consistently showed significantly greater DNA synthesis than controls, it can be concluded that, under the given experimental conditions, GH (ovine or human) does possess slight growth stimulatory effects. This is in accord with the study of LI and YANG[10] who reported that mammary tumor growth of bovine GH treated animals was essentially intermediate to that of controls and PL treated animals. Furthermore, the results of the autoradiographic analyses suggest an additive effect of these hormones as the hormonal combination was significantly greater than either hormone alone. This apparent effect of PL and human GH on DNA synthesis of mammary carcinoma cells is in accord with a previous in vivo study[14] and an in vitro study[15] which have suggested a possible synergism between PL and GH in the regulation of growth of normal[14] and hyperplastic[15] mammary tissue. Thus, the results of these studies further emphasize the key role of prolactin in growth stimulation of carcinogen-induced rat mammary carcinomas, but in addition, provide evidence that growth hormone may also be an influential hormonal factor in this process.

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Muscular and Nervous Systems of the Cubopolyp (Cnidaria)

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Summary. New observations on the morphology, anatomy, asexual reproduction and metamorphosis of the formerly unknown polyp of the tropical Cubomedusae resulted in the conclusion that a new class Cubozoa must be established and positioned between the Scyphozoa and Hydrozoa. This conclusion could be confirmed by the histological investigation of the cubopolyp's muscular and nervous systems by light and transmission electron microscopy.

As the lowest group of true Metazoa, the Cnidaria have always attracted the extensive interest of biologists. In the classes of Scyphozoa and Hydrozoa, there are 2 generations, the sessile asexual polyp and the free-swimming sexual medusa, whereas the class Anthozoa is represented by the polyp generation only. Anatomically, the basic plan of body construction is the tetra-radial symmetry. This is particularly evident in the class Scyphozoa because not only the medusa but also the polyp has a marked tetraradial body plan exhibited by the 4 gastric septa and 4 gastric pockets. Because of their tetramerously constructed body, the Cubomedusae, which are inhabitants of the neritic zones of tropical oceans, have also been grouped by most zoologists with the Scyphozoa, though some authors have pointed to aberrant characteristics by which they differ form 'true' Scyphomedusae (orders Coronatae, Semaeostomeae, Rhizostomeae). The Cubomedusae are famous as 'sea wasps' because of their severe stinging. Some species belong to the most dangerous sea creatures, as they can kill young and sensitive people by the strong venom of their nematocysts.

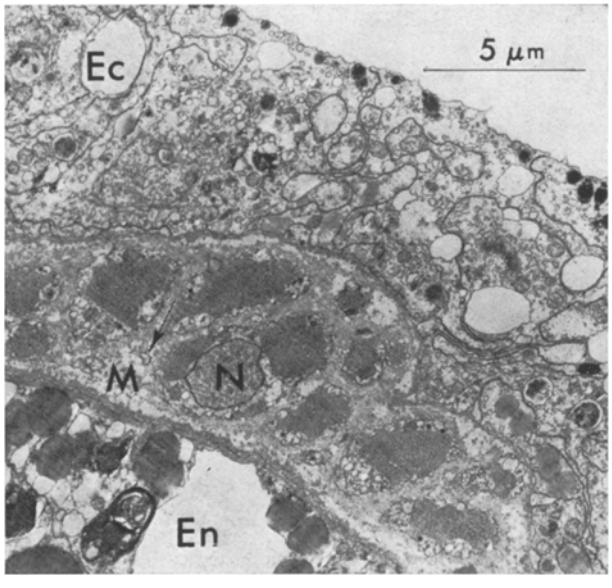

Fig. 1. *Carybdea* spec., transverse section near the base of the polyp. Note how the middle layer is occupied by myocytes. Ec, ectoderm; En, endoderm; M, middle layer; N, nucleus of myocyte; arrow points to a neurite. Fixed in Dorey's chrome-osmic fixative, embedded in Araldite and stained in alcoholic uranium.

New investigations[3–5] have revealed that the systematic position of the Cubomedusae needs to be revised. For the first time, it has been possible to rear the formerly unknown polyp generation to full size, to observe its asexual reproduction by laterally budding off small secondary polyps, and to follow the formation of the medusa, which is unique by the complete metamorphosis of the solitary polyp into one medusa. Through long-term culture experiments, it has been possible to elucidate the complete life cycle of the Caribbean species

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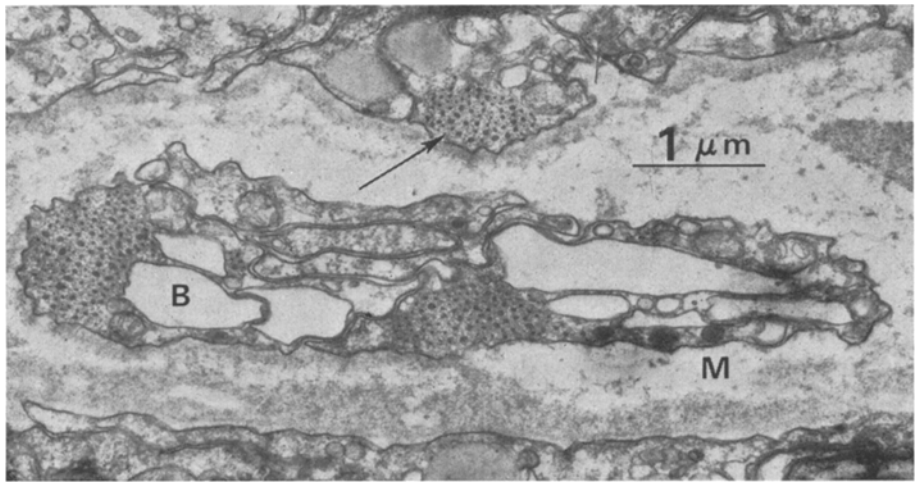

Fig. 2. *Tripedalia cystophora*, transverse section of the polyp's body just proximal to the insertion of the tentacles. The ectoderm is above with a myofibril indicated by the arrow. In the mesoglea (M) a bundle of myofibres occurs. At the bottom is the endoderm. Dorey's chrome-osmium fixation, Spurr embedded, uranium and lead stained.

Tripedalia cystophora and subsequently also of a related species of the genus *Carybdea*. The most important observation made is that the cubopolyp possesses a clear radial symmetry in respect to the morphology of its body, and the number and arrangement of its tentacles. Corresponding to these externally visible characteristics, the internal anatomy is also without any sign of a tetramerous structure, as the 4 gastric septa and 4 gastric pockets are lacking. At a first glance, the cubopolyp does not resemble a scyphopolyp but resembles much more a hydropolyp the morphological and anatomical radial symmetry of which is well known.

Further observations have shown the cubopolyp to possess other features in common with the hydropolyp. This is particularly true when considering the types of nematocysts. On the other hand, the cubopolyp has characteristics which are typical of scyphopolyps. To give a striking example: it can be noted that the epidermis of the polyp of *Carybdea* is equipped with numerous beating flagella which are completely lacking in the hydropolyp's epidermis. All results lead to the conclusion that the cubopolyp represents an evolutionary link between the scyphozoan and hydrozoan polyp.

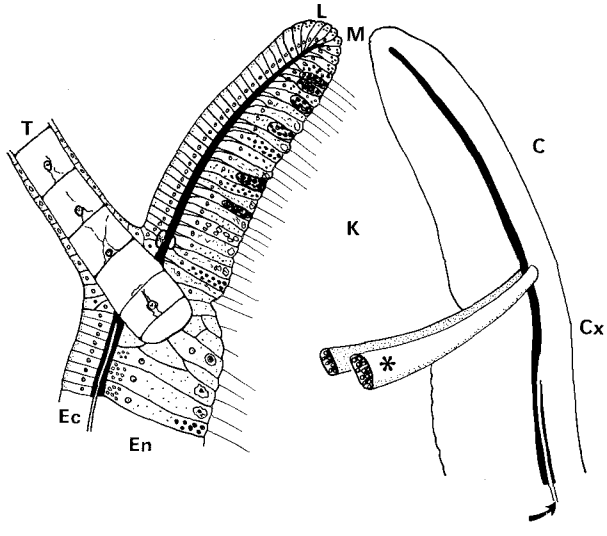

Fig. 3. Longitudinal view of the cubopolyp at the oral end to show the location of the minute nerve rings. The tentacle-bearing side is detailed and the other side plain. The plain side accentuates the ectodermal (*) and endodermal nerve rings in stereo view. The sheath around the nerve rings' neurites does not really exist. The nerve rings on the detailed side face each other across the black mesoglea near the oral side of the tentacle. The arrow points to a mesogleal muscle fibre which is derived from the ectoderm. C, oral cone; Cx, calyx; Ec, ectoderm; En, endoderm; K, coelenteron; L, lip cells; M, mouth; T, tentacle.

From this point of view, it was considered necessary to learn more about the muscular and nervous systems of the cubopolyp, especially as there are striking differences in the muscular systems between the scyphozoan and hydrozoan polyp. The scyphopolyp has 4 interradial muscles which are of ectodermal origin and which are embedded in the mesoglea, the middle layer between ectoderm and endoderm. The muscles consist of numerous myocytes the fibres of which form 4 longitudinal tubular strands localized in the 4 gastric septa. The hydropolyp, on the other hand, has a muscular system which consists of numerous epitheliomuscular cells that form an ectodermal longitudinal layer and an endodermal circular layer around the body.

Both the polyp of *Carybdea* (up to 2.0–3.0 mm body length) and the smaller polyp of *Tripedalia* (up to 1.0 mm) which can shorten to a quarter of their extended length, were studied by light and transmission electron microscopy. The brief description given here focusses on the muscular system of the body beneath the tentacular crown.

These new observations showed the cubopolyp to possess a unique type of muscular system, though there are some differences between *Carybdea* and *Tripedalia*. The muscular system of *Carybdea* (Figure 1) is localized in the middle layer between ectoderm and endoderm, and it is in this point that it is similar to the muscular system found in scyphopolyps. On the other hand, there is a difference in that the muscular system of *Carybdea* does not consist of tubular muscle strands of a fixed number but consists instead of numerous myocytes which are arranged all around the complete body. The cross-sections of the myocytes in Figure 1 demonstrate that they are stratified in several layers. The muscle cells represent 'pure' myocytes which have a slender spindle shape and contain an elongated nucleus with a large nucleolus. Several myocytes are connected at the tapered ends to form longer muscle fibres which run along the main body axis.

The muscular system of the polyp of *Tripedalia* seems to be of an evolutionary more advanced type as there are ectodermal epitheliomuscular cells as in the hydro-

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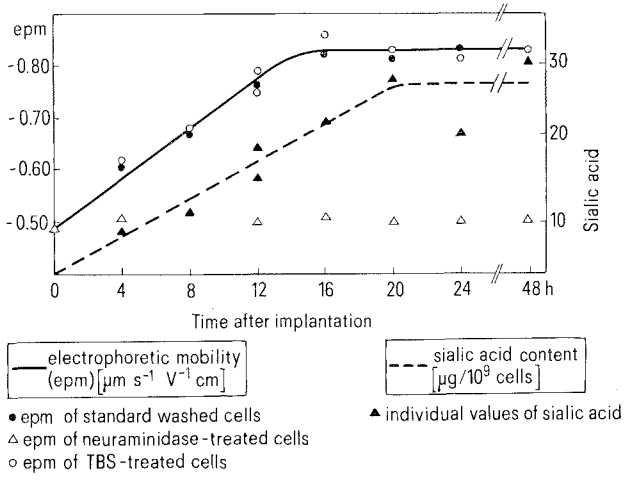
Summary. Cell electrophoretic data and quantitative sialic acid determination show that, 16 to 20 h after i.p. implantation of neuraminidase-treated L 5222 rat leukemia cells, the original sialic acid content at the cell periphery is reconstituted.

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

[1] This investigation was performed within the EORTC Cell Surface Project Group and supported by the Swiss National Science Foundation, Grant No. 3.901.72, and by the Zürich Cancer League.

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