polyp's ectoderm. But there are also ectodermal muscle cells the fibres of which bulge into the mesoglea, and others which are localized completely within the mesoglea thus representing pure myocytes which lack an epithelial part as in *Carybdea* (Figure 2). From this study it becomes clear that the cubopolyp's muscular system has features characteristic of both the scyphopolyp and hydropolyp, yet it is nevertheless unique.

The histological investigation of the polyps of *Carybdea* and *Tripedalia* yielded the other surprizing result that the cubopolyp possesses a nerve ring. Scypho- and hydropolyps have not been reported to possess nerve rings which are characteristic of the medusoid phase of the Cubozoa and Hydrozoa. As is demonstrated in Figure 3, the nerve ring of the cubopolyp is localized near the junction of the oral cone and the tentacular region, and consists of an ectodermal and endodermal nerve ring pair. That a nerve ring could be shown to exist in the polyp generation is important from the point of evolution, as in the phylum Cnidaria the polyp represents the primary generation in which transspecific and macro-evolution has been effective.

All results confirm that a new class Cubozoa must be established and given the systematic and evolutionary position between the basic class of Scyphozoa and the more advanced class of Hydrozoa.

Reappearance in vivo of Neuraminidase-Sensitive Sialic Acid in L 5222 Rat Leukemia Cells[1]

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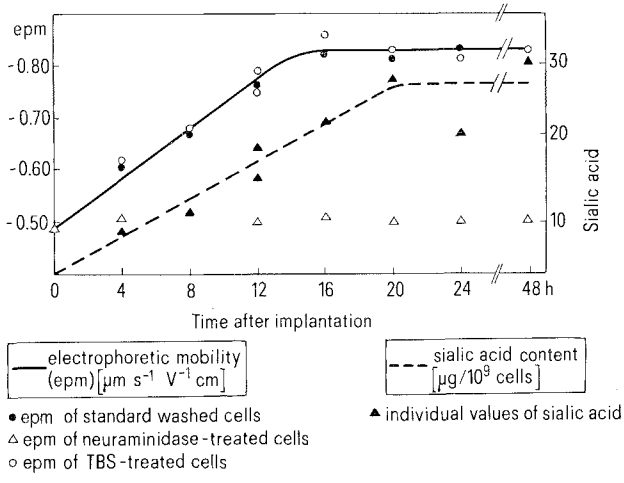
Summary. Cell electrophoretic data and quantitative sialic acid determination show that, 16 to 20 h after i.p. implantation of neuraminidase-treated L 5222 rat leukemia cells, the original sialic acid content at the cell periphery is reconstituted.

The significance of sialic acid moieties as constituents of the cell surface is still largely unknown[2]. One possible role to which particular attention has been focused during the past years is that of masking antigenic sites[3–7]. In all experiments with neuraminidase-treated cells, the time required for regeneration of surface sialic acid has to be taken into consideration. Only a limited amount of relevant data is available, all concerning in vitro-conditions[8–12]. For our studies on the influence of neuraminidase treatment on spread of leukemia cells, information about in vivo-regeneration of sialic acid is indispensable. As the biochemical mechanism of this restitution – resynthesis or utilization of constituents from the environment – is of no immediate importance for our experimental model, we determined the time necessary for restoration of the normal sialic acid complement by implanting neuraminidase-treated leukemia cells into the peritoneal cavity of syngeneic hosts.



Reappearance of sialic acid at the surface of neuraminidase-treated L 5222 leukemia cells after i.p. implantation in the BDIX rat.

Materials and methods. The undifferentiated leukemia L 5222, induced and propagated by IVANKOVIC and ZELLER[13] in the inbred BDIX rat[14], was utilized in this study. 4 to 5 days after the i.p. implantation of 50×10^6 L 5222 leukemia cells, the cells were harvested by rinsing the peritoneal cavity with balanced salt solution (BSS) containing isotonic sodium citrate (9:1 v/v). The cells, termed standard washed, were centrifuged 3 times with 0.145 *M* NaCl for 5 min at 150 g (ratio of washing fluid to packed cells 40:1). Purified neuraminidase (E.C.3.2.1.18) from *Vibrio cholerae* (Behringwerke Marburg/Lahn, West Germany) was used in a final concentration of 0.5 units/10^6 cells. The cells were enzyme-treated for 50 min at 37 °C on a rocker platform. Control cells were incubated in *Tris*-buffered saline (TBS), pH 7.3, under identical conditions. Sialic acid was estimated according to the method of WARREN[15]. Synthetic N-acetyl neuraminic

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acid in its crystalline form, obtained from Sigma Chemical Corporation, St. Louis, Mo., USA, served as standard. The supernatant fluids of neuraminidase- and TBS-treated leukemia cells were analyzed, and the sialic acid content related to the number of cells incubated. Cellular electrophoretic mobility (epm) of cells suspended in 0.145 *M* NaCl, pH 7.2 ± 0.2, was measured with a Zeiss cytopherometer as described before[16].

50×10^6 neuraminidase-treated cells were i.p. implanted in 35 BDIX rats. 5 animals were sacrificed every 4 h up to 24 h, also at 48 h after implantation, and the tumor cells harvested as stated above. Part of the cells were again standard washed, part were treated with neuraminidase and TBS, respectively, and their epm measured. The sialic acid content was determined from the supernatants of the 2 groups of treated cells.

Results. The sialic acid content in the supernatant of L 5222 leukemia cells after treatment with neuraminidase was in the order of 27 $\mu g/10^9$ cells. A second enzyme incubation yielded negligible amounts of sialic acid only, while the supernatants of TBS-treated controls were free of sialic acid. Measurements of the electrophoretic mobility showed that standard washed leukemia cells had values of -0.84 ± 0.05 SD $\mu m\ sec^{-1}V^{-1}\ cm$ (mean of 17 different experiments). Incubation with neuraminidase lowered the mobility by 38% to -0.52 ± 0.05 SD $\mu m\ sec^{-1}V^{-1}\ cm$ (mean of 11 different experiments) if compared to TBS-incubated controls (0.84 ± 0.04 SD $\mu m\ sec^{-1}V^{-1}\ cm$ as mean of 9 different experiments). The gradual reappearance of sialic acid moieties on leukemia cells treated with neuraminidase, reimplanted, and then harvested at the time intervals indicated, is presented in the Figure. Sialic acid and surface charge start to be restituted immediately after cessation of the enzyme treatment. The original sialic acid content is regained after 20 h, while the electrophoretic mobility values are normalized after 16 h.

Discussion. The results presented in this study correspond roughly to published data on in vitro-models and show that cellular alterations induced by removal of neuraminidase-sensitive sialic acid residues are of short duration. This fact is particularly important for attempts to expose cell surface antigens masked by sialic acid moieties. It should be realized that the latter, after cessation of neuraminidase treatment, begin to reappear immediately. Although this may still be compatible with detection of the unmasked antigens by surveillance mechanisms, the situation is uncertain with regard to the ensuing immune reactions. We have to assume that classical antibodies and sensitized lymphocytes will only be available after the target cells have reconstituted their original surface configuration. However, in view of the conflicting results in the literature on the fate of neuraminidase-treated tumor cells, the operation of natural antibodies to 'cryptic' membrane antigens unmasked by neuraminidase treatment[17,18] should be taken into account.

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Further Studies on Neural Tube Defects Caused by Concanavalin A in Early Chick Embryos[1]

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Summary. Neural tube defects caused by concanavalin A in chick embryos are consequences of change in the cell surface of developing neuroepithelium.

Many physicochemical agents have been found to interfere with neurulation of the chick embryo when applied during the early stages of development. Despite new information concerning the etiology of central nervous system abnormalities, much is yet to be learned about the underlying mechanisms responsible for neural tube closure. Recent evidence has suggested that driving forces for closure of the neural tube arize, at least in part, from the constriction of microfilaments and elongation of cells under the influence of microtubules[2-4]. Our previous study[5] showed that concanavalin A (Con A), a plant agglutinin that binds to cell surface terminal glycosyl and mannosyl residues of glycolipids and glycoproteins[6,7], selectively inhibited neurulation and interkinetic nuclear migration in explanted early chick embryos. These findings suggest that closure of the neural tube is strongly influenced by the cell surface. The objective of this study was to elaborate further causative mechanisms of Con A action in producing neural tube defects in chick embryos.

Materials and methods. Two experimental series were carried out. In the first series, fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 4 of development[8]. Embryos were explanted by NEW's[9] technique. Thin albumen (nutrient medium) with or without 16 μg/ml Con A, a concentration which strongly inhibits neurulation of chick embryos[5], was added outside the glass ring. After 24 h of incubation, neural tissue was isolated from randomly selected control and experimental embryos, fixed for 1 h in 3% phosphate-buffered glutaraldehyde, rinsed several times in buffered sucrose solution, and postfixed for 1 h in 1% phosphate-buffered osmium tetroxide. After fixation, specimens were dehydrated in graded ethanol series and embedded in a mixture of Epon and Araldite. Thick sections stained with toluidine blue were prepared for light microscopy.

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