Transfer of D-C^{14} glucose from compartment A to compartment B

Time (min)	Compartment A_0	Compartment B_0	Compartment A_1	Compartment B_1	Compartment A_2	Compartment B_2
0	2010	26	2055	30	1822	122
5	1980	31	1791	131	1100	648
10	1895	35	1331	554	1095	813
15	1880	40	1195	646	748	1062
20	1840	98	622	1151	76	1778
25	1830	110	371	1204	68	1792
30	1790	130	58	1768	73	1759

Results are expressed as cps/0.1 ml. 0 = Black film with bombesin; 1 = biomembranes without bombesin; 2 = biomembranes with bombesin 10 µmoles.

Picofluor (Packard) and counted in a Beckman LS 100β counter. The above experiment was repeated with the addition of bombesin, in compartment A, in 3 different concentrations: 5, 10 and 20 µmoles.

Results. The results are summarized in the table only for bombesin 10 µmoles; No effects could be resolved below 5 µM; above 20 µM the response was saturated.

Plotting the values of the table on a graph: log (cps/0.1 ml) versus time we can calculate, i.e. from compartment A, the $t_{1/2}$. So we find that $t_{1/2}$ is 16 min 15 sec for the control and 12 min 30 sec for bombesin 10 µmoles. At present we are trying, with other peptides and other methods[9], to see if the increase in transport may be due to an action of the bombesin on the 'expansion' of the contractile GBP.

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Modification of radiation response by agents that elevate the intracellular c-AMP level[1]

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Summary. A study has been made of the effects of drugs known to elevate c-AMP level on radiation-induced damage in thymocytes. The test used was the ability of the cells to exclude dye. β-receptor stimulation and phosphodiesterase inhibition were found to induce radioresistance. The possible importance of the plasma membrane in connection with cytoplasmic factors is briefly discussed.

It has been reported that an elevated concentration of intracellular c-AMP correlates with a modification of the radiation response of mammalian cells in culture conditions[3]. The correlation is principally given by an elevation of the extrapolation number with increased intracellular c-AMP concentration. For bone marrow cells, an increased radioprotection by β-mercapto ethylamine has been reported in cells treated with agents known to increase c-AMP cellular levels[4,5]. Similarly, Scaife has obtained an inhibition of division delay induced by irradiation in thymic lymphocytes[6], although using a similar model in a mould, contradictory results have been reported[7]. The experiments quoted suggest that c-AMP is at least an indicator of metabolic situations that interfere with the radiation response of cellular systems. The present investigation was undertaken to test whether agents known to elevate c-AMP levels can interfere with mechanisms leading to radiation-induced loss of the capacity of thymocytes to exclude Trypan blue.

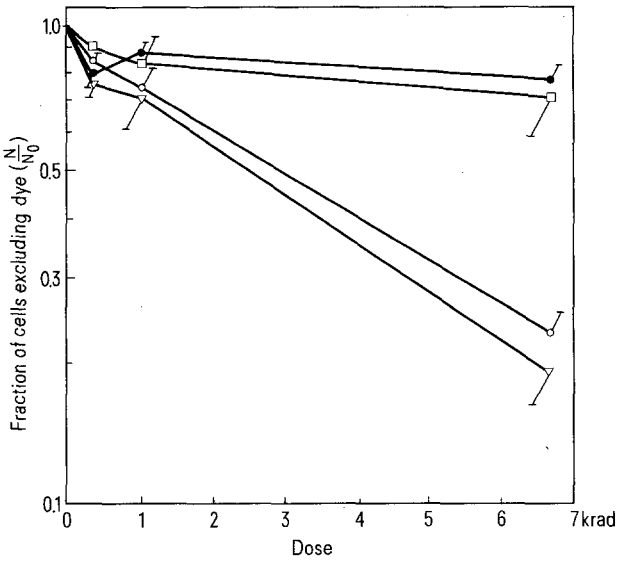
Thymic lymphoid cells from normal adult RK mice, 6 weeks old, from our colony, were used throughout the experiments. The cells were obtained by teasing the organs in sterile cold Hanks solution containing a low concentration (0.4 mM) of $CaCl_2$ and stabilized at pH 7.4 with Hepes buffer (N-2-hidroxyethylpiperazine-N-2-ethanesulfonic acid, Calbiochem., San Diego, California, USA). Different doses of irradiation were given with a Co^{60} gamma source at the dose rate of 200 rad/min as determined by ferrous sulfate dosimetry; the whole procedure was carried out in an ice bath and at a cell concentration of 2×10^6 cell/ml. After irradiation aliquots (including nonirradiated controls) were incubated at 37 °C for 30 min in the same saline medium plus Bovine Serum Albumin (BSA Miles Lab., USA).

Different pharmacological agents were added at the end of this period and incubated for 7 additional hours. Finally the function of the cell membrane was evaluated by the Trypan blue exclusion test. The drugs used in order to elevate the c-AMP level were Isoproterenol (Isuprel, Winthrop Labs, USA) 6×10^{-5} M and Aminophyllin (Aminofilina, Lab-Chile, Chile) at a concentration of 10^{-3} M. In control experiments, the treatment with isoproterenol was preceded by 5 min incubation with Propanolol (Inderal, ICI Macclesfield, Cheshire, U.K.) 10^{-4} M, in order to annul the stimulatory effect of isoproterenol by blocking the beta receptor sites.

As can be seen from the figure the beta-receptor stimulator Isoproterenol shows a marked protective action on irradiat-

ed thymocytes. As this can be abolished by the specific blocker Propanolol, it is suggested that the protective action of Isoproterenol is mediated by the β-adrenergic receptor. To test whether c-AMP is involved in this action, we tested the effect of the c-AMP phosphodiesterase inhibitor Aminophyllin on the radiation response of thymocytes; this substance shows the same effect as Isoproterenol.

Since the first common biochemical effect of both substances tested is an elevation of c-AMP levels, it is suggested that c-AMP, or biochemical processes linked to c-AMP, interfere with the processes leading to radiation-induced loss of the capacity to exclude dye. This test is thought to reflect radiation-induced interphase death, where the membrane plays an important role probably connected with events taking place in the cytoplasm. Regarding the membrane role in the process, a recent observation has been reported where irradiation affects the structural immunoglobulins of lymphocyte plasma membranes[8]. The above statements, together with the high radiosensitivity reported for peroxidation of artificial phospholipid membranes[9], suggest that the cell membrane may be important as the primary target for some radiobiological effects on lymphocytes. Great differences in the oxygen enhancement ratios for cortisone sensitive and cortisone resistant thymocytes[10] may be interpreted, according to Alper[11], as a further indication of the cell membrane participation in radiobiological effects on lymphoid cells. In this context, the present experiments showing the correlation between induction of high c-AMP levels and radioresistance are in line with the participation of membranes, although they emphasize the interdependence of membrane and cytoplasm with regard to the cellular radiobiological response.



Fraction of thymocytes able to exclude Trypan blue after irradiation and incubation in medium containing Isoproterenol (□), Aminophyllin (●), Propanolol-Isoproterenol (△), Control without additions (○). All points are referred to a corresponding treatment without irradiation. These controls have a percentage of cells excluding dye of at least 85%.

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2 We thank Dr E. Ortega for the irradiation facilities.
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Presence of auxin protectors in *Eriophyes* induced *Zizyphus* stem galls

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Summary. 3 auxin protectors, which were o-dihydroxyphenols of high molecular weight, were isolated from *Zizyphus* gall tissues. An increase in polyphenol oxidase activity in the gall tissue was observed which probably led to the production of high levels of auxin protectors. This prevented IAA oxidation, resulting in hyperauxinity and auxin-autotrophy.

There are many conflicting reports regarding the reasons for abnormal growth in plants, and the responses they show, in terms of altered hormone metabolism[1]. However, one feature which is common to almost all tumors is the simplification of growth requirements and the presence of high levels of endogenous growth substances. This communication is aimed at suggesting that unregulated synthesis of auxin protectors is responsible for hyperauxinity and auxin-autotrophy in *Zizyphus* gall tissues. Auxin protectors were first reported in *Pharbitis nil*[2]. In the present studies 3 auxin

Polyphenol oxidase activity (assayed by the method of Ponting and Joslyn[8]) in normal and gall tissues

	Normal	Gall (days)				
		10	20	30	40	50
Polyphenol oxidase activity (A/min/g fresh wt)	2.0±0.01	2.2±0.02	2.8±0.02	3.2±0.01	2.6±0.02	2.2±0.02

Values are means ± SE.