Cross reactivity of purified IgG from anti-I_2Tyr-I_2Tyr antisera. 5×10^{-12} g I_2Tyr-I_2Tyr in 0.1 ml of 0.05 M phosphate buffer (pH 7) was incubated in hemolysis tubes with 0.1 ml of purified IgG from L_{10} antisera at the dilution 1:5000, 0.1 ml of 0.05 M phosphate buffer (pH 7) containing 5% ligand-free rabbit serum, and 0.1 ml of 0.05 M phosphate buffer (pH 7) containing 5×10^{-10} to 5×10^{-8} g of thyroxine (T_4), 3,5,3′ triodo-l-tyrosine (T_3), 3-iodo-l-tyrosine (ITyr) or 3,5-diiodo-l-tyrosine (I_2Tyr). The incubation and the separation of the free antigen from the bound antigen were performed as described previously.

Results. Binding capacity was measured on whole antisera. Four rabbits (L_1, L_3, L_4 and L_{10}) gave significant amounts of anti (I_2Tyr-I_2Tyr) antibodies. The immunoglobulins were separated by elution on DEAE-Sephacel from these four whole antisera. Binding capacity was measured for each class of immunoglobulin. The values given in table 1 are expressed in g of I_2Tyr-I_2Tyr per g protein. The binding capacity for the whole antisera ranges from 0.93×10^{-11} for the rabbit L_3 to 0.68×10^{-7} for the rabbit L_{10}. The difference reaches 10^4. The 1st fraction obtained from DEAE-Sephacel with each antiserum shows the best binding capacity for I_2Tyr-I_2Tyr. The 2nd fraction has a weaker binding capacity. The 3rd fraction does not bind specifically to the I_2Tyr-I_2Tyr. Table 2 shows the affinity contants (K_a) for different antisera, which range from 9.2×10^{10} (M^{-1}) for rabbit L_4 to 6.2×10^{13} (M^{-1}) for L_{10}. The difference is about 3×10^3 (fig. 1). Cross-reactivity with other iodinated amino acids was measured. The specificity of the antibodies was determined by measuring the displacement of the binding of the $^{125}I_2$Tyr-$^{125}I_2$Tyr to the immunoglobulins by nonlabeled I_2Tyr-I_2Tyr, I_2Tyr, T_4, T_3 and ITyr. Results are expressed in figure 2. So, more (10^5) I_2Tyr had to be added and more (10^6) ITyr, T_4 or T_3 than the nonlabeled dipeptide to reach the same inhibition level.

Discussion. The difference in the binding capacity in g of I_2Tyr-I_2Tyr per g protein reaches 10^4, between the four whole antisera tested. This corresponds to the heterogeneity obtained in the response when rabbits are immunized. However, the purification of immunoglobulins by ion exchange chromatography on DEAE-Sephacel allows a considerable increase in the binding capacity of IgG to I_2Tyr-I_2Tyr. These results are obtained for the four antisera. L_3 and L_{10} antisera have the best binding capacity in the IgG fraction, about 10^{-5} of I_2Tyr-I_2Tyr per g protein. The second fraction obtained on DEAE-Sephacel contains IgG, IgA and serum albumin. The third fraction contains the IgM. These two last fractions have a lower binding capacity with I_2Tyr-I_2Tyr than the first fraction which corresponds to most of the eluted IgG. The affinity constants obtained for whole antisera and for the first and second immunoglobulin peaks eluted on DEAE-Sephacel were identical. The whole antisera affinity constants seem to correspond to the IgG eluted in peak 1, and, secondly, to the IgG eluted in peak 2. An affinity constant from the third peak could not be determined because the eluted IgM had too weak a binding capacity with I_2Tyr-I_2Tyr. The antisera obtained by use of this original carrier were very specific for the I_2Tyr-I_2Tyr and the purification of immunoglobulins improves their specificity. Displacement studies using iodothyronines and iodotyrosines have not shown cross-reactivity that would be incompatible with the dosing of this hapten in biological media, where this compound is present in low concentrations, as opposed to the I_2Tyr, ITyr and iodothyronines.

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3 Michelot, J., Madelmont, J.C., and Meyniel, G., C.r. Acad. Sci. *276* (1973) 1357.
4 Bernard, D., Nicolas, C., Maurizis, J.C., and Bétail, G., J. immun. Meth. *61* (1983) 351.
5 Maurizis, J.C., Nicolas, C., and Michelot, J., J. Label. Compd Radiopharmaceuticals *17* (1979) 901.
6 Mollison, P.L., Blood transfusion in clinical medicine. Blackwell Scientific Publications, Oxford 1975.
7 Lowry, Q.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
8 Scatchard, G., Ann. N.Y. Acad. Sci. *51* (1949) 660.

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Lectin receptors in snail proteogalactans. II. *Archachatina marginata* and *Achatina achatina* (preliminary report)

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Summary. The lectin receptor sites on the proteogalactans from the albumin glands of West African land snails, *Archachatina marginata* and *Achatina achatina* have been studied by precipitin reactions using the agar-gel double diffusion technique with various lectins. The proteogalactans from both snails have predominantly terminal β-D-galactose structures; but they show characteristic differences in the topographical features at the surfaces of the carbohydrate structures presumed to be compatible with the combining site for these lectins.

Key words. Snail, land; *Archachatina marginata; Achatina achatina;* albumin gland; lectin receptors; proteogalactans.

Different receptors for heterophile lectins of plant, invertebrate and microbial origin have been identified on the proteogalactans extracted from the albumin glands of the snail *Achatina fulica*[1-3]. Most of these lectins are galactose specific[4]. This paper reports preliminary investigations on the proteogalactans from other related snail species, *Archachatina marginata* and *Achatina achatina.*

The proteogalactans were extracted from the albumin gland tissue with saline and 4 M guanidinium hydrochloride, then fractionated as described earlier[5]. The results of agarose double-diffusion experiments with lectins and the semi-purified proteogalactans are shown in the table.

Strong precipitin lines were formed by the lectins from *Bauhinia purpurea* alba seeds and peanut, against the proteogalactans from both snail species. The precipitin lines exhibited close identity indicating similar combining sites for these lectins. Whereas, the disaccharide D-Gal β(1→3) D-GalNAc has been considered to be the dominant receptor structure for the peanut lectin[6], the structure, D-Gal β(1→3) D-GalNAc β(1→3) D-Gal was proposed for the *Bauhinia purpurea* lectin[7].

This finding suggests that the proteogalactans in these snail species contain predominantly ($\beta 1 \rightarrow 3$) galactosidic linkages. The lectin from *Triticum vulgaris* which binds D-GlcNAc specificically gave strong precipitin lines against the proteogalactan from *Archachatina marginata* but failed to react with that of *Achatina achatina*. The amino acid analysis of the former proteogalactan (carried out on the amino acid autoanalyzer model Durrum D-500) confirmed the presence of glucosamine in the sample: this constituted roughly 1.1% on a molar basis. However, only trace amounts of this hexosamine (0.1%) were found in *Achatina achatina* proteogalactan. Moreover, the lectins from *Ricinus communis* I, and *Phaseolus vulgaris* which are also specific for β-galactosyl groups formed visible precipitin lines against the proteogalactan from *Arachachatina marginata* only. Whereas, these lectins failed to react against *A. achatina* proteogalactan, the lectin *Abrus precatorius* which is specific for D-Gal $\beta(1 \rightarrow 4)$ gave strong precipitin lines against the proteogalactan. Unfortunately, other non-galactose specific lectins were not available for further investigation.

The precipitin lines do represent the lectin-glycoconjugate complexes. The unspecific reactions which may occur in this Ouchterlony agar-gel technique, as discussed by Uhlenbruck and co-workers[8], have been excluded in this investigation by control experiments. Moreover, highly purified lectins from commercial sources (Medac) were employed in this investigation. Accordingly, the interaction between the lectin and the proteogalactan can be assumed to be specific.

The results obtained in this investigation therefore indicate that the proteogalactans from the 2 snail species most probably differ structurally, as shown by the partial differences in the lectin receptor site topography. These findings await confirmation from a detailed chemical analysis of the proteogalactans which is now in progress.

Precipitin reactions in agar-gel of proteogalactans from the albumin glands of *Archachatina marginata* and *Achatina achatina* with various lectins

Lectin	Origin	Specificity	Reaction with proteogalactan from *A. marginata*	*A. achatina*
Arachis hypogaea (peanut)	P	D-Gal β (1→3) D-Gal NAc	+ +	+ +
Bauhinia purpurea	P	D-Gal β (1→3) D-Gal NAc β (1→3) D-Gal	+ +	+ +
Viscum album	P	D-Gal β (1→?)	0	0
Abrus precatorius	P	D-Gal β (1→4)	0	+ +
Helix pomatia (HPA)	I	D-GalNAc	0	0
Ricinus communis I (RCA_{120})	P	D-Gal β (1→?)	+	0
Phaseolus vulgaris	P	D-Gal β (1→4)	+	0
Triticum vulgaris (wheat germ agglutinin)	P	D-GlcNAc	+ +	0

+ +, Precipitin lines; 0, no visible reaction; P, plant; I, invertebrate.

1 Uhlenbruck, G., Steinhausen, G., and Kareem, H.A., Z. Immun-Forsch. exp. Ther. *152* (1976) 220.
2 Uhlenbruck, G., Steinhausen, G., Geserick, G., and Prokop, O., Comp. Biochem. Physiol. *59B* (1978) 285.
3 Okotore, R.O., and Uhlenbruck, G., Experientia *38* (1982) 507.
4 Uhlenbruck, G., Naturwissenschaften *68* (1981) 606.
5 Okotore, R.O., Klein, P.J., Ortmann, M., and Uhlenbruck, G., Comp. Biochem. Physiol. *70B* (1981) 469.
6 Lotan, R., Skutelsky, E., Danon, D., and Sharon, N., J. biol. Chem. *250* (1975) 8515.
7 Wu, A.M., Kabat, E.A., Gruezo, F.G., and Allen, N.J., Archs Biochem. Biophys. *204* (1980) 622.
8 Uhlenbruck, G., Chatterjee, B.P., and Shuldes, U., Z. Naturf. *33C* (1978) 442.

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Membrane resting potentials in cultured mouse neuroblastoma cells

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Summary. Membrane resting potentials (MRP) were measured systematically in cultured mouse N_2A neuroblastoma cells: 1) in the logarithmic growth phase; 2) in subconfluent cultures; 3) in confluent cultures; 4) after dBcAMP had induced morphological differentiation. Neurite extension was accompanied by a significant increase in MRP as compared to the appropriate controls. No significant differences in MRP were observed with regard to the different growth phases.
Key words. Mouse neuroblastoma culture; membrane resting potential; neurite extension.

The presence of a membrane resting potential (MRP) is one of the essential features of living excitable cells. Adequate measurements in vivo, however, are often very difficult[1]. The search for accessible working models has led to the introduction of the mouse neuroblastoma cell lines in neurophysiological research[2]. These cell lines are derived from a malignant neuronal tumor, and are rather easily grown in vitro in monolayer culture[3]. Their use in morphological and physiological studies raises the question as to how far results obtained with these malignant cells can be extrapolated to normal neurons. This report presents the results of a systematic study of the MRP in a neuroblastoma cell line in relation to cellular growth and differentiation.

Materials and methods. The N_2A neuroblastoma cell line was grown in costar flasks (25 cm^2) in a single batch of DMEM-medium (5% fetal calf serum) buffered with 5% CO_2 in 100% relative humidity containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Antibiotics were omitted for 24 h prior to the experiment. Some of the cells were cultured in the presence of 1 mM dBcAMP dissolved in serum free DMEM, added 24 h