compares well with the value of 74° measured by X-ray in crystalline L-cystine[8]. A more difficult task is to gain pieces of information on the sign of the dihedral angle χ (CS-SC), i.e. on the chirality of disulfide bond. Indeed, when χ (CS-SC) is nearly 90°, 2 skewed conformations, named M (minus) and P (plus), are expected for a disulfide molecule[3,4]. Unfortunately, Raman bands seem to be insensitive to disulfide chirality, and on the other hand CD data are not totally unambiguous[2]. However, the ellipticity of GSSG ($[\Theta]_{260} = -1300$ deg/cm^2 decimole^{-1}) compares quite well with that of cystine ($[\Theta]_{253} = -1600$), suggesting that there is probably some predominance of the M conformation in solution at room temperature[3].

At this point a short comment is also necessary on the conformation about the C-S bond. A study on the methyl ethyl disulfide[11] and an investigation of several proteins[8] have indeed shown the existence of 3 rotational isomers, with similar dihedral angles χ (CS-SC) - about 90° - but with the different dihedral angles χ (SS-CC). These rotamers - designated A (with χ ((SS-CC) $\simeq$ 20-30°), B(90°) and T (180°) - can be partially distinguished by their ν (SS) frequency, since for the A conformation SS stretching appears at about 525 cm^{-1} and for the B and T conformations ν (SS) is observed in the range 510-520 cm^{-1} [8]. As we mentioned above, in GSSG ν (SS) is found at about 513 cm^{-1} and no Raman peak appears at 525 cm^{-1}, so we conclude that A rotamers are absent both in the solid and in solution, while neither B nor T conformations can be excluded. Finally we mention another correlation, proposed by Lord and Yu[6], between the Raman intensities of ν (CS) and ν (SS) bands and the bond angle CŜS. According to these authors an intensity ratio $I_{CS}/I_{SS} \lesssim 0.1$ indicates an angle CŜS $\simeq 115°$ while a ratio $\gtrsim 0.5$ corresponds to a bond angle of about 105°. For GSSG we observed $I_{CS}/I_{SS} \simeq 0.4$, which suggests that CŜS $\simeq 105°$. As the correlation is based only on a small number of model disulfides, this value has to be taken with caution but, on the other hand, it is characteristic of cystine-related compounds[8].

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Effect of bombesin on glucose transport system in biomembranes

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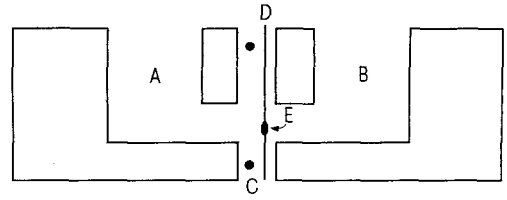
Summary. Bombesin is able to stimulate the glucose transport system supported by a contractile glucose binding protein (GBP) in biomembranes. The increase of glucose transport indicates that bombesin affects GBP.

Previous results indicate that the glucose transport system in biological membranes is supported by an expanding and contracting glucose binding protein (GBP)[1-3] according to the theoretical model of Blumenthal and Katchalsky[4]. The aim of the present communication is to study whether bombesin interacts with GBP in biomembranes.

Bombesin is a tetradecapeptide occurring in the skin of the European discoglossid frogs *Bombina bombina* and *B. variegata*. This polypeptide displays a complex spectrum of effects on gastric and pancreatic secretions, on gallbladder motility, and on gut electrical and mechanical activity. The peptide is also active on other target organs and tissues outside the gastrointestinal tract[5,6]. All these actions suggest a probable effect of bombesin on GBP.

Materials and methods. The experiments were performed with the perspex apparatus shown in the figure, where A = starting compartment, volume 3.0 ml; B = migration compartment, volume 3.0 ml; C = 'O' ring; D = polyethylene disk (PE disk = 1.5 cm diameter with 1 mm central hole). Black films or biomembranes were deposited in the central hole of the PE disk. The formation of black films (bimolecular lipid membranes in aqueous solutions) was obtained, according to the experimental methods first introduced by Mueller et al.[7], from a 0.5% solution of phosphatidyl inositol and a 1.5% solution of phosphatidyl choline in n-decane. Biomembranes (black membrane plus GBP) were obtained by stratifying GBP (aqueous solution, 10 mg protein/ml) on the surface of the black film according to the techniques of Mueller and Rudin[8]. Optical controls were performed previously to verify that the membrane remained black, or black plus GBP, during the course of the experiment[1,3]. The disk was now assembled with the compartments A and B. In compartment A 2.5 ml of phosphate buffer and 50 µl of an aqueous solution of D-C^{14} glucose were introduced. In the compartment B 2.5 ml of phosphate buffer without D-C^{14} glucose was introduced. Samples (0.1 ml) were simultaneously collected from both compartments, at 0, 5, 10, 15, 20, 25, 30 min, dissolved in



Perspex apparatus used for transport experiments. A = Starting compartment; B = migration compartment; C = 'O' ring; D = poliethylene disk; E = central hole.

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-