On the Presence of Periodic Acid Schiff Positive Substances in the Paraphysis Cerebri, the Choroid Plexuses and the Neuroglia of Ambystoma Mexicanum*

In former papers[1], the development of the paraphysis and its general and cytological structure were described, using ordinary fixatives and staining methods. The cytology of the paraphyseal epithelium was compared with that of the epithelium of the choroid plexuses. It appeared that in amphibians the paraphysis is a compound racemose tubular organ, developing from the posterior part of the tela chorioidea telencephali medii. The anterior lamina of the velum transversum is secondarily involved. The tubules are surrounded by venous sinusoids, the endothelium of which is, for the most part, in immediate contact with the basal membrane of the single-layered low cylindrical paraphyseal epithelium. The lumina of the tubules communicate by means of a short common duct with the ventriculus impar telencephali.

In the paraphyseal cells, vacuoles were often observed, also by VAN DE KAMER[2]. These cells show a thin cuticula lining the free border, have no cilia, and do not take up India ink particles after intraventricular ink injection. The cells of the plexus epithelium, on the other hand, never show vacuoles. They have a striated cuticular membrane, sometimes cilia and take up black particulate matter. Especially on the evidence of its vacuoles and of its incapacity to absorb ink particles, it was suggested that the paraphyseal epithelium might be concerned in the production of cerebrospinal fluid, or at least in the production of one or more of the chemical components of this fluid, as yet unknown in character.

The present paper gives a short account of the results obtained after staining brains of *Ambystoma* for polysaccharides and mucopolysaccharides according to the method of MCMANUS. Only the paraphysis, the choroid plexuses and the neuroglia in general will be considered.

The brains were fixed in ROSSMAN's fluid in the refrigerator, after dehydration embedded in paraffine and cut in sections of 6 μ in thickness. The sections were stained according to the McManus technique with Schiff's reagent and counterstained with Ehrlich's haematoxylin. Part of the sections were first treated with 1% diastase before subjecting them to the periodic acid Schiff (PAS) procedure.

The results may be summarized as follows. The cells of the paraphyseal epithelium in series stained without using diastase were crowded with red-staining, different-sized droplets (Fig. 1). Such droplets could be observed likewise along the ventricular walls, especially so in the lateral ventricles where most of them were evidently included in the cytoplasm at the ventricular side of the ependymal gliocytes. Strikingly, many of these same PAS positive droplets were found along the long and slender processes of these ependymal gliocytes, which, in general, run in a direction perpendicular to the longitudinal axis of the brain into the nervous parenchyma. In this way, veritable rows of droplets are formed (Fig. 2). At many places red-staining droplets were likewise present along the end feet of the glial processes, constituting the external limiting membrane. In the optic tectum, the type of ependymal gliocyte is somewhat different. They show rather short conical cytoplasmatic processes which also stained PAS positive. Likewise, similar droplets were found scattered at many places in the nervous parenchyma. It looks rather more probable that they are situated along the meshes of the glial reticulum than that their location is dictated by the course of the nerve fibres.

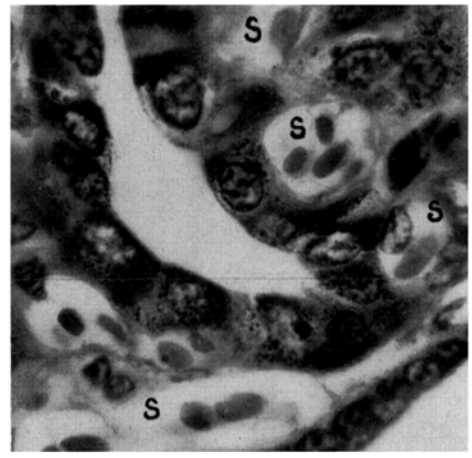

Fig. 1.–Paraphyseal epithelium crowded with PAS positive droplets. S = venous sinusoids. 600:1.

Moreover, in many epithelial cells of the choroid plexuses PAS positive droplets were present, although much less than in the cells of the paraphyseal epithelium. They could be observed likewise in a number of epiplexus cells (cells of KOLMER) that are evidently phagocytotic[3].

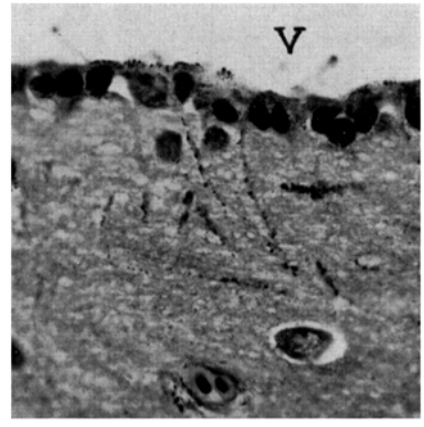

Fig. 2.–PAS positive droplets (glycogen) in ependymal gliocytes and along their processes, running into the nervous parenchyma. V = ventricle. 360:1.

After treating sections with 1% diastase before subjecting them to the periodic acid Schiff procedure, the droplets were quite absent in the epithelium of the

* This investigation was aided by a grant from the Health Organization of the Society for Applied Scientific Research, The Netherlands.

[1] J. ARIËNS KAPPERS, Exper. 5, 162 (1949); J. comp. Neurol. *92*, 93 (1950).

[2] J. C. VAN DE KAMER, *On the Development, Determination and Significance of the Epiphysis and the Paraphysis of the Amphibians*, Thesis (Utrecht 1949).

[3] J. ARIËNS KAPPERS, Z. Anat. Entw.-Gesch. *117*, 1 (1953).

choroid plexuses as well as in the epiplexus cells. This is proof that they consist of glycogen. In the neuroglia they were also lacking.

In the cells of the paraphyseal epithelium the amount of PAS positive substance was diminished after diastase treatment of the sections. In many cells vacuoles were now visible, quite similar to those formerly observed in preparations fixed and stained according to ordinary methods (Fig. 3). From the present investigation it follows clearly that presence of these vacuoles is due to the diastase which has caused the disappearance of conglomerates of glycogen droplets, being PAS positive in McManus preparations without preceding diastase treatment. That these vacuoles were likewise visible in preparations, described and illustrated in my former papers, is evidently caused by the fact that the glycogen present had neither been fixed nor stained by the ordinary methods then used.

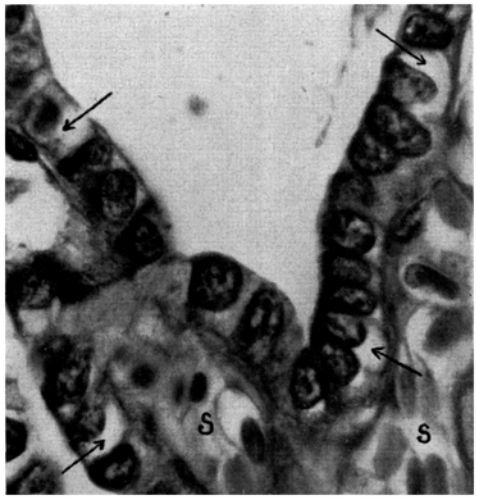

Fig. 3.–Paraphyseal epithelium, stained with the PAS procedure after treatment with diastase. Vacuoles, due to digestion of glycogen, indicated by arrows. The cells still contain some non-glycogeneous PAS positive substance. *S* = venous sinusoids. 600:1.

That many cells in the paraphyseal epithelium still showed PAS positive substance notwithstanding the treatment with diastase, proves that they contain a certain amount of mucopolysaccharides in addition to glycogen. As could be expected, the basal membrane of the paraphyseal epithelium and of the plexus epithelium, the cuticular borders of both epithelia and the endothelium of all vessels remained PAS positive because they consist of mucopolysaccharides, as is well known. The same is true for the nervous parenchyma which showed a light red tinge all over. The PAS reaction is also positive for muco-, glyco- and lipoproteins and for glyco- and phospholipids, which are all diastase fast[4]. In the present investigation, however, no attempt was made to determine the exact nature of the PAS positive substance which is still present after diastase treatment.

Discussion.—The structure of the paraphysis in *Ambystoma* being glandular, and its cells having been found crowded with PAS positive substances in young adult specimens as well as in old ones, the provisional working hypothesis seems justified that these substances are produced by the paraphyseal cells and secreted into the ventricular system. The exact way in which this secretion happens is still questionable. Once circulating in the ventricular cerebrospinal fluid, these products are probably taken up by the ependymal gliocytes and transported along their processes into the nervous parenchyma. This seems to be true at least for the glycogen.

That ependymal gliocytes are indeed able to transport substances present in the cerebrospinal fluid, is, for instance, proved by experiments in which India ink was injected intraventricularly. Following unpublished data, I observed ink particles along the meshes of the glial reticulum and especially along the end feet of glial processes in *Ambystoma* under this condition. ARNVIG[5] and the author[3] found black particles in the ependymal cells as well as subependymally in *Cavia* in similar experiments. These data, as well as the results of the present investigation, suggest the possibility of a transport of non-physiological as well as of physiological substances from the cerebrospinal fluid into the nervous parenchyma by means of ependymal gliocytes.

The presence of differing quantities of glycogen droplets in many cells of the plexus epithelium would be explained either by local production of this substance or by its absorption from the cerebrospinal fluid. Certainly plexus cells are able to absorb many substances, also glycogen, from this fluid, as is experimentally proven[3]. A local production of glycogen in the plexus epithelium, however, cannot be wholly ruled out, because in mammalian embryos at least this is crowded, during a certain period of development, with glycogen which is probably of local origin. Other PAS positive substances are evidently absent in the cytoplasm of the epithelial cells of the choroid plexuses in *Ambystoma*. They occur exclusively in their basal membranes and cuticular borders.

Glycogen being a source of energy and a building stone for more complex chemical compounds, its transportation into the nervous parenchyma stands to reason. Here it may serve metabolic as well as structural purposes. The fact that in urodele amphibians, the nervous parenchyma is exclusively vascularized by simple capillary loops (HORNE CRAIGIE[6]), may perhaps be correlated with the existence of an organ, i.e. the large paraphysis, producing a functionally basic chemical substance which is transported to the nervous parenchyma not by way of the blood and the gliocytes but by the cerebrospinal fluid and these same cells. This tentative suggestion, however, will have to be proved by comparative investigation, both along morphological and chemical lines. The present investigation has at least pointed out the probable importance of the gliocytes for the metabolism in the brain of *Ambystoma* and the probable function of the paraphysis in this connection.

J. ARIËNS KAPPERS

Department of Anatomy and Embryology, State University at Groningen, The Netherlands, December 21, 1955.

Zusammenfassung

Mittels Färbung mit der histochemischen Methode nach MCMANUS lässt sich eine grosse Menge Glykogen in der *Paraphysis cerebri* von *Ambystoma* nachweisen.

[4] A. E. G. PEARSE, *Histochemistry* (Churchill Ltd., London 1953).

[5] J. ARNVIG, *Cerebrospinalvaeskens produktion og resorption*, Thesis, (Copenhagen 1948).

[6] E. HORNE CRAIGIE, Proc. amer. philos. Soc. *78*, 615 (1938); *82*, 395 (1940).

Glykogentropfen finden sich weiter im Epithel des *Plexus chorioideus* und vor allem auch in den ependymalen Gliozyten, deren Ausläufer richtige Strassen zum Glykogentransport zu bilden scheinen.

Es wird eine Arbeitshypothese aufgestellt, nach der das Glykogen, eine wichtige Energiequelle wie ein Baustein mehr komplexer chemischer Verbindungen, gebildet in der bei Amphibien mächtig entwickelten Paraphyse, in die ventrikuläre Cerebrospinalflüssigkeit gelangt, um von dort aus von den ependymalen Gliozyten aufgenommen und in das nervöse Parenchym hinein transportiert zu werden, wo es eine Rolle bei der Metabolie und dem strukturellen Aufbau spielen kann.

Befunde aus der Frühentwicklung des Rattenkeimes als Beitrag zur Rhythmik der Entwicklung

Rhythmik und Polarität sind zwei tragende Prinzipien der Entwicklungsphysiologie. Im Zuge der Untersuchung der Entwicklungspotenz des Rattenkeimes *in vitro* ergaben sich Befunde, die als Beitrag zur grundsätzlichen Gültigkeit des Prinzips der Rhythmik von Bedeutung erscheinen. Die Arbeit am Säugetierkeim hat den Vorteil, dass alle Anlagen unter streng gleichen Bedingungen und unter Einwirkung gleichartiger Umweltfaktoren zur Entwicklung kommen. Die Abweichungen der Keime eines Muttertieres bis zu 48 h Entwicklungszeit von der «Norm» (wobei die Norm den groben Durchschnitt, wie er etwa in Normentafeln festgelegt ist, darstellen soll) sind bekannt (NICHOLAS[1]). Überraschend ist die Sachlage jedoch bei der Untersuchung von Stadien der Frühentwicklung bei der Ratte.

Die konventionelle Bestimmung gibt für den Rattenkeim etwa folgende Verhältnisse: am 2. Tag finden sich 2-Blastomerenkeime, am 3. Tag 4-Blastomerenkeime, im Laufe des 4. Tages 8–16-Zeller, am 5. Tage beginnt die Blastocoelbildung, um am 7. Tag ins Eizylinderstadium überzugehen. Abweichungen von dieser Norm sind natürlich nicht überraschend. Die starken Abweichungen der Keime eines Tieres gegeneinander sind jedoch so auffallend, dass eine Erklärung dafür zu suchen ist.

Die Tabelle zeigt eine Zusammenstellung der Stadien, in welchen Rattenkeime bei der Isolierung vom 2. bis 6. Tag gefunden wurden.

Als 1. Tag der Entwicklung gilt derjenige, an welchem der Vaginalausstrich der Ratte morgens Sperma zeigt. Der Begattungszeitpunkt wird für 2 Uhr der vorhergehenden Nacht $\pm$ 6 h angenommen. Es ergibt sich, dass in dem Entwicklungszeitraum vom 2. bis 6. Tag eine erhebliche Verschiebung in der Rhythmik der Furchungsteilung eintritt. Die Unterschiede betragen etwa am 4. Tage innerhalb der Keime eines Tieres bis zu 5 Tagen der konventionellen Bestimmungsweise. Schon am 6. Tage sind die gewonnenen Stadien wieder einheitlich; alle am 7. Tag der Entwicklung untersuchten Keime ergeben übereinstimmend Eizylinderstadien völlig gleicher Differenzierungshöhe. Das bedeutet, dass alle Keime zwischen dem 2. und 7. Tag die gleiche Anzahl von Furchungsteilungen zurücklegen, dass jedoch die zeitliche Aufeinanderfolge dieser Teilungsschritte sehr unterschiedlich sein kann.

[1] J. S. NICHOLAS, Anat. Rec. *53*, 71 (1932).

Es sei erwähnt, dass der untersuchte Rattenstamm durch hohe Produktivität mit durchschnittlich 10 Jungen je Wurf ausgezeichnet ist. Es handelt sich um Wistarratten, die seit 5 Jahren im hiesigen Institut gezüchtet werden. Die Zahl der gewonnenen Keime liegt infolge der Präparationsschwierigkeiten unter dem Durchschnitt, so dass angenommen werden kann, dass alle Keime zur Entwicklung gelangt wären. Angaben über die Schwankungen früherer Stadien der Rattenkeime innerhalb eines Muttertieres finden sich selten.

Gewinnung durch	Tag der Entwicklung	Norm	Anzahl der aufgefundenen: 2-Zeller	4-Zeller	8–16-Zeller	Morulae	Blastocoelkeime	Eizylinder	Grösste Abweichung gegeneinander in Tagen	Zahl der untersuchten Muttertiere
Präparation der Eileiter	2.	2-Zeller	8						1	5
			7	1						
			4							
			6							
			7	1						
	3.	4-Zeller	1	3	4				3	5
				5	4	1				
			9							
			7	2						
			2	3						
Präparation der Eileiter bzw. Uterusspülung	4.	8–16-Zeller			2	1	4		5	10
							4			
			1				4			
						1	8			
			2	1	1	1	2			
						1	5			
						1	4			
							8			
						5	2			
			1	2			2			
Uterusspülung	5.	Morula | Blastocoelstad.			2	3	3		3	5
					1	2	3			
						1	4			
					1	2	3			
						4	5			
	6.	Blastocoelstad.					7		0	5
							7			
							8			
							5			
							4			
Präparation bzw. Serienschnitt des Uterus	7.	Eizylinder						93	0	10