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.

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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

after seeding. Cells cultured without any additive served as control. Differentiation was maintained for 48 h. Cell numbers in the flasks were obtained by counting the attached cells within the surface of a photo ocular. At least 10 fields per flask were counted. For electrophysiological measurements, flasks were rinced thoroughly with serum free medium and then broken, leaving the side with the cells intact. This side was positioned on an inverted microscope (Zeiss invertoscope D, phase contrast). MRP's were measured at room temperature after penetrating the cells with conventional glass microelectrodes filled with 3 M KCl solution (resistance of 8–10 MΩ) connected to an operational amplifier. The resulting signals were recorded on a Kipp recorder. For calculations, stable values for the membrane potential, obtained 30 sec after penetration of the cell, were used.

In order to check the stability of the measurements, MRP's in every 10th cell were registered for 10 min (first control). The effect of MRP measurements on stability was examined by repenetrating every 10th cell four times (second control). Selection of cells used for the measurement of the MRP was based on cell size. For this purpose, the mean cell diameter in a given population was estimated using a calibrated photo ocular. Only cells with a diameter deviating less than 20% from this mean value were used.

During a series of measurements the glass electrodes were replaced after 2–3 registrations in order to avoid artefacts due to the development of tip potentials as a consequence of microfractures at the tip of the electrode.

Viability of the cells was monitored by trypan blue exclusion. The values recorded are expressed as mean ± SEM. The differences between mean values were analyzed by Student's t-test for paired data and were considered significant when p-values were less than 0.005.

Results. It was found that N_2A cells, 24 h after seeding, proliferated according to a 'logarithmic growth curve' and possessed a MRP value of 28.4 ± 3.7 mV (n = 25). 24 h later, i.e. 48 h after seeding, a 'subconfluent' culture was obtained and a slightly lowered MRP value was measured (24.4 ± 0.6 mV, n = 60). 72 h after seeding, a 'confluent' culture was obtained. In this culture, the MRP was increased to 34.3 ± 1.8 mV (n = 38), which is significantly higher than the MRP at subconfluency ($p < 0.005$) but not significantly different from the MRP of cells in the logarithmic growth phase. Adding 1 mM dBcAMP to the growth medium of a subconfluent N_2A culture at the end of the logarithmic growth phase, i.e. 24 h after seeding, provoked a marked inhibition of proliferative activity as evidenced by the number of cells in the culture flask (fig. 1). At that time, characteristic neurite formation is present (fig. 2B). 24 h after the addition of dBcAMP, cells were morphologically characterized as 'subconfluent and fully differentiated' and possessed a significantly increased MRP value (43.7 ± 3.8 mV, n = 27) as compared to a culture with a comparable degree of confluency but without dBcAMP.

Leaving the electrode in place for 10 min (first control) yielded stable values (less than 5% variation of the value measured after 30 sec).

Penetrating the same cell four times (second controls) yields nonsignificantly different MRP values as compared to first penetration values ($p < 0.05$) irrespective of the number of experiments, which varies from n = 5 in cultures of subconfluent and fully differentiated cells to n = 20 in subconfluent cultures. No difference was found between the percentage (usually more than 98%) of cells excluding trypan blue at the beginning and at the end of MRP measurements.

Discussion. It is known that rapidly dividing ('logarithmic growth phase') neuroblastoma cells in a nonconfluent culture tend to have lower MRP values than the same cells in a confluent culture or than slowly dividing differentiated cells[4–6].

In this study we analyzed systematically the evolution of the membrane resting potential in the well-characterized N_2A neuroblastoma cell line during several stages of proliferation, taking into account that this value is subject to modification by a number of factors.

Firstly, the MRP value can be influenced by changes in culture conditions[7]. In our growth or differentiation experiments, all conditions were kept constant during the time of the experi-

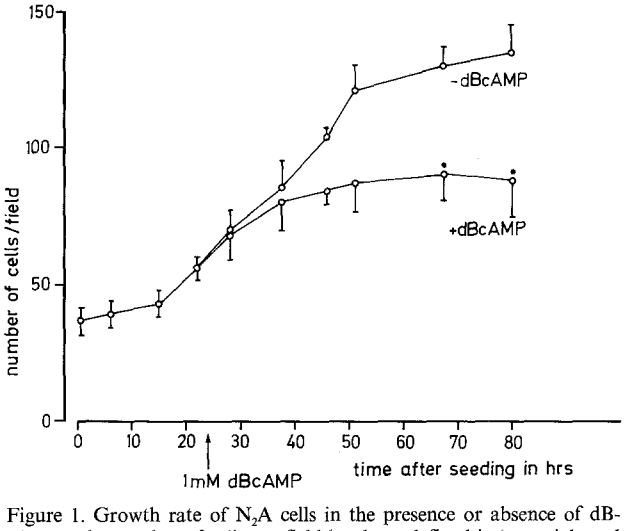

Figure 1. Growth rate of N_2A cells in the presence or absence of dBcAMP. The number of cells per field has been defined in 'materials and methods'. *Significant at the $p < 0.05$ level.

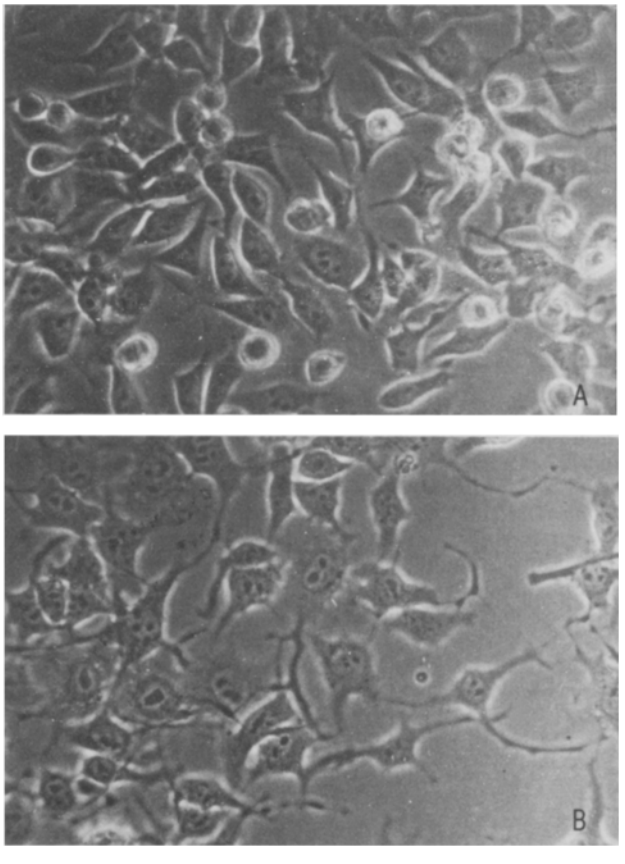

Figure 2. Light microscopic aspect of neuroblastoma cells (N_2A) without (A) and with (B) 1 mM dBcAMP. Magnification in both cases: × 400.

ment including temperature, humidity, percentage of CO_2 in the atmosphere, number of cells in the inoculum and time of harvesting. Also, differences in batches of fetal calf serum were avoided by preparing the complete medium in advance and freezing it down (−70 °C) in aliquots.

Furthermore, leakiness of the membranes due to the penetration of the cell is a frequent cause of lowered MRP[8]. In our experiments this possibility was apparently excluded since repeating or prolonging the penetration of the cell yielded reproducible results, showing that cells survive the treatment and that the MRP values recorded can be considered as real. The same suggestion can be made on the basis of the trypan blue test which showed no difference in viability before and after MRP measurements. It has to be pointed out, however, that exclusion of trypan blue is indicative of cell death but is only of limited value for testing the integrity of the cells.

As for the electrophysiological measurements, low resistance electrodes were used throughout our experiments. Although high resistance electrodes are known to cause less damage to the cell membrane, we prefer to use low resistance electrodes as they yield less artifactual measurements due to relatively lower and more stable tip potentials[9].

Finally, one should consider the possibility that the more accessible larger cells exhibit higher MRP values than the smaller cells which are more difficult to penetrate[9]. In our experiments, care was taken to avoid this selection by excluding arbitrarily both the very large and very small cells. Accordingly, it was concluded that the MRP values obtained represent real values. The MRP value for confluently grown cells is significantly higher than for cells in the subconfluent state. Logarithmically growing cells show in our experiments a higher MRP value as compared to the subconfluent cells, although this difference is nonsignificant. A similar phenomenon has been described by others[10] and is thought to be due to the initiation of the cell cycle.

The differentiation-induced increase in MRP probably reflects a true functional alteration of the tumor cell membrane.

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Colchicine-induced appearance (proliferation) of smooth sarcoplasmic reticulum in arterial smooth muscle cells

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Summary. Colchicine treatment resulted in the appearance and proliferation of smooth sarcoplasmic reticulum in some smooth muscle cells of the aortic and pulmonary trunk walls in the rabbit. The significance of cytoplasmic microtubules and/or membrane-bound tubulin for the morphogenesis, functioning and control of smooth endoplasmic reticulum in different kinds of cells is discussed.

Key words. Rabbit aorta; smooth muscle cells, arterial; sarcoplasmic reticulum; colchicine; tubulin; microtubules.

We have previously reported that treatment with a microtubule-disassembling agent, colchicine, resulted in an accumulation of secretion granules[1,2] and lysosomes[3], and vacuolar dilation of rough endoplasmic reticulum[1,2], in smooth muscle cells (SMC) from rabbit aortic arch, thoracic aorta and pulmonary trunk. During the course of these studies, we found that some but not all of the SMC responded with an evident increase in content of the smooth sarcoplasmic reticulum (SSR), which, from the ultrastructural point of view, resembled the well-known phenobarbital-induced proliferation of the smooth endoplasmic reticulum in hepatocytes. We think that this effect of colchicine, previously undescribed, may not only be of interest for our understanding of arterial SMC, but may also be of more general importance for several reasons. Firstly, because cytoplasmic microtubules and/or membrane-bound tubulin may be involved in the functioning and control of 1) contraction-relaxation cycle of SMC[4], and 2) microsomal drug-metabolizing enzymes in hepatocytes[5,6], and secondly because phenobarbital may share some microtubule-disassembling properties[7]. Moreover, the observation of this effect raises the intriguing question of whether some microtubule-disassembling agents are able to induce alteration(s) in the function of the smooth endoplasmic reticulum in different kinds of cells (SMC, hepatocytes, steroidogenic cells, skeletal muscle cells, cardiomyocytes, etc.).

Materials and methods. Colchicine (0.125 mg/100 g b.wt) was injected i.p., and 4 h later the animals were sacrificed. Untreated animals were used for controls. Longitudinal strips were taken from aortic arch, thoracic aorta and pulmonary trunks of rabbits aged 20 days, both from control and colchicine-treated groups. The strips were immediately immersed in cold 3% glutaraldehyde and postfixed in cold 1% osmium tetroxide, both in 0.1 M phosphate buffer, pH 7.4. Dehydration was in alcohols and acetone, and embedding in Durcupan ACM (Fluka). Ultrathin sections were stained with uranyl acetate and lead citrate. A JEM 7A electron microscope was used.