It might be properly argued that the increased distensibility produced by collagenase action was the result of destruction of the sarcolemma by the enzyme, but there was no microscopical evidence of such destruction. More important, however, is the fact that trypsin activity, which indeed produced tissue maceration, failed to produce any significant change in distensibility. Thus it would appear that the effect seen was due to degradation of extracellular collagen and not solely to destruction of the sarcolemma. The collagen content of hearts treated with collagenase was 0.077 ± 0.011 (SE_m) g soluble collagen/100 g wet tissue while the collagen content of the control group was 0.118 ± 0.017 g soluble collagen/100 mg wet tissue. The difference between the 2 means was significant at the 0.05 level (Student's t test).

No chemical evaluation of the efficacy of elastase or trypsin activity was undertaken other than the histological sections mentioned previously. There was some histological evidence of beading of elastin and of tissue maceration in those hearts subjected to elastase and trypsin respectively. Concentration levels of the enzymes used were in excess of those known to produce significant enzymatic effects[5-8].

Zusammenfassung. Isolierte Hasenherzen wurden im Inkubator in 3 Gruppen von Enzymgemengen behandelt, ihre Ausdehnungscharakteristika durch Druckvolumen-Messungen bestimmt. Von den verwendeten Enzymen Collagenase, Elastase und Trypsin, erzeugte nur Collagenase einen Wechsel in der Ausdehnung.

L. J. O'Brien and C. M. Moore

Medical College of Georgia, Augusta, Georgia and Civil Aeromedical Research Institute, Oklahoma City (Oklahoma, USA), July 15, 1966.

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Experimentally Produced Ependymal Inclusions in the Brain of *Xenopus laevis*

In the course of an investigation on the influence of telencephalic injuries on hypothalamic neurosecretion in *Xenopus laevis*[1], it was noted that portions of the lateral ventricles, partially or completely isolated from the remainder of the ventricle system, develop strongly chrome hematoxylin (Gomori) positive inclusions in the ependymocytes. These Gomori positive inclusions (GPI) are present in the perikarya and prolongations of the ependymocytes, being frequently found even in the most distal parts of the prolongations which form meningeal end-feet at the brain surface (Figures 1–3).

In this study similar lesions were performed as those previously made in the telencephalon of adult specimens of *X. laevis*[1]. As the result of the operation, the olfactory lobes were isolated from the rest of the brain. The cut caudal end then healed; a small isolated ventricle remaining in the posterior part. Sometimes only a trace of a ventricle was seen; but in every case where ependymocytes were present, GPI developed. In the olfactory lobes, GPI were also seen in glial cells (Figure 6). The caudal extremities of the hemispheres, when isolated, were also frequently the site of GPI. Most frequently, GPI were situated basally with respect to the cell nucleus (Figures 2 and 4). However, at sites where they were particularly abundant, GPI could be seen also in the apical portions of the ependymocyte perikarya. In such cases, the lumen of the isolated ventricle showed the presence of intensely Gomori positive amorphous precipitates (Figure 1). GPI were never found in the ependyma of a normal brain.

GPI are oval, spherical, or bean-like shaped granules, 0.5–2.0 μ in size, situated in the cytoplasm. They frequently form aggregates which coalesce into large Gomori positive masses (Figures 2 and 6). In preparations stained with Gabe's aldehyde fuchsin, GPI are also strongly positive. They are strongly performic acid-Alcian blue positive but negative or only weakly positive in the PAS procedure. In unstained preparations GPI are colourless. They do not contain acid mucopolysaccharides and are distinct from lipofuscin, as shown by the negative result of the staining with 0.1% Alcian blue in 3% acetic acid and the negative outcome of the Hueck and Lillie methods. Thus, GPI are probably of protein character with a cystine content of over 4%.

As shown by the results presented above, the GPI in the ependymocytes of the isolated ventricles of the *X. laevis* brain are, at least with all the methods so far employed, histochemically similar to the Gomori positive inclusions normally found in the periventricular glial cells of the hypothalamus and some other brain areas in

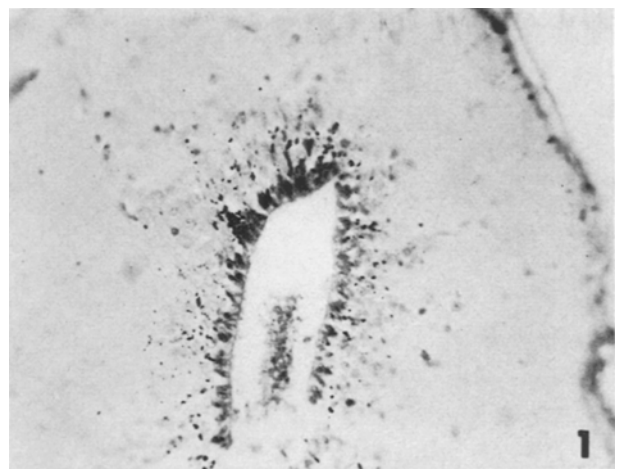

Fig. 1. Isolated ventricle with ependymal Gomori positive inclusions (GPI) in a hemisphere remnant. In the lumen Gomori positive precipitate can be seen. Chrome hematoxylin-phloxin (CHP). × 110.

[1] Z. Srebro, Folia biol. Kraków *13*, 397 (1965).

mammals[2-7] and the more recently described inclusions in some cells situated in the most caudal part of the septal nucleus of the telencephalon of the amphibian *Rana esculenta*[8-10].

The nature of the GPI is unknown. Their presence only in *Xenopus* and in the isolated ventricles suggests that they arise in this case as a result of impaired cerebrospinal fluid (CSF) flow. The ependyma of amphibia, which is the counterpart of the more specialized astrocytes in mammals, probably plays an important role in water and/or electrolyte transport from the CSF into the brain tissue. The long ependymocyte (tanycyte) prolongations which form meningeal end-feet contain large mitochondrion-like bodies – the gliosomes[11,12]. The presence of numerous longitudinally oriented mitochondria in the apical portions of the tanycyte perikayrya[13] and the gliosomes in their prolongations may also indicate a transport role of the ependyma, which is known to be energy-dependent. In the isolated ventricles, the transport of water and/or electrolytes from the CSF into the brain tissue is certainly more difficult owing to disturbances or lack of CSF flow. Thus an additional functional demand is laid upon the transport capability of the ependyma. This in turn is bound to cause a hypertrophy of cellular structures involved in the transport mechanism. Thus, until more information is obtained from further studies, among which an electron microscopic one seems to be the most promising, GPI may be regarded as hypertrophied cytoplasmic structures or accumulated material involved in water and/or electrolyte transport.

However, a different explanation of the nature of the GPI is possible; namely, that they are a secretory product of the ependyma. Moreover, this supposition explains satisfactorily all the facts observed. Supposing that the ependyma, or rather a specialized region of it, elaborates a 'choriotropic hormone' with a stimulatory action on the production of the CSF by the choroid plexuses and that the secretion of this hormone is influenced in turn by the composition of the CSF, ependymal hypersecretion is to be expected in the isolated ventricles. As mentioned above, the fluid in the lumina of the isolated ventricles certainly has a different composition from the CSF with

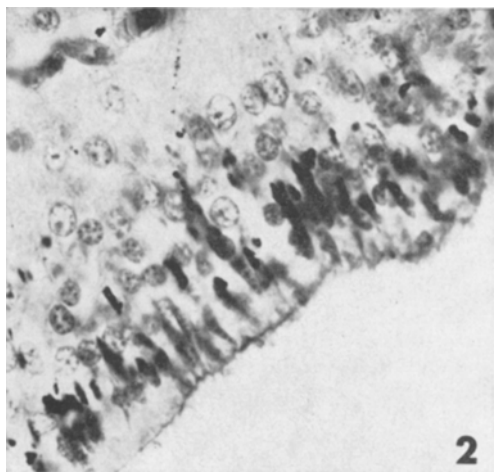

Fig. 2. Ependyma with GPI at higher magnification. The GPI are situated basally with respect to the cell nuclei (CHP). × 550.

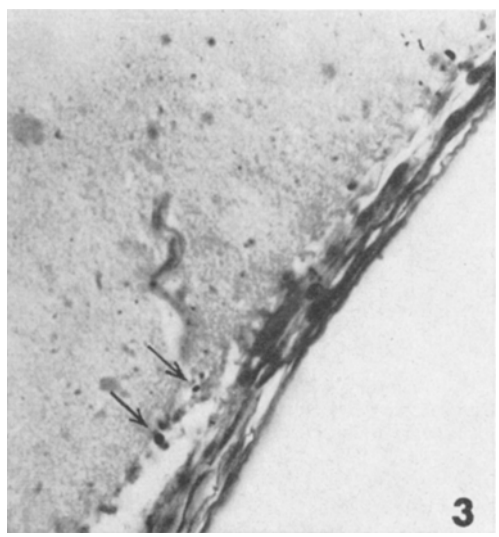

Fig. 3. GPI at the brain surface (arrows) (CHP). × 550.

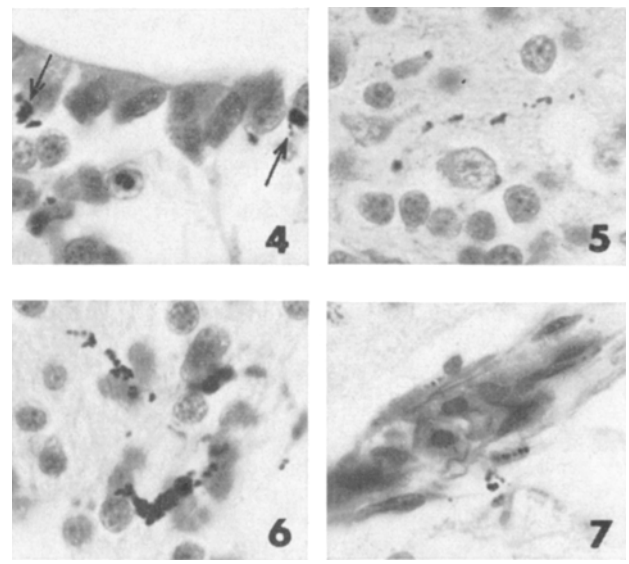

Fig. 4. Fragment of the ependymal lining of an isolated ventricle in the olfactory lobe. Arrows indicate GPI (CHP). × 840.

Fig. 5 and 6. GPI in perikarya and prolongations of glial cells in an isolated olfactory lobe (CHP). × 840.

Fig. 7. GPI at a capillary wall (CHP). × 840.

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which the ependyma is in contact in the normal brain. This change is a stimulus for increased ependymosecretion, i.e. production of more of the 'choriotropic hormone'. The morphological expression of this hypersecretion is the appearance of the GPI. The presence of Gomori positive precipitates in the lumina of the isolated ventricles and the apparent transport in the prolongations and release in the meninges of the Gomori positive material are also in favour of the supposition that GPI are an ependymosecretory material. The role of ependymosecretion in the control of the production of CSF by the choroid plexuses, influenced in turn by the composition of the CSF, if proved, will be another example of a simple feed back control of a physiological process.

Zusammenfassung. Verletzungen des Endhirns beim erwachsenen Krallenfrosch (*Xenopus laevis*) führen zur Entstehung von Ventrikelteilen, die vollständig oder teilweise vom Ventrikelsystem des Gehirns isoliert sind. Das Ependym solcher Ventrikelteile zeigt stark Chromhämatoxylin-positive cytoplasmatische Einschlüsse (GPI), die im Perikaryon und in den Zellfortsätzen der Tanycyten lokalisiert sind. Charakter und Bedeutung der GPI werden diskutiert.

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Die zentralnervöse Rangstufe des Blauwals (*Sibbaldus musculus* Linnaeus)[1]

Der Blauwal kann eine Körperlänge von 30 m und ein Gewicht von 150 Tonnen erreichen. Somit ist diese Walart das grösste und schwerste Tier der Erde und auch fossile Saurier und Säuger haben dieses Maximum niemals erreicht. Das Tier ist kosmopolit, monogam, und erreicht ein Alter von etwa 30 Jahren (NORMAN und FRASER[2]). Das von uns untersuchte Gehirn (T. Nr. 260) stammt von einem ♀ Blauwal, der während der Fangsaison 1961–1962 vom Thor/Dahl im südlichen Eismeer harpuniert wurde. Die Körperlänge betrug 88 englische Fuss, das Körpergewicht ist unbekannt. Das Hirngewicht, ohne Dura, beträgt nach Formolfixation 6500 g. Die allgemeinen Formverhältnisse sind aus den Figuren 1–4 ersichtlich. Die laterale Hemisphärenausladung ist ziemlich ausgeprägt, hingegen ist die Rotation des Schläfenlappens in basaler Richtung unvollständig (Figur 2). Die Sylvische Furche ist fast vertikal gerichtet und kurz. Die Furchung des Neopalliums, die Form der palaeo- und archicortikalen Strukturen und des Kleinhirns sind für Mysticeten charakteristisch. Der Thalamus hat, im Gegensasz zum Seiwal (PILLERI[3]), eine relativ breite Massa intermedia. Der Hypothalamus ist schmal, vertikal entwickelt. Die Hirnstammformationen haben eine gemeinsame Achse die von der Mitte der Massa intermedia zur unteren Olive verläuft. Die Medulla oblongata schliesst mit dem Zervikalmark einen dorsal offenen Winkel von 120° ein.

Vergleichend-anatomisch betrachtet weist das Gehirn des Blauwals die für Mysticeten typischen Merkmale auf. Mit den übrigen Bartenwalarten verglichen und in Anbetracht der zentralnervösen Ranghöhe, nimmt es eine Mittelstellung zwischen dem primitiveren Gehirn des Right Whale (*Eubalaena australis* Desmoulins; siehe PILLERI[4]) und dem des Buckelwals (*Megaptera novaeangliae* Borowski; siehe PILLERI[5]). Höher als dieses letztere steht das Zentralorgan des Seiwals (*Balaenoptera borealis* Lesson; siehe PILLERI[3]). Dabei sind diese Rangstufen vom Körpergewicht unabhängig, auch wenn man

[1] Durchgeführt mit Unterstützung des Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung (Gesuch Nr. 3883/1966).

[2] J. R. NORMAN und F. C. FRASER, *Giant Fishes, Whales and Dolphins* (Putnam, London 1948).

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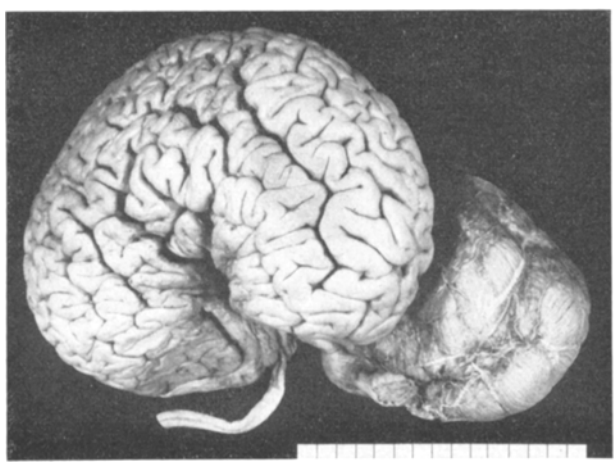

Fig. 1. Laterale Aufnahme des Gehirns des Blauwals (T. 260, Sammlung Dr. G. PILLERI).

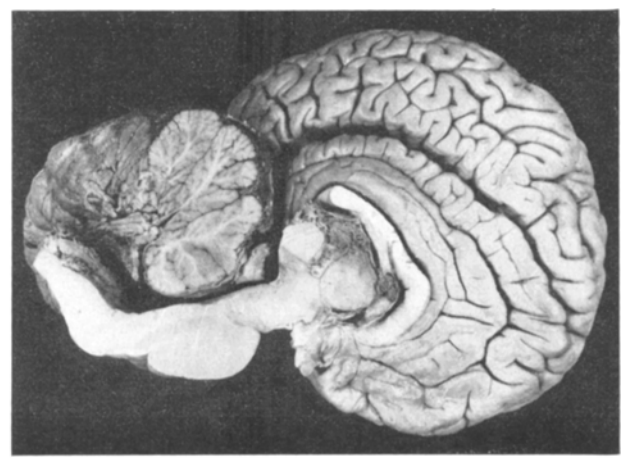

Fig. 2. Mediale Aufnahme des Gehirns des Blauwals.