wong and Govindjee (1976) tried lead ions on photosystem I in isolated chloroplasts. There they have used the Tricine-NaOH buffer. Tricine 20 mM and 50 mM solutions were made in which 10 mM $Pb(NO_3)_2$ salt was dissolved and the pH had been adjusted by using very dilute NaOH to 6.8. Now from the above stock solutions, 5 mM and 2.5 mM $Pb(NO_3)_2$ solutions were made.

Gamma-Irradiation:

10 tissue slices were put in each conical flask of 50 ml capacity with 10 ml of Tris-HCl buffer. In few sets, Tris buffer containing calcium (100 mM) was used and then subjected to gamma-irradiation.

The source of γ-irradiation was 4000 CO^{60} (5500ci) gamma chamber supplied by the Bhabha Atomic Research Centre, Trombay, Bombay. The dose rate of the gamma irradiation plant was determined by using Fricke-Ferrous Sulphate dosimetry (1967). The dose rate was 3.259 K. rads/minute (Fig. 11).

In the initial stages, irradiations for 2, 5, 10, 30, 60, 90 and 120 minutes have been tried but was found to be inadequate for the leaching out of betacyanin from the tissue. The tissues were then subjected for 150 minutes and 180 minutes to irradiation. After irradiation the temperature of the solution in the conical flasks was noted and were subjected to a temperature of 37 ± 1°C for 120 minutes. Another set after irradiation was kept at a temperature of 29 ± 2°C (room temperature).

<u>Irradiation in 'dry' and 'wet' conditions:</u>

In another experiment, the slices were dried using a tissue paper and then these were irradiated without buffer. After irradiation 10 ml of buffer was added in each 50 ml of conical flask consisting of 10 dried irradiated slices. Again one set was kept at 29 ± 2°C and another set at 37 ± 1°C for 120 minutes. The above two experiments have been repeated by using $CaCl_2.2H_2O$ (100 mM).

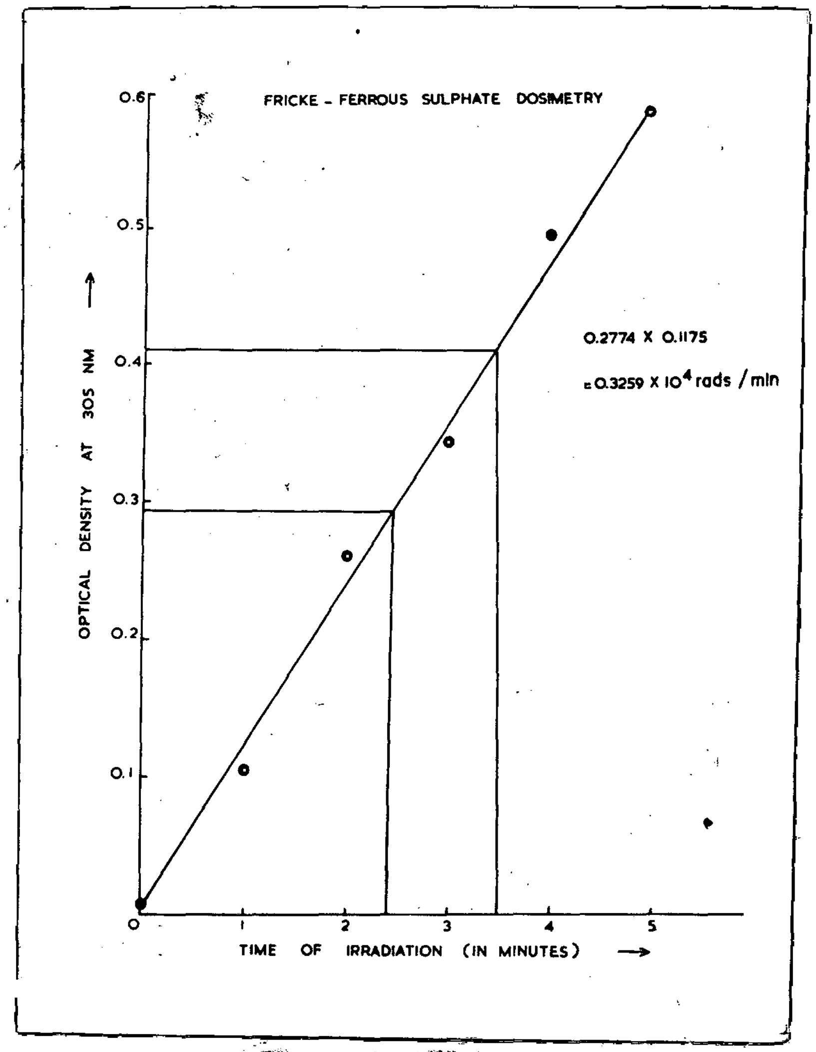

Figure 11. Determination of dose-rate of the gamma source by Fricke-ferrous sulphate method.

During irradiation, there was rise in temperature. So one set was tried by putting conical flasks containing the tissue slices in a jar containing crushed ice and then irradiated. After the irradiation was over, one set was kept at 12°C ± 1°C and another set at 37°C for two hours and then the efflux of betacyanin was measured.

C H A P T E R - III

RESULTS

The beet root tissue cylinders were cut into slices and put under running tap water. Betacyanin, the red pigment of the tissue, leaked out for about 4 - 5 hours. So, before transferring the tissue slices into buffer whatever betacyanin that has been released from the tissue due to the mechanical stress was thoroughly washed out.

From Table 1 and Figure 1, it is seen that at 25°C, clearly there was no efflux of betacyanin from the tissue slices. But when the tissue slices have been kept at 27°C for about 90 minutes there was some leakage of betacyanin, but that was very little. For tissue slices kept at 30°C and 37°C, the efflux was moderate after 2 hours. But the release of betacyanin was higher at temperatures 45°C, 55°C and 60°C. The betacyanin leaked from the tissue when it was subjected to a temperature, 45°C for 120 minutes when the O.D. was 0.16. This was 53% lesser than the O.D. that was observed when the tissue was kept at 60°C for 30 minutes only (i.e., 0.34 O.D.) Fig. 1). Efflux of betacyanin has been taken into consideration because it happens to be a fairly big molecule and its release into the surrounding system does definitely mean the release of other macromolecules like proteins and carbohydrates from inside the cell into its surroundings.

Table 1

S.No.	Temperature	O.D. at various intervals Time in minutes					
		30	60	90	120	150	180
1.	25°C	0	0	0	0	0	0
2.	27°C	0	0	0.005	0.006	-	-
3.	30°C	0.007	0.013	0.020	0.022	-	-
4.	37°C	0.018	0.023	0.028	0.035	-	-
5.	45°C	0.030	0.046	0.100	0.167	0.300*	0.378*
6.	55°C	0.081	0.340	0.420	-	-	-
7.	60°C	0.340	0.470	0.600	-	-	-

*Readings have not been shown in the figure.

Efflux of betacyanin at temperatures 25°, 27°, 30°, 37°, 45°, 55° and 60°C. The O.D. was measured for every 30 minutes interval at 535 nm. The readings were the mean determinations of 4 values (Figure 1).

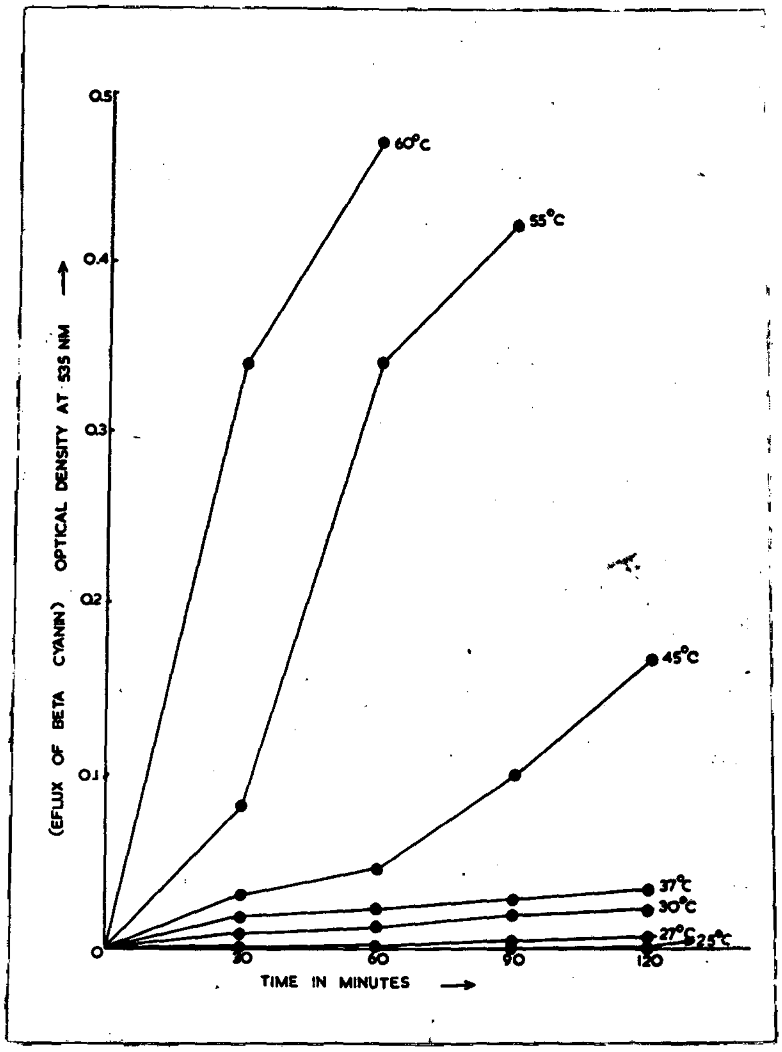

Figure 1. Efflux of betacyanin for every 30 minutes interval at various temperatures.

After treating the tissue at a temperature of 45°C for 30, 60, 90, 120 and 150 minutes, the slices have been transferred to a temperature of 25 ± 2°C. The efflux of betacyanin was more or less constant when the tissue was kept at 45°C for 30, 60 and 90 minutes and then transferred after each interval to 25 ± 2°C. But the efflux started increasing when the tissue was incubated at 45°C for 120 minutes and 150 minutes (Table 2) and transferred to 25 ± 2°C (Fig. 2).

To see the effect of Ca^{++}, the experimental tissue slices were placed in calcium chloride solution of varying concentrations - 12.5, 25, 50, 100 and 200 mM, and these were subjected to a temperature of 45°C. It was observed (Table 3) that there was a reduction of betacyanin efflux from the tissues when $CaCl_2$ was present in the incubating medium. Solutions of 50 mM and 100 mM protected the tissue from thermal damage more than either 200 mM or 12.5 and 25 mM solutions (Fig. 3).

When tissues were heated in presence of zinc sulphate and magnesium chloride solutions, it was found that with zinc ions (Table 3), the efflux has been inhibited more than that with magnesium ions. Moreover, with increase in the concentration (upto 200 mM) tried) of magnesium ions the protection was more whereas with zinc ions it was not the same. 50 mM $ZnSO_4$ solution was found to be more effective in inhibiting the efflux of betacyanin(Fig. 4).

Table 2

Tissue slices were kept at 45°C for 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes. Then they have been transferred to 25 ± 2°C and kept there for 120 minutes. The reading were taken for every 30 minutes interval. The readings were the mean determination of 4 values (Figure 2).

45°C O.D.	O.D. Temperature 25 ± 2°C (Time in minutes)			
	30	60	90	120
0.03 (After 30 mts.) A	0.030	0.031	0.031	0.031
0.046 (After 60 mts.) B	0.048	0.048	0.052	0.052
0.100 (After 90 mts.) C	0.100	0.100	0.100	0.105
0.160 (After 120 mts.) D	0.180	0.190	0.190	0.190
0.300 (After 150 mts.) E	0.345	0.400	0.430	0.470

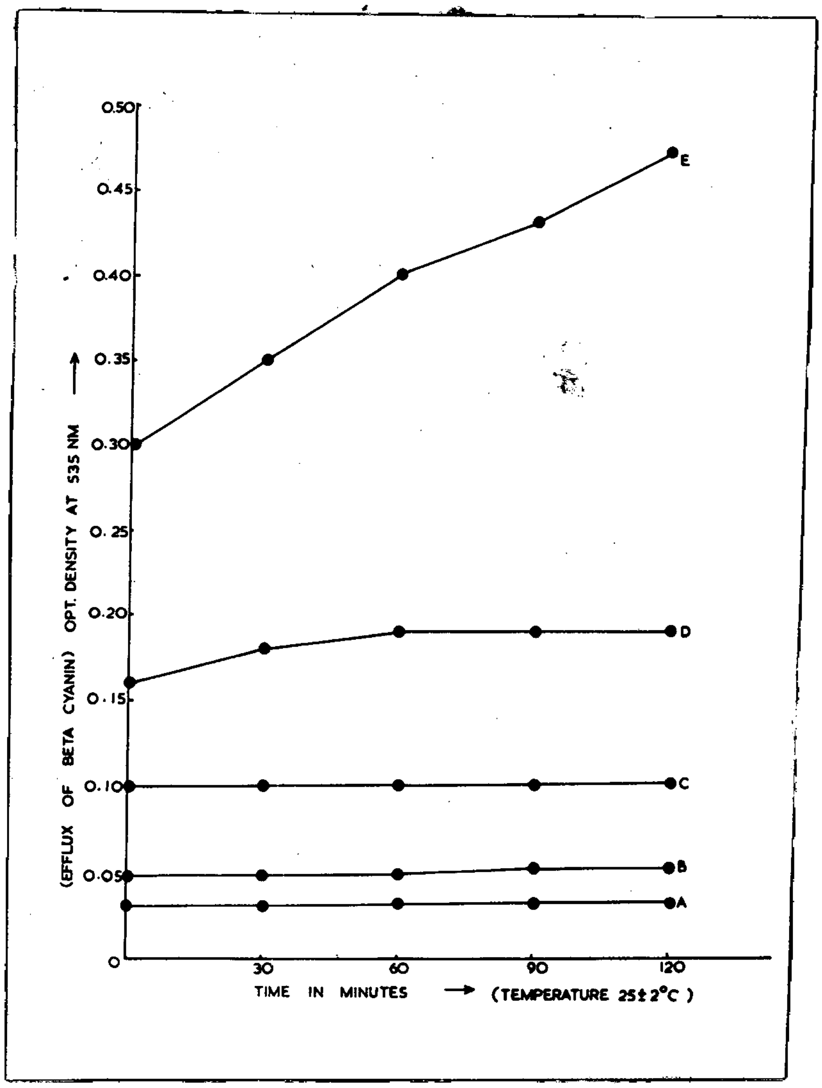

Figure 2. Tissues kept at 45°C for different time periods (A- after 30 minutes, B- after 60 minutes, C- after 90 minutes, D- after 120 minutes, and E- after 150 minutes.) and then transfer to 25 ± 2°C to observe the efflux of betacyanin at different intervals of time.

Table 3

S.No.	Name of the salt solution	OD at various concentrations (in mM)				
		12.5	25	50	100	200
1.	$CaCl_2 \cdot 2H_2O$	0.093	0.073	0.040	0.045	0.063 (Fig. 3)
2.	$MgCl_2 \cdot 4H_2O$	-	-	0.375	0.340	0.325 (Fig. 4)
3.	$ZnSO_4 \cdot 7H_2O$	-	-	0.025	0.037	0.050 (Fig. 4)

The tissue slices have been incubated at a temperature of 45°C for 180 minutes along with the various salt solutions to find the effect of metallic ions Ca^{++}, Zn^{++} and Mg^{++} on the efflux of betacyanin at that temperature. The readings were the mean determination of 4 values (Figures 3 and 4).

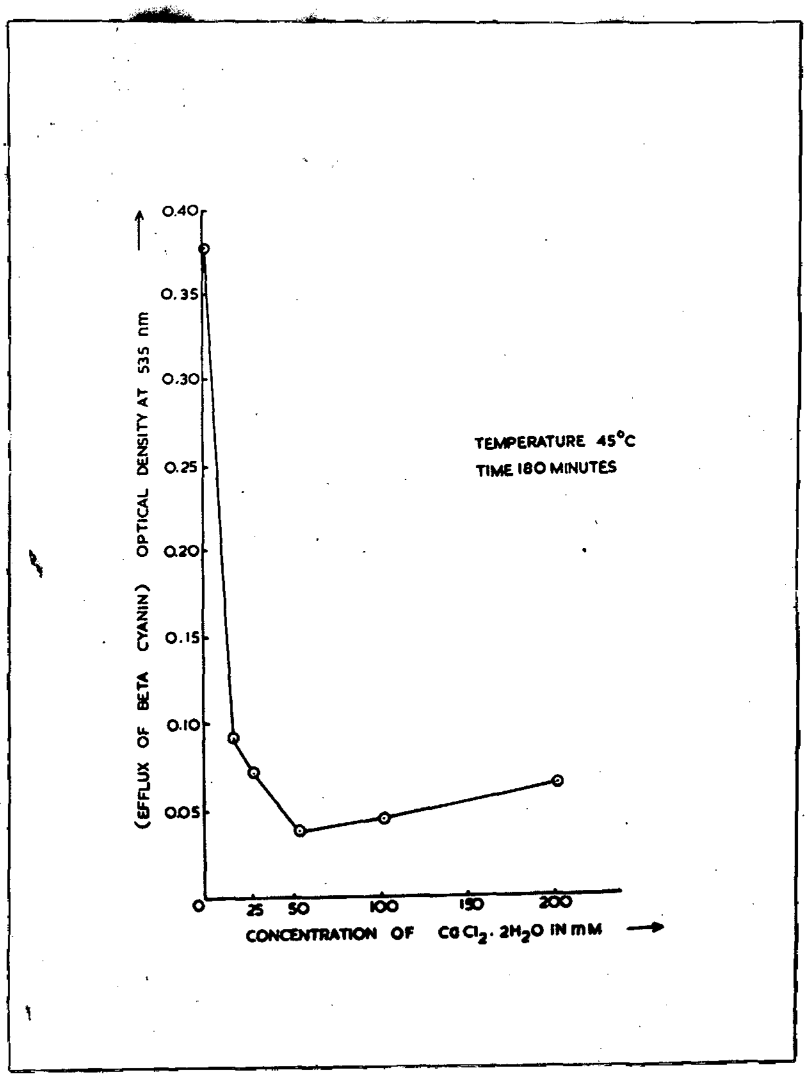

Figure 3. Effect of $CaCl_2.H_2O$ on the efflux of betacyanin from tissue slices kept at 45°C for 180 minutes.

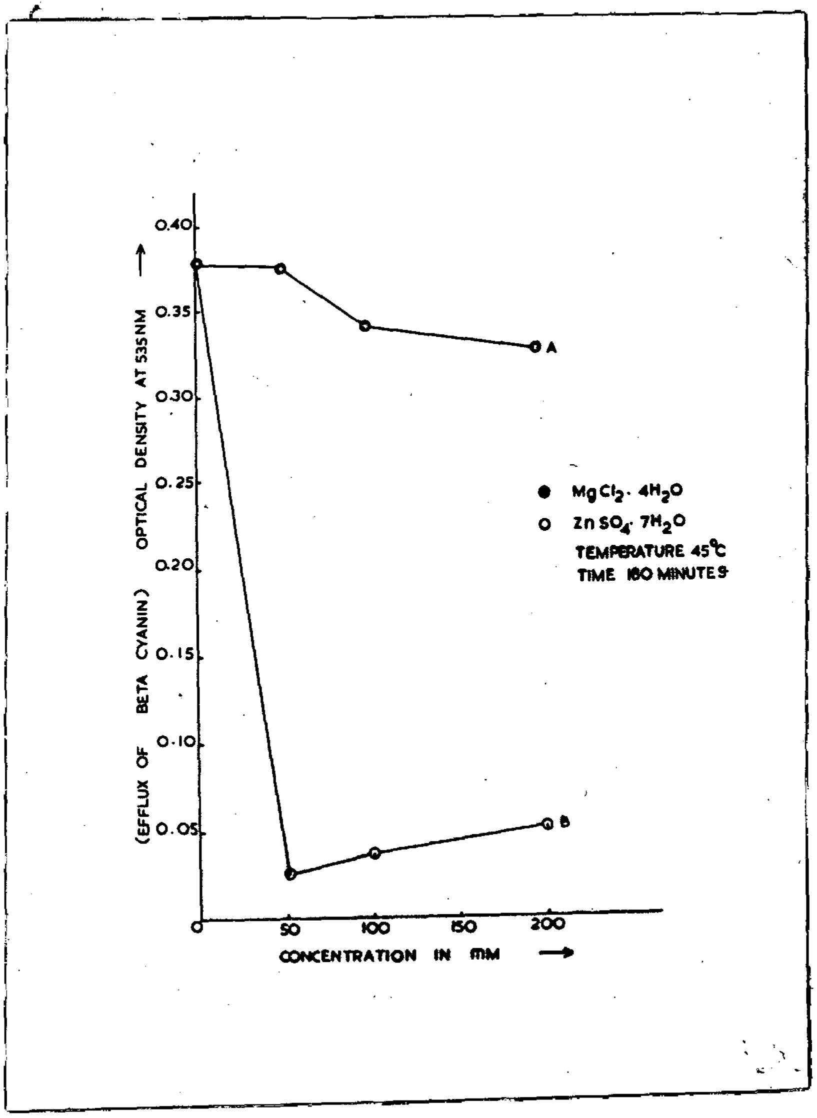

Figure 4. Effect of different concentrations of $ZnSO_4$ (○) and $MgCl_2$ (●) on the efflux of betacyanin from tissue slices, after 180 minutes of incubation at a temperature, 45°C.

The increasing presence of Pb^{++} as a pollutant in the environment is of considerable concern, and effect of lead ions was also of interest for a comparative investigation with other cations. It was observed that lead ions considerably reduced the efflux of betacyanin when the tissue slices have been incubated at 45°C for 180 minutes in the presence of Pb^{++} (Table 4 and Fig. 5). In fact, the inhibition was more pronounced than that observed with calcium ions. Varying concentrations of $Pb(NO_3)_2$ (2.5, 5 and 10 mM) solutions were made using the tricine buffer (pH 6.8) in a 1:2 and 1:5 ratio respectively. This was to check if any binding was taking place between lead and tricine or not, then it should get reduced with the increase in the concentration of tricine. From the data (Table 4 and Fig. 5) it is seen that the inhibition of betacyanin efflux was slightly more when the ratio of $Pb(NO_3)_2$ to buffer was 1:5 than 1:2.

Data collected on the efflux of betacyanin from gamma-irradiated beet root tissue slices clearly showed that a dose of 293.3 K. rads for 90 minutes damaged the membrane very little and very little efflux of betacyanin was noticed (Table 5). Later, when tissue slices were irradiated for longer periods - 150 and 180 minutes - with a dose of 3.259 K. rads/minute, then the efflux of betacyanin was immediate (Fig. 6 & 7). A factor to be noted here is that the temperature in the conical

Table 4

Control: 0.105 (O.D.)				
S.No.	Buffer/Salt	O.D. at various concentration of $Pb(NO_3)_2$		
		2.5 mM	5 mM	10 mM
1.	Tricine + $Pb(NO_3)_2$ (2:1)	0.017	0.012	0.015
2.	Tricine + $Pb(NO_3)_2$ (8:1)	0.006	0.007	0.007

Effect of various concentrations of $Pb(NO_3)_2$ solution on the efflux of betacyanin from the tissue slices which were incubated at 45°C for 150 minutes. The readings were the mean determination of 4 values (Figure 5).

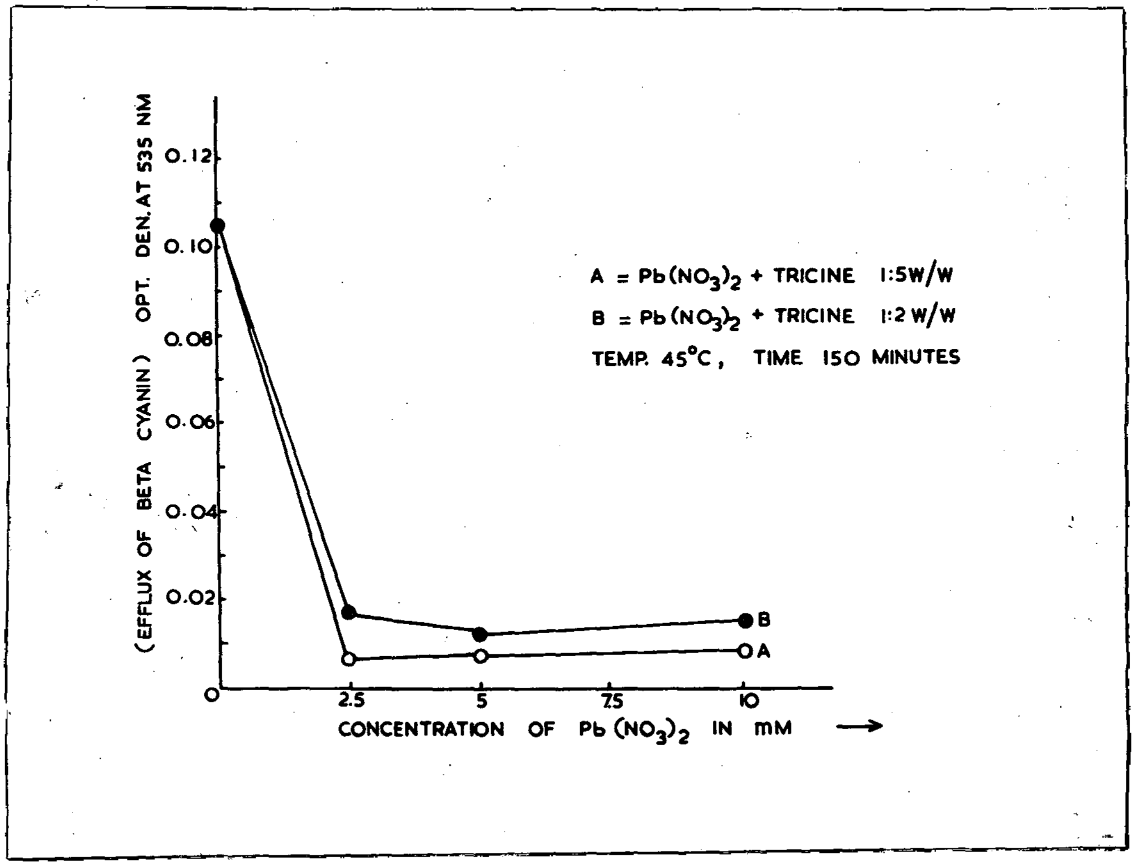

Figure 5. Effect of Pb^{++} on the efflux of betacyanin at a temperature, 45°C after 150 minutes of incubation.

flasks containing the buffer and tissue slices went upto 35°C after 90 minutes, 37°C after 150 minutes and 38.5°C after 180 minutes of irradiation.

Effect of irradiation:

The leakage of betacyanin observed for the tissues irradiated for 150 minutes and 180 minutes and then incubated at 37°C ± 1°C for 120 minutes was 10 times and nearly 13 times more respectively than the efflux observed for the set kept at 37°C for 120 minutes without previous irradiation (Fig. 1 & 6; Table 1 & 5).

Dose Effect:

An increase in the dose of γ-irradiation enhanced the damage. The efflux was 47% more for the set irradiated without calcium for 180 minutes and then kept at 29 ± 2°C for 120 minutes, than the set irradiated for 150 minutes. Similarly, the rise was 29% more in the set irradiated for 180 minutes and then incubated at 37 ± 1°C for 120 minutes than the corresponding set irradiated for 150 minutes (Fig. 6 and Table 5).

Presence of Calcium during irradiation:

Presence of calcium (100 mM $CaCl_2 \cdot 2H_2O$) during irradiation (or after irradiation) markedly reduced the irradiation damage thus reducing the efflux of betacyanin. The efflux was reduced by 78.6% in the set irradiated for 180 minutes and

Table 5

Buffer or Buffer along with Ca^{++} ($CaCl_2.2H_2O$ (100 mM) was present during γ-irradiation.	Minutes of γ-irradiation		
	180	150	90
	O.D. Values		
Buffer+γ-irradiation+($-Ca^{++}$)+29±2°C	0.235	0.160	0.035
Buffer+γ-irradiation+($-Ca^{++}$)+37±1°C	0.455	0.350	0.045
Buffer+γ-irradiation+($+Ca^{++}$)+29±2°C	0.015	0.020	0.005
Buffer+γ-irradiation+($+Ca^{++}$)+37±1°C	0.057	0.055	0.010

Buffer or Buffer along with Ca^{++} ($CaCl_2.2H_2O$, 100 mM) was present after γ-irradiation.	Minutes of γ-irradiation		
	180	150	90
	O.D. Values		
γ-Irradiation+Buffer+($-Ca^{++}$)+29±2°C	0.120	0.097	0.045
γ-Irradiation+Buffer+($-Ca^{++}$)+37±1°C	0.260	0.200	0.046
γ-Irradiation+Buffer+($+Ca^{++}$)+29±2°C	0.070	0.079	0.010
γ-Irradiation+Buffer+($+Ca^{++}$)+37±1°C	0.160	0.097	0.020

Effect of -irradiation in the presence of buffer or buffer with calcium and without buffer or calcium (they are added after the irradiation) on the efflux of betacyanin. The irradiated discs were kept at a temperature, either at 37±1°C or at 29±2°C for 120 minutes and then the efflux of betacyanin was noted. During the irradiation, no cooling arrangement was made. The readings were the mean determinations of 4 values (Figures 6 and 7).

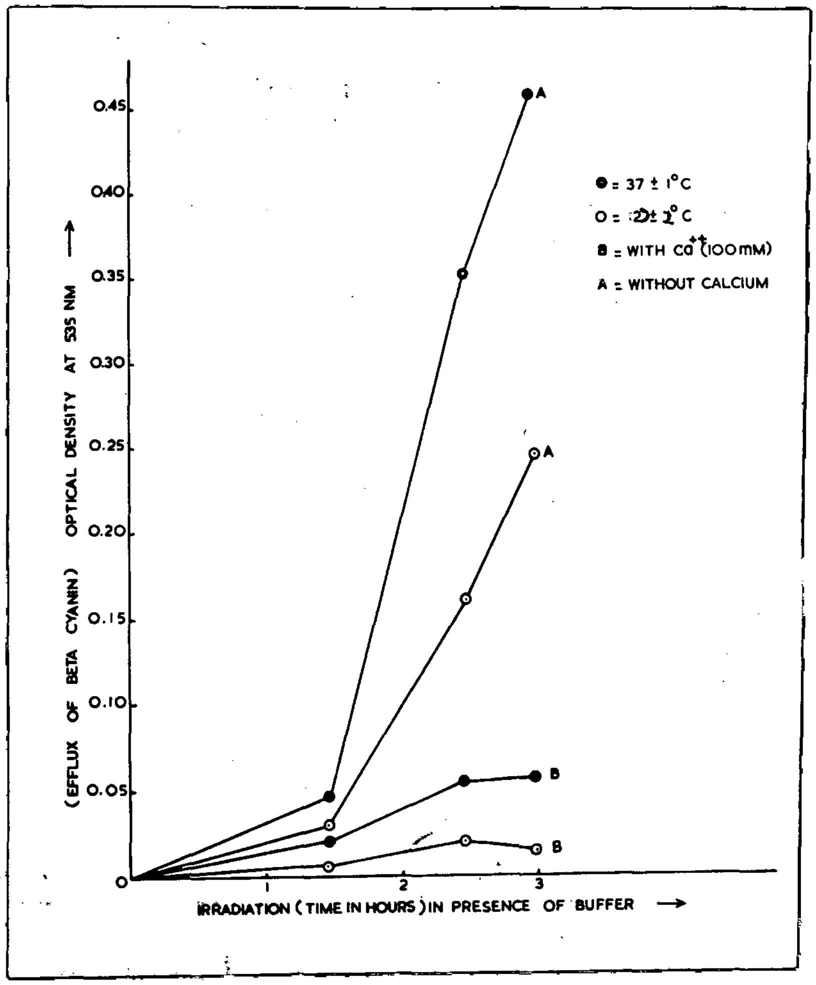

Figure 6. Effect of γ-irradiation in the presence of buffer (No cooling arrangement during the irradiation of the tissues). Efflux was measured after 2 hours of incubating the irradiated tissues either at 37±1°C or at 29±2°C.

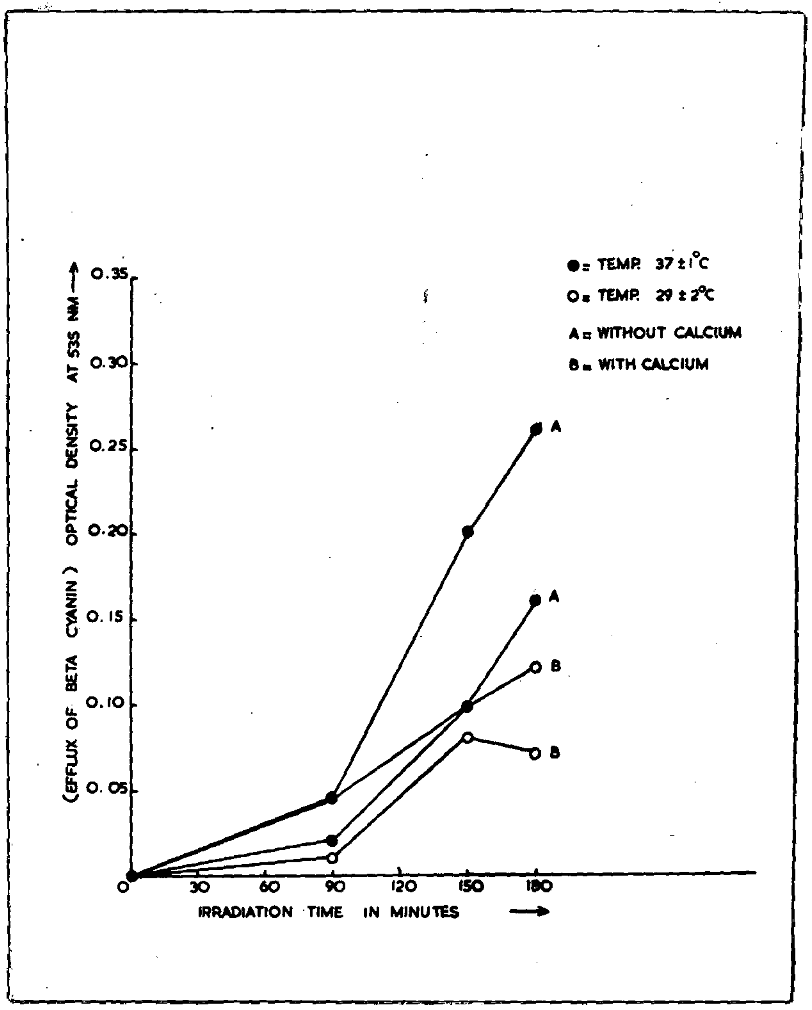

Figure 7. Effect of γ-irradiation in the absence of buffer (No cooling arrangement during the irradiation of tissues). Efflux was measured after 2 hours of incubating the irradiated tissues either at 37±1°C or at 29±2°C.

kept at 29 ± 2°C for 120 minutes with calcium than the corresponding set which has been irradiated without calcium (calcium was added after the irradiation), (Fig. 6 and 7, and Table 5). Similarly the rise in the efflux of betacyanin in the set without calcium and irradiated for 180 minutes and then incubated at 37 ± 1°C for 120 minutes, was nearly 8 times more compared to the corresponding set containing the calcium ions during irradiation (Fig. 6; Table 5).

Addition of Calcium after irradiation:

Calcium ions have decreased the efflux more when they were present during the irradiation than their presence after the irradiation. The decrease was 76.36% in the set containing the tissue slices irradiatied in the presence of calcium for 150 minutes and kept at 37 ± 1°C for 120 minutes than in the corresponding set in which calcium was added immediately after the irradiation. This trend was ... found in other conditions also (Table 6; Fig. 6 & 7).

Presence of buffer during irradiation:

Presence of buffer during irradiation enhanced the damage and thus the corresponding leaching of the efflux of betacyanin has also been enhanced. Thus the increase was almost doubled when the tissue slices were irradiated in the presence of buffer and without calcium for 180 minutes and then kept at 29 ± 2°C for 120 minutes compared to the efflux observed in the corresponding set irradiated without buffer (Fig. 6 & 7 and Table 5). The leakage was 75% more when the tissue slices were irradiated in the presence of buffer and without calcium for 180 minutes and kept at 37 ± 1°C for 120 minutes than the efflux noticed from the tissue slices irradiated without buffer (Fig. 6 & 7; Table 5).

In order to reduce the rise in temperature in the conical flask during irradiation and to see the effect of gamma irradiation on the membrane permeability, the conical flasks containing the tissue slices were kept in a jar containing crushed ice and then the tissue slices were irradiated with buffer, without buffer, with calcium and without calcium. Irradiations were given for 120, 150 and 180 minutes and temperature rise was noted. The temperature was 33°, 34° and 34.5°C respectively after 120, 150 and 180 minutes of irradiation. The rise in temperature must be a gradual process. The efflux of betacyanin was significantly lowered in this set up compared to the previous set up where no arrangement was made to control the rise in temperature in the conical flasks during irradiation. The irradiation effect was clearly observed in the tissue slices which have been incubated at 37 ± 1°C for 2 hours after 150 and 180 minutes of irradiation without calcium (Fig. 8 & 9; Table 6). The effect of

Table 6

Buffer or Buffer along with Ca^{++}. $2H_2O$, (100 mM) was present during γ-irradiation.	Minutes of γ-irradiation		
	180	150	120
	O.D. Values		
Buffer+γ-irradiation+($-Ca^{++}$)+12±1°C	0.170	0.009	0.008
Buffer+γ-irradiation+($-Ca^{++}$)+37±1°C	0.202	0.035	0.031
Buffer+γ-irradiation+($+Ca^{++}$)+12±1°C	0.005	0.004	0.002
Buffer+γ-irradiation+($+Ca^{++}$)+37±1°C	0.041	0.019	0.012

Buffer or Buffer along with Ca^{++}. $2H_2O$ (100 mM) was present after γ-irradiation.	Minutes of γ-irradiation		
	180	150	120
	O.D. Values		
γ-Irradiation+Buffer+($-Ca^{++}$)+12±1°C	0.010	0.007	0.007
γ-Irradiation+Buffer+($-Ca^{++}$)+37±1°C	0.170	0.023	0.020
γ-Irradiation+Buffer+($+Ca^{++}$)+12±1°C	0.007	0.006	0.004
γ-Irradiation+Buffer+($+Ca^{++}$)+37±1°C	0.040	0.012	0.008

Effect of γ-irradiation in the presence of buffer or buffer with calcium and without buffer or calcium (they are added after the irradiation) on the efflux of betacyanin. The irradiated discs were kept at a temperature, either at 37±1°C or at 12±1°C for 120 minutes and then the efflux of betacyanin was noted. During the irradiation, the conical flasks containing the tissue slices were put in a jar containing crushed ice. The readings were the mean determinations of 4 values (Figures 8, 9 and 10).

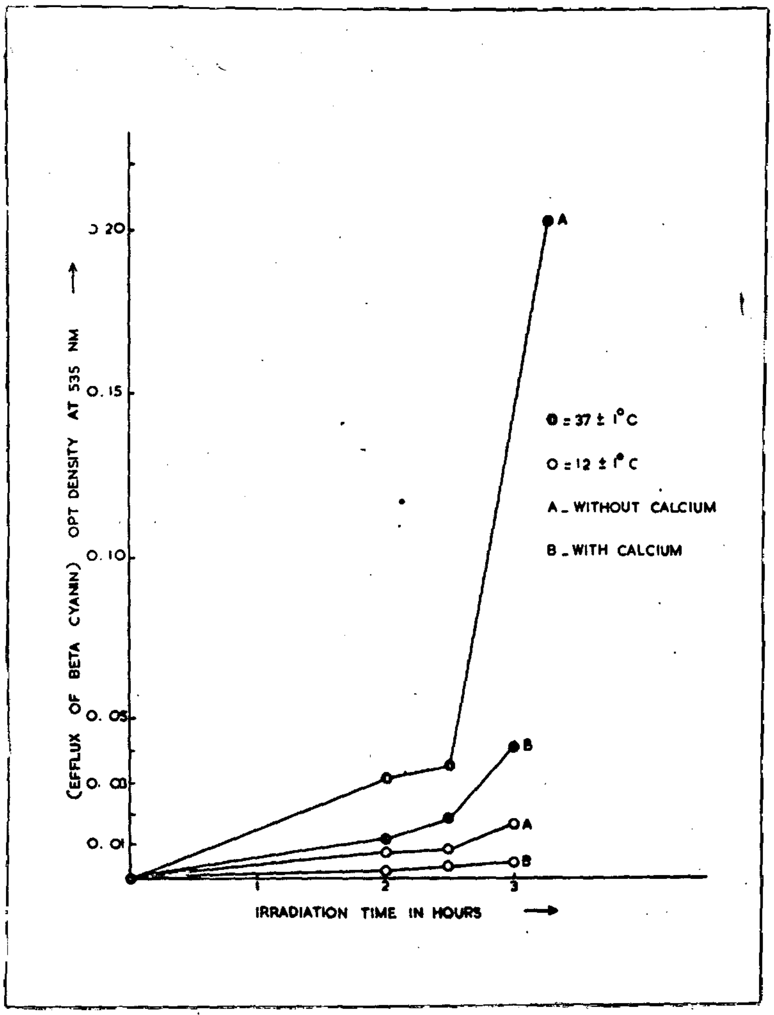

Figure 8. Effect of γ-irradiation in the presence of buffer (Partial cooling arrangement was made during the irradiation of the tissues). Efflux was measured after 2 hours of incubating the irradiated tissues either at 37±1°C or at 12 ±1°C.

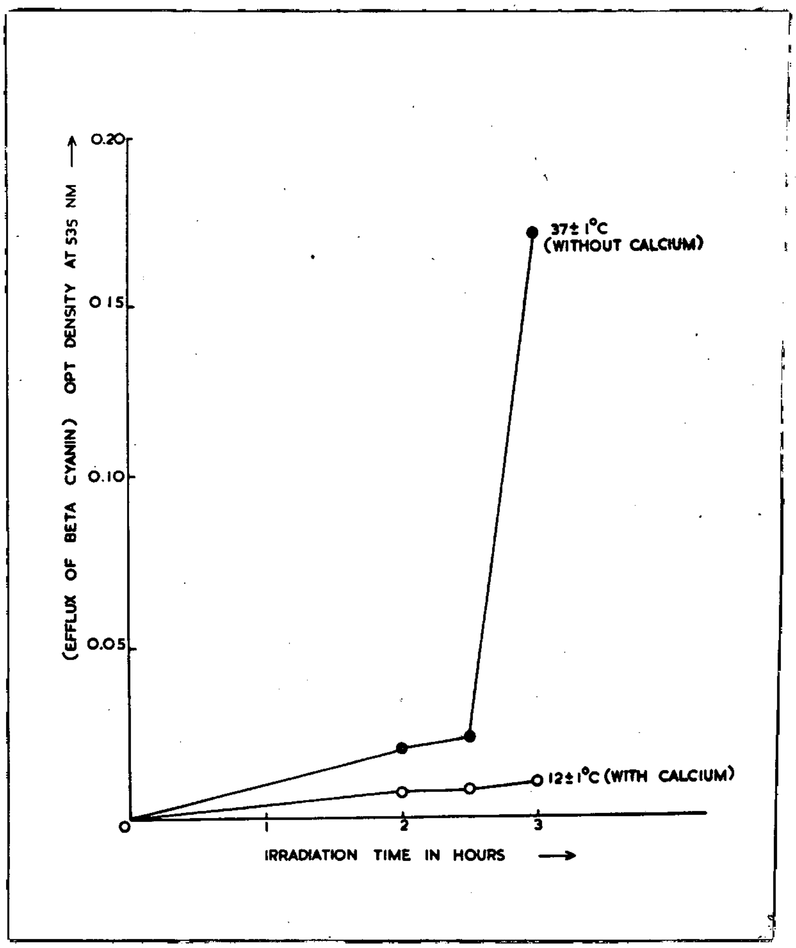

Figure 9. Irradiation without buffer. No Calcium Chloride was added after the irradiation (Partial cooling arrangement was made during the irradiation). Efflux was measured after incubating the irradiated tissue slices either at 37±1°C or at 12±1°C for 120 minutes.

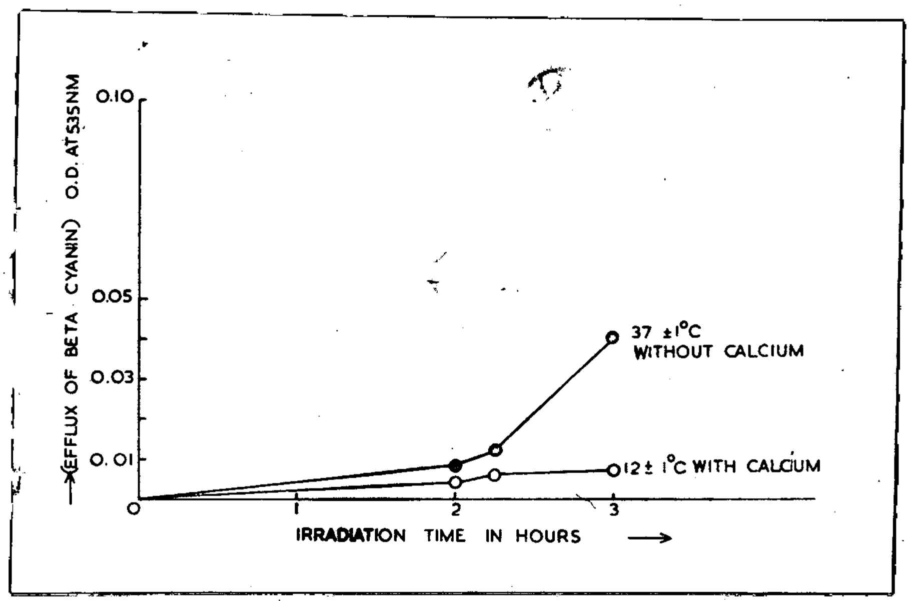

Figure 10. Irradiation in the absence of buffer (Partial cooling arrangement was made during the irradiation). $CaCl_2.2H_2O$ (100 mM) added after the irradiation. Efflux was measured after incubating the irradiated tissue slices either at 37±1°C or 12±1°C for 120 minutes.

irradiation was very low in the set kept at 12°C for 120 minutes immediately after the irradiation (Fig. 8, 9 & 10 and Table 6). Presence of calcium reduced the efflux of betacyanin and similarly irradiating the slices in the absence of buffer ('dry' irradiation) and adding buffer after irradiation reduced the effect of irradiation (Table 6; Fig. 8, 9 & 10).

CHAPTER - IV

DISCUSSION

The discussion has been divided into two parts under two main headings (i) temperature effects on membranes and (ii) effects of radiation on membranes. Under both the headings the interaction of ions with membranes and their ability to restore the lost semipermeability of membranes either due to the temperature stress or γ-irradiation, has been discussed.

Temperature effects on membranes:

Plenty of data are available on the effects of temperature on individual organisms but reports on the effects of temperature at the cellular level or molecular level, molecular thermobiology as it is termed, are not many. The discussion will focus the effect of heat on the permeability of beet root tissue slices. It will show how the observed effects are probably related to phenomenon occurring in or at the cell membrane level. And, also to elucidate the mechanism by which the metallic ions are stabilising the membranes against the thermal damage.

From the present experimental data (Table 1), it is evident that at elevated temperatures membranes of beet root tissues were getting disintegrated. This is because of the various secondary forces as evidenced by the possible solubilisation of membranes in certain solvents; hydrogen bonding, ionic and dipole interactions and Vanderwals forces that hold membrane components in place would be first destroyed at elevated temperatures. In comparison, the structure of individual proteins is stabilised by similar forces as well as by covalent links such as peptide bonds and disulfide bridges. It is to be expected that forces stabilising a special configuration of the polypeptide backbone of individual protein molecules are at least as strong as, if not stronger than those responsible for the mutual attachment of membrane components. From these considerations, the first step of heat injury could be the destruction of membrane integrity and not the denaturation of individual proteins. So from the present experimental results it is very obvious that with increasing temperature, membranes of the beet root discs were disintegrated. The disintegration of membranes resulted in the efflux of betacyanin. Efflux of betacyanin has been taken as an index to measure the integrity of the membrane.

The rate of haemolysis of erythrocytes in one group of animals (cat, dog, pig, sheep, ox) is shown to be a function of temperature. With a slight rise in temperature the time taken for haemolysis rapidly decreased (E.J. de Gier et al 1967). Bogen (1948) observed the loss of colour from Rhoeo discolor cells before any protoplasmic coagulation occurred. Lepeschkin (1935) showed than an increase in temperature damaged firstly, the membrane permeability and then coagulation of protoplasmic proteins occurred. Daniell et al (1969) have shown that soybean and elodea exposed to sublethal temperatures showed a loss of chlorophyll and swollen chloroplasts. Thermal shocks have been found to disintegrate the membranes of embryonic axes excised from red Kidney bean (Phaseolus vulgaris) (Siegel 1974). Studies on the thermal effects of beet root tissue have been conducted by Siegel (1969). Thus the integrity of membrane can be measured by the efflux of substances.

In the present study it has been observed that the efflux of betacyanin from the tissue slices was a function of both temperature and the time. From Figure 1, it was very clear that the damage caused to the tissues at higher temperatures in shorter period (about 30 minutes) would certainly be more severe, and injurious than the damage caused at lower temperatures for longer durations of time. The O.D. of betacyanin recorded when the tissue kept at 45°C for 120 minutes was 0.16 and that was 53% lesser than the O.D. of betacyanin recorded when the tissue was subjected to a temperature of 60°C for 30 minutes (i.e. 0.34 O.D.). Similarly, the rise in the efflux was slow at lower temperatures, whereas it was noticed that at higher temperatures the efflux of betacyanin was very fast (Fig. 1; Table 1).

Elevated temperatures damage the membrane in a different way than the freezing temperatures. At low temperature formation of ice crystals (either intracellular or extra cellular) is possible and thus the cells will be exposed to mechanical stress caused by the pressure or shearing force produced by growing ice crystals. Moreover, solutes become more and more concentrated and this increase in the salt concentration has a deleterious effect on the sensitive membrane. The situation is much simpler if cells are killed by heat. During brief heating, mechanical, dehydration and concentration effects can be ruled out leaving only the temperature increase. Thus the question may be limited to which of the cell constituents are sensitive and which are insensitive to elevated temperatures.

Low molecular weight cell constituents are not sufficiently heat labile to warrant extensive consideration. That does not necessarily exclude the temperature induced phase transitions which also involve low molecular weight material. But in the membranes mainly proteins, lipids and a little of carbohydrates are present. Among the high molecular weight cell constituents polysaccharides are rather thermostable as nucleic acids.

From the present experimental data (Table 2 and Figure 2) the efflux of betacyanin has become inhibited and was more or less constant when the tissue slices were treated at a temperature of 45°C for 30, 60 and 90 minutes and transferred to 25 ± 2°C. But the efflux started increasing and it was not constant when the tissue slices which have been incubated at 45°C for 120 and 150 minutes and transferred to 25 ± 2°C (Figure 2). Thus membranes receive a plastic strain (irreversible physical or chemical change) when they are subjected to a high temperature stress for a short time or low temperature stress for longer duration. Whereas a low temperature stress for short time may produce an elastic strain only (i.e. reversible change).

In order to explain these changes due consideration will be given here, as to how temperature could possibly alter the structure of proteins and lipids. In earlier literature, various theories like coagulation of protein (Lepeschkin 1912) denaturation of protein have been put forward to explain the nature of heat injury. It is well known that proteins are denatured by heat. A very high activation energy is needed for protein denaturation. The sensitivity of different proteins to high temperature varies and it is never the same. Alexandrov (1969) has found that the resistance to heat denaturation of the enzyme which perform the same function in different organisms varies according to the thermotropic adaptation of these organisms. Levitt (1962) observed that heat injury resulted in an increased SS content of the proteins at the expense of SH groups. When germinated wheat was heated to 40 - 80°C, the level of SH groups in the water soluble protein decreased and the SS:SH ratio increased. Morre (1970) has shown that proteins of soybean hypocotyl show maximum thermostability after 4 hours of incubation of the tissue with 10^{-5}M, 2-4D* and this was correlated with the decrease in the SH content, and therefore a decrease in ability to form intermolecular SS bonds on heating.

Chapman (1967) assumes that heat injury is related to the melting point of cellular lipids. It was shown by earlier workers, (Howell and Collins 1957; Fraenkel and Hopt 1940; and Terroine et al 1930) that in plants, insects and in microorganisms the proportion of unsaturated fatty acids was more when they were grown at low temperatures. Homeothermic organisms also contain greater proportion of unsaturated fatty acids in the surface of lipids if the environmental temperature is low. A study has been made by Johnston and Roots (1964) on the lipid fatty acids of gold fish acclimatised to various temperatures and they have observed that the total amount of lipids increased and there was an overall tendency for the degree of unsaturation of the fatty acids to increase. Such

*Dichlorophenoxy acetic acid.

studies suggest that acclimatisation involves the ability to control the degree of unsaturation of cellular lipids to maintain a specific liquid-crystalline state of cell membranes. This intermediate phase is stable over a characteristic temperature range. The information obtained on the effects of heat on pure phospholipids from various physical studies like infrared spectroscopic studies, differential thermal analysis studies, x-ray studies, NMR studies, electron microscopic studies has been summarised by Chapman (1967). From such studies he stated that "they all undergo a transition from crystalline to the liquid crystalline phase many degrees below the capillary melting point."

From the above discussion, it may be concluded that the rise in temperature no doubt damages the membrane permeability as has been observed in the beet root tissue slices with increasing temperatures. The damage is reversible when the tissues are subjected to a temperature of 45°C for 30 to 60 and 90 minutes and transferred to 25 ± 2°C. So this may be because of the phase transitions of the phospholipids i.e. from crystalline phase which shows a high degree of order to a liquid-crystalline phase. A loss of semipermeability at higher temperatures may be due to either excessive fluidity of the lipid bilayer or denaturation and aggregation of the membrane proteins leading to holes in the membrane. The membrane permeability may be irreversibly damaged when aggregated proteins are formed from denatured proteins (Levitt 1962) at higher temperatures. So the damage due to low temperature may be due to the changes in the organisational nature of the lipids and this is reversible whereas at high temperatures both lipids and proteins will be affected and the damage becomes irreversible. In general, the stress acts at a point where there is least resistance and the victim may be either a protein or lipid or both.

In the present study, it was found that the efflux of betacyanin which started with the rise in temperature from 25°C onwards could be inhibited by using various strengths of $CaCl_2.2H_2O$ solution. It has been noticed that calcium definitely reduced the membrane damage caused due to the rise in temperature. But the ionic strength of calcium in the medium also makes a difference. Solutions of 50 mM and 100 mM protected the tissue slices when the latter are incubated at 45°C for 180 minutes, from thermal damage more than either 200 mM or 12.5 and 25 mM solutions (Fig. 3). Moreover, besides the concentration of ions, the nature of ions (whether it was Ca^{++}, Zn^{++} or Mg^{++}) is also important because in the present study it has been noticed that among all the three essential elements Zn^{++} was found more protective than Ca^{++}. Magnesium was the least protective than either Zn^{++} or Ca^{++} against thermal damage of the membrane. Thus the observation tallies with various other reports where it was shown that various inorganic solutes alter the membrane permeability of roots and leaf tissues. Cations like calcium have been shown to restore the natural level of permeability to K -ions after EDTA treatment in the beet root tissue slices (Van Steveninck 1965). It has been observed by Siegel *et al* (1964, 65 & 70), Poovaiah *et al* (1976) that polycations NH_3 andalcohols induced leakiness in beet root membranes and the leakiness has been reverted to certain extent by calcium ions. Membrane damage caused due to the increased temperature stress and ultraviolet light are also shown to be considerably reduced with the use of $CaCl_2.2H_2O$ (Siegel 1969 & '74). Considerable evidence has also been accumulated to show that zinc increases the stability of various membranes (Chvapil 1973). Von Hippel and Wong (1964) have defined the relative effects of wide range of cations and anions altering the characteristics of proteins and other macromolecules. The solutes may involve in surface charges on the macromolecular surface, and charges in the water lattice interacting with the macromolecule.

So from the experimental data and the mechanisms proposed by others it is evident that at low temperature the state of lipids will be changed whereas at higher temperatures the denaturation and thus conformational changes in protein can be brought about which ultimately affect the membrane structure. So the addition of divalent cations like Zn^{++}, Mg^{++}, Ca^{++} and Pb^{++}, interact with proteins or lipids or with both of them to certain extent and stabilise the membrane.

Urbina and Chapman (1971) have investigated the interaction of different divalent cations on the endothermic phase transition of dipalmitoyl-lecithin and ox-brain phospatidyl serine. From monolayer and Liposome experiments Uranyl ions (UO_2^{++}) are known to act stoichiometrically with the polar groups of lecithin and to bind in the membrane surface of different biological membranes, presumably to phosphate groups. Chapman et al (1971) confirmed that strong binding of this ion is specifically to the phosphate groups by means of infrared spectroscopy. Hauser et al (1969) have also shown that phosphatidyl serine forms stoichiometric complex with Ca^{++} and other divalent ions. Those interactions have very strong effects on the phase transitions of both lipids i.e. dipalmitoyl lecithin and phosphatidyl serine. This is important because it shows how the temperature enhances the degree of lipid charge fluidity and thus related permeability of a lipid bilayer forming part of a membrane could be affected by metal-ion interaction with the polar head group. Gary-Bobo (1970) also showed that calcium could markedly reduce the permeability in the synthetic membrane of cephalin. Levin et al

(1973) have made synthetic vesicles with lecithin and through the use of NMR resonance measurements, they found that calcium and stronger destabilising solutes could greatly depress the permeability of vesicles, apparently through alterations of the lecithin and its interactions with surrounding water lattice.

Binding of calcium ions with proteins has been shown by Kretsinger (1976). Ljunger (1970) studying thermophilic bacteria concluded that calcium protects enzymes and other essential proteins of the cell against heat denaturation by forming links with protein molecules. Besides binding, there are also reports available where calcium was found useful in maintaining the stability of the enzyme, thermolysin against temperature stress.

In the present experiments it has been observed that magnesium and zinc are also found to be protecting the cell membrane from the thermal damage. Warren et al (1966) postulated that zinc stabilises the membrane reacting with the SH-groups of the membrane protein to form stable mercaptides. They arrive at this conclusion because membrane stabilisation is obtained by using other heavy metals and SH blocking agents. Levitt (1962) has shown that heat injury results in an increased SS content at the expense of SH groups. Petinov et al (1964) sprayed zinc sulphate on leaves and believed that foliar sprays of $ZnSO_4$ increase the heat resistance by lowering the respiratory quotient in causing accumulation of organic acids. Apart from these reports, there are not many evidences of zinc or calcium on the thermostabilisation of proteins or lipids. In case of magnesium it may be mentioned that although it is not as stabilising as Ca^{++} or Zn^{++} but it has certain amount of thermostabilising ability.

Pb^{++} ions have also been tried expecting they would enhance the damage of the membrane permeability at higher temperatures. But when the damage was measured in terms of the efflux of betacyanin, contrary to expectation, Pb^{++} (concentrations 2.5, 5 and 10 mM tried) inhibited the efflux of betacyanin and thus the thermal damage of the membranes was very much reduced. It may be the non-specific effect rather than the specific effect. When the buffer tricine to $Pb(NO_3)_2$ ratio was maintained 5:1, then the efflux was more inhibited than when it was 2:1. So possibly there may be moderate binding of Pb^{++} with tricine and the freely available ions to the tissue slice, would be less in the first case than in the second case. So far reports of lead on thermostabilisation of membranes have not been noticed.

So it may be concluded that these metallic cations Ca^{++}, Mg^{++}, Zn^{++} and Pb^{++} may form links with the RSH groups, thus, reducing the formation of SS bonds during heating and increase the thermostability of proteins. Similarly, it may also be possible that at lower temperatures, metallic cations may form links with the surface charges of the lipid molecules and bring stabilisation.

Radiation damage to membranes:

Research into the radiation induced lesions of biological membranes is being carried out not only in isolated structures but also at the cell and tissue levels and also in entire organisms. Some reports and reviews have appeared on the direct effect of radiation-induced damage to the membrane permeability (Singh et al 1974; Myers 1970; Wallach 1972; Pollard 1967 and 1968).

From the present study it is clear that beet root tissue slices are much more radioresistant than other animal tissues as the membranes could be disintegrated only when they have received a dose of 489 K. rads or more (Table 5) of γ-irradiation. Thus the result agrees with that of other workers who have shown that plant materials like onion scale (Biebl and Pape 1951) liverworts (Biebl and Url 1963), cyanophycean algae (Krans 1969) and carrot tissues (Echandi and Massey Jr. 1970) are more radioresistant than animal tissues and need high doses of irradiation to damage the membrane.

The leakage of betacyanin was immediate when the tissue was irradiated for 150 minutes and 180 minutes with a dose of 3.259 K.rads/minute. But with lower doses the effect was not immediate as it was found by Takamori et al (1968). They have found that release of lactate dehydrogenase from thymocytes irradiated at a dose of 1000 r takes place not immediately but after 3 hours of incubation of the cells in buffer at 37°C.

The leakage of betacyanin was almost doubled in the case of tissues which have been irradiated in the presence of buffer for 150 minutes and then incubated at 37°C for 120 minutes than the corresponding set kept at 29 ± 2°C. Moreover, the efflux was very much reduced when irradiation was done with some cooling device (Table 6, Figures 8, 9 and 10). So from this it is obvious that irradiation in presence of elevated temperatures would certainly be more damaging than with irradiation itself. It may be additive or synergistic.

Presence of buffer during the irradiation doubled the efflux of betacyanin in the set irradiated for 180 minutes and kept at 29 ± 2°C than its corresponding set which has been irradiated without buffer for 180 minutes and kept at 29 ± 2°C for 120 minutes (Table 6 and 7; Figures 6 - 10). Here the question of indirect effect is to be considered as most probably free radical formation takes place in the presence of the buffer.

Moreover the efflux of betacyanin from the beet root tissue to an aquoous medium was found to be both dose and dose-rate dependent as it was observed by Echandi and Massey Jr. (1970) in the carrot root tissues (Table 5 & 6).

Presence of $CaCl_2 \cdot 2H_2O$ (100 mM) at the time of irradiation definitely protected the membrane from radiation damage and leaching of the betacyanin has been considerably reduced (Table 5 and 6; Figures 6 - 10). Again, addition of calcium after the irradiation was also protective but relatively less so for the tissues, from radiation damage, then its presence during the time of irradiation.

It has been found by Biebl and Url (1968) that substances like $CaCl_2$ and KCl when applied after the irradiation protected the onion epidermis and decreased the injury due to the irradiation. Sab (1964) observed changes in the permeability of plasma membranes revealed by the plasmolysis of epidermal cells of γ-irradiated and non-irradiated bulbs of *Allium cepa* (L). In the presence of electrolytes (1 M and 0.5 M KNO_3) plasmolysis of irradiated cells was very slow than compared to the controls whereas plasmolysis of irradiated cells was more rapid in the presence of non-electrolytes than in the controls. In the case of *Neurospora* conidia, x-irradiation led to a post irradiation leakage of ^{32}P which was largely prevented by including calcium gluconate in the distilled water in which they were irradiated (Weijer 1961). Less leakage occurred after irradiation with 7200 R in the presence of calcium than its absence after irradiation with 240 R only. Rixon and Whitfield (1958) when working with the survival of x-irradiated rats, treated with parathyroid extract, stated that protection against radiation death might be due to the ability of hormones to increase serum calcium. Calcium was also found protecting the beet root tissue membranes from UV-damage (Siegel 1974).

In the case of irradiating the slices in the absence of buffer i.e. in the 'dry' state, the efflux was less which is consistent with the data of previous workers (Mookerjee 1959; Lea 1955). From the above studies, whether the effect was direct or indirect the simple method of distinguishing between the two was by the greater speed of the former. The effect may be direct in the tissues which have been irradiated without buffer because immediately after the irradiation when the buffer was added efflux of betacyanin has been noticed which increased with increase in the time of incubation at 37 ± 1° or 29 ± 2°C. The effect may be indirect in the tissues which have been irradiated in the presence of buffer because by the action of ionising radiation on water, free radical formation is possible. These intermediates are capable of diffusing through the cell and inactivating the vital molecules. Thus, direct injury may result from an instantly fatal plastic physical strain eg. a change in state leading to loss of semipermeability and indirect injury may be due to a slower chemical metabolic strain, that required the build up of an excess or a deficiency of a substance before becoming injurious. Siebl et al (1963b, 1968) called the rapid injury "direct" assuming that it damaged the cytoplasm directly and the slow injury "indirect" assuming that it injured the nucleus leading to a gradually increasing injury to the cell's metabolism. Based on the dose also it is possible to say whether the injury is direct or indirect. Low doses might be expected to cause direct injury if the irradiations were captured specifically by the essential components of protoplasm eg. proteins, nucleic acids or lipids. But this cannot happen because the ionising radiations are not absorbed specifically since all substances absorb them essentially radiolyse the water molecules and thus peroxide radicals will be formed. The latter are responsible for the damage of cell membrane. Larger doses of radiation have more probability of hitting the right components and bring the damage directly by changing them. The most likely type of direct injury both on the basis of speed and the large dose required to induce it, is a loss of semipermeability. Besides radiation, sometimes the rise in temperature during irradiation (secondary stress)is also inevitable)

In erythrocytes it has been shown that radiation induced increase in permeability is due to changes in protein-SH groups. Irradiation decreased SH-groups. That change is truly in passive permeability and not in active absorption as indicated by its occurrence when erythrocytes are irradiated (2000 - 20000 R) at 4°C (Shapiro et al 1966). Using SH blocking agents like PCMBS (Parachloromercury Benzene Sulfonic acid) produces similar effects as radiation. Shapiro et al (1966) suggested that the protein-SH groups on the cell surface and inside the cell are the radiation targets and that MEG (Mercapto ethyl guanidine) may reverse the radiation effect by reducing protein disulfide formed by radio-active oxidation of protein-SH groups. Peroxidation of lipids would also accur. This might precisely be the reason for more injury i.e. more efflux of betacyanin when the tissue slices were irradiated in presence of the buffer. The peroxide radicals formed due to the radiolysis of water must have reactedwwith lipids of the tissue membranes and the formation of lipid peroxides would have been the reason for the enhanced damage observed in the set containing the tissue slices irradiated along with buffer. But the mechanism by which calcium ions could protect the membrane from radiation damage is not very clearly known. It may be possible the ions would form links with the RSH groups and thus reduce the formation of disulfide groups at the expense of SH groups of the proteins and protect from radiation damage. Calcium may also prevent lipid peroxidation during radiation just like zinc which has been noticed by Chavapil _et al_ (1973) to inhibit the lipid peroxidation. Thus the relation of lipid peroxidation to the integrity of the membrane may also be determined using betacyanin leaking as an index of membrane integrity.

SUMMARY

Beet root tissue slices were subjected to various temperatures 25°, 27°, 30°, 37°, 45°, 55°, 60°C and has been noticed that with the rise in temperature from 27°C onwards the beet root cell membranes have been disintegrated which resulted in the efflux of betacyanin. The first step is the loss of semipermeability due to either elevated temperature or radiation. It is attributed to the loss of integrity of the membrane components rather than denaturation of proteins. The efflux of betacyanin has been taken as an index to measure the damage occured to the cell membrane either due to elevated temperatures or high radiations. The damage was more pronounced when the tissues were heated for a short period at high temperatures or at low temperatures for longer periods. To a certain extent at low temperature the damage occurred to the membrane is repairable and reversible and thus stress brings an elastic strain. This reversible change is attributed to the lipid phase transitions from crystalline to liquid crystalline state. Whereas at higher temperatures the damage of the membrane becomes irreversible and the stress produces a plastic strain. So the damage at higher temperatures may be due to either excessive fluidity of the lipids leading to disruption of the lipid bilayer or denaturation and aggregation of the membrane proteins leading to

holes in the membrane. The membrane permeability may be irreversibly damaged when formation of aggregated proteins result from denatured proteins.

Tissues were also subjected to γ-irradiation for 2 minutes to 3 hours with a dose rate of 3.259 K. rads/minute. Lower doses was found in effective to damage the membrane. From a dose of 489 K. rads onwards, the dose was found to be effective in damaging the membrane permeability instantaneously. The damage was more when the tissue slices were irradiated with buffer than without buffer.

Thermal damage or radiation damage to certain extent can be prevented or delayed by metallic cations like calcium, zinc, magnesium and lead ions. But the nature of the metallic cation, its ionic strength and its binding strength to the components of the membrane are important properties that are to be considered in order to achieve maximum protection against thermal damage or radiation damage of cell membranes. So any such agent which prevents or delays the damage to occur may play a decisive role in keeping cells alive during a temporary period at elevated temperatures or radiation.

The nature of the binding of the metallic cations to the intact membrane components and the exact mechanism of heat or radiation on the reversible and irreversible changes in membrane characteristics is not clearly known. But for few reports no definite mechanisms have been established as to how thermostabilisation or effect on the radiation induced damage of membrane phospholipids or proteins are occurring. There are few reports where cations like calcium have been shown as essential for maintaining the thermal stability of certain enzymes.

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