1 We thank Prof. Y. Ishida, Gunma University School of Medicine, Dr K. Iwata, Kochi Prefectural Central Hospital, Dr M. Motoi, Okayama University Medical School, Dr H. Enzan, Kochi Medical School, for supplying the material.

2 Moore, B.W., Biochem. biophys. Res. Commun. *19* (1965) 739.
3 Nakajima, T., Yamaguchi, H., and Takahashi, K., Brain Res. *191* (1980) 523.
4 Stefansson, K., Wollmann, R.L., Moore, B.W., and Arnason, B.G.W., Nature *295* (1982) 63.
5 Cocchia, D., Michetti, F., and Donato, R., Nature *294* (1981) 85.
6 Takahashi, K., Yamaguchi, H., Ishizeki, J., Nakajima, T., and Nakazato, Y., Virchows Arch. B Cell Path. *37* (1981) 125.
7 Rausch, E., Kaiserling, E., and Goos, M., Virchows Arch. B Cell Path. *25* (1977) 327.
8 van Ewijik, W., Verzijden, J.H.M., van der Kwast, Th.H., and Luijcx-Meijer, S.W.M., Cell Tissue Res. *149* (1974) 43.
9 Silberberg, I., Baer, R.L., and Rosenthal, S.A., J. Invest. Dermat. *66* (1976) 210.
10 Veldman, J.E., Ph. D. Thesis, Groningen 1970.
11 Sternberger, L.A., Hardy, P.H., Ciculis, J.J., and Meyer, H.G., J. Histochem. Cytochem. *18* (1970) 315.
12 Goos, M., Arch. derm. Res. *255* (1976) 215.
13 Janossy, G., Thomas, J.A., and Habeshaw, J.A., J. Histochem. Cytochem. *28* (1980) 1207.

Specific interaction between soybean agglutinin and lipid bilayers containing the GM_1 ganglioside

M. Rochus, G. Kayser, M. Deleers and J.M. Ruysschaert[1]

Laboratory of Macromolecules at Interfaces, Faculty of Sciences, Bd du Triomphe, and Laboratory of Experimental Medicine, Faculty of Medicine, Brussels University, B-1050 Brussels (Belgium), 14 December 1981

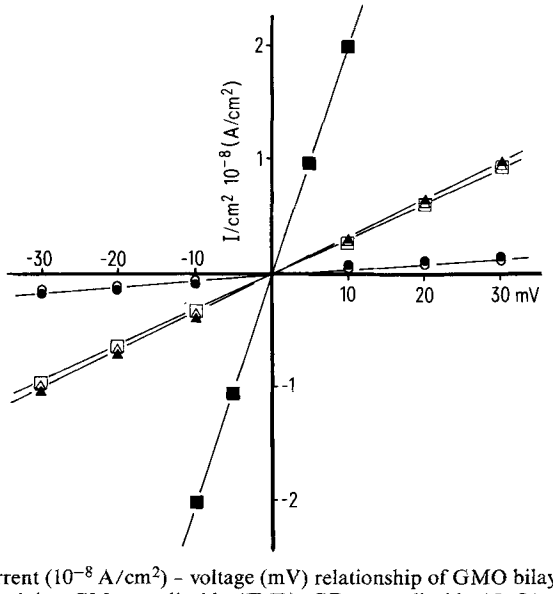
Summary. A specific interaction was demonstrated between glycerolmonoleate bilayer membranes containing the GM_1 ganglioside and soybean agglutinin. Electrical conductance changes are discussed in terms of ganglioside clustering in the bilayer.

Plant lectins agglutinate cells by binding to the plasma membrane and forming cross-bridges between cells[2,3]. Significant progress has been made in recent years in the isolation of glycoproteins containing receptors for sugar-specific plant lectins. However, it has recently been suggested that glycolipids could be implicated in this recognition process[4-8]. We demonstrate here that soybean agglutinin interacts specifically with GM_1 ganglioside incorporated into a planar lipid bilayer by measuring the electrical conductance changes observed after addition of the lectin in the bathing solution. This permeability change may initiate membrane events (activation processes) by providing hydrophilic pores for the influx of cations.

Material and methods. Soybean agglutinin (SBA) (type VI), glycerolmonoleate (GMO) were purchased from Sigma Chemical Co. GM_1 ganglioside (galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide), GD_{1a} ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide) and GT_1 ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl-N acetylneuraminyl)-galactosylglucosylceramide were Supelco products. The mixtures of GMO-gangliosides (98/2 molar ratio) were dissolved in $CHCl_3$-methanol-decane (30/5/65). The bilayers were formed on a 1.3 mm diameter aperture in a Teflon cell separating 2 aqueous phases (2.5 cm^3), as described elsewhere[9-11]. The membrane conductances were determined by measuring the specific current (I) as a function of imposed potential differences (V). The aqueous phase contained $CaCl_2$ 1 mM, Tris-HCl 10^{-2} at pH 7.2.

Results and discussion. The figure shows a marked change in lipid membrane conductance before and after addition of soybean agglutinin in the aqueous phase bathing the bilayer containing GM_1 ganglioside. No conductance changes were observed when GD_{1a} and GT_1 were incorporated into the bilayer. This effect is not the consequence of a nonspecific penetration of the agglutinin into the lipid bilayer. Indeed, soybean agglutinin does not affect the conductance of a pure GMO bilayer.

It has been shown[7] that agglutination of liposomes containing erythrocyte lipids is inhibited in the presence of N-acetylgalactosamine. Our results indicate that the neuraminic acid attached to the terminal galactose strongly inhibits the recognition of the N-acetylgalactosamine present in the GT_1 and GD_{1a} gangliosides. A similar observation was made several years ago by Novogrodsky et al.[12] with cells treated with neuraminidase. Indeed, after neuraminidase treatment, they had an increased number of receptors. A possible explanation of this conductance change is that the ganglioside-lectin interaction modifies the lectin conformation and induces its penetration into the lipid bilayer. It



Current (10^{-8} A/cm^2) - voltage (mV) relationship of GMO bilayers containing GM_1 ganglioside (□ ■), GD_{1a} ganglioside (○ ●) and GT_1 ganglioside (△ ▲), in the absence (open symbols) or in the presence (filled symbols) of soybean agglutinin (200 µg/ml).

must be admitted that this insertion of an hydrophobic lectin segment in the bilayer could be reversed by addition of a specific inhibitor like N-acetylgalactosamine. Indeed, as demonstrated by Rendi et al.[7] liposome agglutination could be reversed. Alternatively, our results could be explained in terms of ganglioside organization in the lipid matrix. It has been demonstrated that gangliosides are randomly distributed in a fluid lipid matrix[13]. Soybean agglutinin may induce ganglioside clustering and the permeability change may simply result from the formation of hydrophilic pores of GM_1 gangliosides without penetration of the lectin into the lipid bilayer. The lateral mobility of the gangliosides will determine the reversibility of the agglutination process. In model membranes, ganglioside clustering strikingly increases the membrane permeability. Moreover, it has been recently proposed that lentil lectin induces the existence of reversible microclustering of membrane receptors in HeLa cells[14].

Finally, although GM_1 ganglioside appears to be the receptor of cholera toxin[15,16] and of *Ricinus communis* toxin[8], it would be premature to attribute GM_1 as a lectin receptor before demonstration that cells lacking GM_1 could be made agglutinin-responsive after incorporation of GM_1 in their membranes.

1 Reprint requests to J.M.R., Faculté des Sciences-Chimie-Physique des Macromolécules aux Interfaces, Université libre de Bruxelles, Campus Plaine-Accès 5, Blvd. du Triomphe, B-1050 Bruxelles (Belgium).
2 Lis, H., Sharon, N., and Katchalski, E., Biochim. biophys. Acta *192* (1969) 364.
3 Inbar, M., and Sachs, L., Proc. natl Acad. Sci. USA *68* (1971) 274.
4 Surolia, A., Bachhawat, B.K., and Podder, S.K., Nature *257* (1975) 802.
5 Redwood, W.R., and Polefka, T.G., Biochim. biophys. Acta *455* (1976) 631.
6 Deleers, M., Poss, A., and Ruysschaert, J.M., Biochem. Biophys. Res. Commun. *72* (1976) 709.
7 Rendi, R., Kuettner, C.A., and Gordon, J.A., Biochem. biophys. Res. Commun. *72* (1976) 1071.
8 Kayser, G., Goormaghtigh, E., Vandenbranden, M., and Ruysschaert, J.M., FEBS Lett. *127* (1981) 207.
9 Poss, A., Deleers, M., and Ruysschaert, J.M., FEBS Lett. *86* (1978) 160.
10 Chatelain, P., Deleers, M., Poss, A., and Ruysschaert, J.M., Experientia *35* (1979) 334.
11 Deleers, M., Chatelain, P., Poss, A., and Ruysschaert, J.M., Biochem. biophys. Res. Commun. *89* (1979) 1102.
12 Novogrodsky, A., and Katchalski, E., Proc. natl Acad. Sci. USA *70* (1973) 2515.
13 Delmelle, M., Dufrane, S.P., Brasseur, R., and Ruysschaert, J.M., FEBS Lett. *121* (1980) 11.
14 Schreiber, A.B., Hoebeke, J., Vray, B., and Strosberg, A.D., FEBS Lett. *111* (1980) 303.
15 Cuatrecasas, P., Biochemistry *12* (1973) 3547.
16 Tosteson, M.T., and Tosteson, D.C., Nature *275* (1978) 142.

Colicin E3 enhances the oxidoreductive activity of guinea-pig leucocytes

J. Lokaj, J. Šmarda[1] and J. Mach

Department of Microbiology and Immunology and Department of Biology, Medical Faculty, J.E. Purkyně University, CS-66243 Brno (Czechoslovakia), and Institute of Sera and Vaccines, CS-10103 Praha (Czechoslovakia), 19 March 1981

Summary. Colicin E3 increased the capacity of peritoneal exudate leucocytes to reduce iodo-nitro-tetrazolium-chloride to formazane. This effect was directly dependent on its concentration within the range 10^3-10^5 lethal units per cell. In control experiments, dectran C exerted no influence on the rate of this acitivity and colicin E3 did not convert INT to formazane.

Colicin E3 evokes distinct biological effects on mammalian cells in vitro. At suitable concentrations, it kills human epithelial cells of the line HeLa and mouse fibroblasts of the line L[2]. It also decreases incorporation of radioactively-labeled precursors into DNA and RNA in murine leukemia cells of the line P388[3]. Furthermore, it profoundly inhibits the concanavalin A-promoted mitogenic activation of mouse T-lymphocytes and interferes with the homing of murine lymph node lymphocytes to the lymph nodes, without altering their migration to the spleen and liver[4]. At a low concentration, colicin E3 stimulates the proliferation of the murine leukemia cells P388[3].

In the present experiments, the action of colicin E3 on phagocytes was checked. We chose these model cells for 2 reasons: 1. they react very sensitively even on subtle changes in their medium, these reactions being detectable by rather simple methods; 2. the influence of colicin E3 on them would stimulate the consideration of a possible role of bacteriocins in inflammatory lesions in vivo.

Materials and methods. Exudate cells were gained by rinsing the peritoneal cavity of guinea-pigs with Hanks solution (Oxoid), following 18 h after i.p. appplication of 20 ml sterile 1% glycogen solution in physiological saline. Cells were washed 3 times in Hanks solution and resuspended in Hanks at 1×10^7 cells per ml. Viability was determined with trypan blue; differential counts were performed in preparations stained with methyl green and pyronine according to Unna-Pappenheim.

Colicin E3, a highly purified substance (product of the Institute of Sera and Vaccines, Praha), was used. Its purity was checked as described previously[2]. The freeze-dried preparation was dissolved and diluted in distilled water (for injections) immediately before each experiment. The activity of stock solution (containing 17.5 mg colicin substance per ml) was 10^5 arbitrary units (i.e. about 2×10^{12} lethal units per ml), using the sensitive strain *Escherichia coli* C6 as indicator.

Tetrazolium-reductase activity was determined by INT-test[5]. To 0.2 ml of 0.1% iodo-nitro-tetrazolium-chloride Lachema (INT) solution in phosphate buffer (pH 7.6), 0.1 ml of colicin solution of given concentration (or solution of a control substance), 0.5 ml of Hanks solution and 0.2 ml of cell suspension were added. The activity of colicin E3 ranged from 1×10^0 lethal units per cell to 1×10^5 lethal units per cell. After 45 min and 90 min incubations at 37 °C (in a water bath, with occasional stirring), the reaction was