Zur Entwicklung der Gefässe im Herzmuskel

Die Entwicklung der Herzgefässe wurde an 379 Wistar-Ratten beiderlei Geschlechts vom 9. Embryonaltag bis zum 6. Lebensmonat histologisch und mit Hilfe von Tusche-Gelatineinjektionen studiert. Neu ist die Beobachtung von 3 unterschiedlichen Phasen der Herzernährung: 1. bis zum 12. Embryonaltag durch Diffusion, 2. etwa bis zum 17. Embryonaltag durch eine rein venöse Zirkulation, 3. danach koronar. Der Übergang von dem einen zum anderen Versorgungstyp erfolgt nie abrupt, vielmehr gibt es stets einen Zeitraum, in dem altes und neues Versorgungsprinzip nebeneinander bestehen.

Besondere Aufmerksamkeit wurde der Periode der rein venösen Zirkulation gewidmet, weil in dieser Zeit der grösste Teil des herzeigenen Kreislaufs aufgebaut wird. Ausgangspunkt sind die zwischen den Trabekeln der Kammerwand gelegenen Sinusoide. Hier bilden sich am 13. Embryonaltag an der Herzbasis und am Kammerseptum Endokardeinstülpungen aus, die zum Teil durch die Herzsulze hindurch das Myokard erreichen. Es handelt sich um die ersten Anlagen der Kapillaren, die bald mit den ersten Herzvenen in Verbindung treten, die als Knospungen aus dem Sinus coronarius hervorgehen. So wird die Strombahn geschlossen, und das Blut gelangt aus der Kammerhöhle durch die Sinusoide, Kapillaren und Venen in den Sinus coronarius. Es liegt eine sinusoido-venöse Zirkulation vor. In der Folgezeit breiten sich die Kapillaren über das ganze Herz aus. Die Venen entwickeln sich ausschliesslich auf der Rückseite des Herzens und greifen erst später auf die Vorderseite über. Die Umgestaltung der sinusoido-venösen Zirkulation in die definitive koronare beginnt mit der Anlage der Koronararterien (16. bis 17. Embryonaltag), die aus den Sinus Valsalvae aussprossen. Diese treten zu den vorhandenen Kapillaren in Beziehung, die sich teilweise zu Arteriolen, Arterien, Venen und Venulen umwandeln. Diese Beobachtung steht im Gegensatz zu bisherigen Mitteilungen[1–3], in denen behauptet wird, dass die Koronararterien in ganzer Länge Neubildungen seien. Ausserdem wird das Kapillarsystem als Ganzes umgestaltet. Die Kapillaren werden zahlreicher, bilden schliesslich ein feinmaschiges Netzwerk und orientieren sich ähnlich wie die Muskulatur in den Kammerwänden aussen und innen längs, in der Mitte zirkulär[4]. Wichtig für die Ausbildung der koronaren Zirkulation ist ferner die Umwandlung der Sinusoide. Im Gegensatz zu der Annahme von BENNETT[2] verschwinden die Sinusoide nicht. Sie behalten stets Verbindung mit dem Kapillarsystem des Myokards. Pränatal sind die Sinusoide noch verhältnismässig plump, später schmal und langgezogen. In ihrer Gesamtheit bilden die ehemaligen Sinusoide im erwachsenen Herzen die thebesianischen Gefässe (thebesianische Venen, kapilläre gefässluminäre Anastomosen). Weitgehend abgeschlossen ist die Ausbildung der koronaren Strombahn am Ende der 1. Lebenswoche[5].

Summary. In the developing rat heart 3 periods may be distinguished with regard to the nutrition of the myocardium: (1) Nutrition by diffusion (up to the 12th embryonic day). (2) Period of venous circulation in which the blood passes from the ventricles through sinusoids, capillaries and veins to the coronary sinus. (3) The coronary circulation, which starts with the formation of coronary arteries. By the end of the first week of life, the cardiac circulating system is fully developed.

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Anatomisches Institut der Universität, 87 Würzburg (Deutschland), 19. Mai 1969.

[1] F. T. LEWIS, Anat. Anz. *25*, 261 (1904).
[2] H. S. BENNETT, Am. J. Anat. *60*, 27 (1937).
[3] J. B. GOLDSMITH und H. W. BUTLER, Am. J. Anat. *60*, 185 (1937).
[4] B. OŠŤÁDAL, Z. RYCHTER und O. POUPA, Folia morph. *16*, 116 (1968).
[5] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

An Electron Microscopical Demonstration of the Permeability of Cerebral and Retinal Capillaries to Ions

The blood-brain and blood-retinal barriers to large molecules have been known for a long time; only recently their structural bases have been demonstrated by the electron microscope. They are not caused by simple lack of perivascular spaces[1], nor by the glial-investment[2–6], but by the cerebral and retinal vascular endothelial cells being completely surrounded by tight junctions (zonuale occludentes), which are impermeable to quite small proteins (e.g. peroxidase, mol. wt. ~43,000), and by the paucity of small vesicles in the cells[5,6]. (Elsewhere, junctions often permit the passage of small proteins, e.g. peroxidase[7,8], and the numerous vesicles slowly carry large molecules across the cells[9–15].)

While the barriers prevent the passage of large molecules, they are permeable to small ones[16]. Some workers consider it likely that small molecules must pass through the endothelial cells, which would impute great transport and regulatory powers to them[5,6]. Yet the endothelium has little resemblance to cells which actively secrete or transport material, and vascular permeability is generally held to be an essentially passive process[4,12,13,15,17].

To study this problem further, it was decided to investigate the passage of ions through the cerebral and retinal barriers using a technique which has proved its value before – in showing that ions do pass through other junctions[18–20].

Materials and methods. Wistar rats (~200 g) were anaesthetized with Nembutal and ether. Intravitreous injections[21] of 0.05–0.1 ml of $Na_4Fe(CN)_6 \cdot 10H_2O$ (16.2 g/l, plus NaCl, 4.75 g/l), or of Na_2SO_4 (7.75 g/l, plus NaCl, 4.75 g/l) were given at 39 °C, 5 and 10 min before fixation. Intrathecal injections of 0.1 ml of one of the same solutions were given, 7 min before fixation, after a small portion of the skull had been carefully removed from over a cerebral hemisphere. The animals were fixed by perfusion with fresh 4% glutaraldehyde (pH 7.2) in Caulfield's medium[22] at 20 °C and 100 mm Hg pressure, after flushing-out with just the buffer for $^1/_2$–1 min. The fixative also contained $FeCl_3$ (7.05 g/l, neutralized with 0.1 *M* NaOH) when the ferrocyanide was used, and $BaCl_2$ (8.75 g/l) when the sulphate was injected. Perfusion was continued for 15 min and then the tissues were removed,

cut into 1 mm³ blocks and placed in more of the fixative and ion mixture for 12 h. Control material was obtained using the above techniques but omitting one or other of the ion-pairs; this was stained by adding 0.5% uranyl acetate to the dehydrating alcohol (70%) for 30 min. When the Prussian blue reaction was being used, the

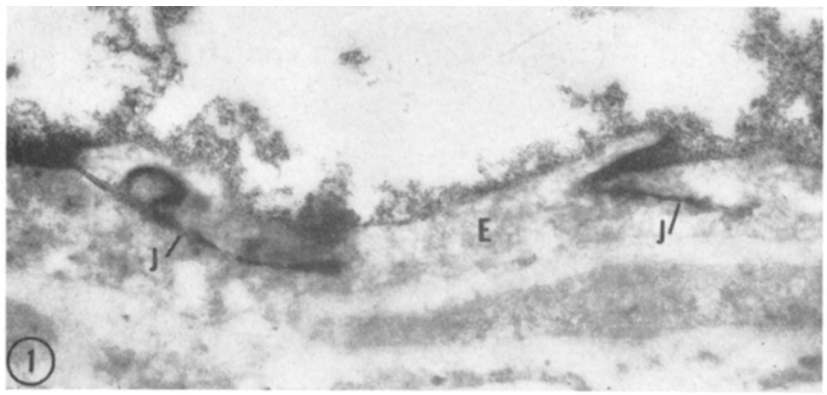

Fig. 1. Retinal capillary. Deposits of the Prussian blue reaction are visible in the lumen and in 2 intercellular junctions (J). The endothelium itself (E) and an external glial cell show very little internal detail because the section was examined unstained. × 45,000.

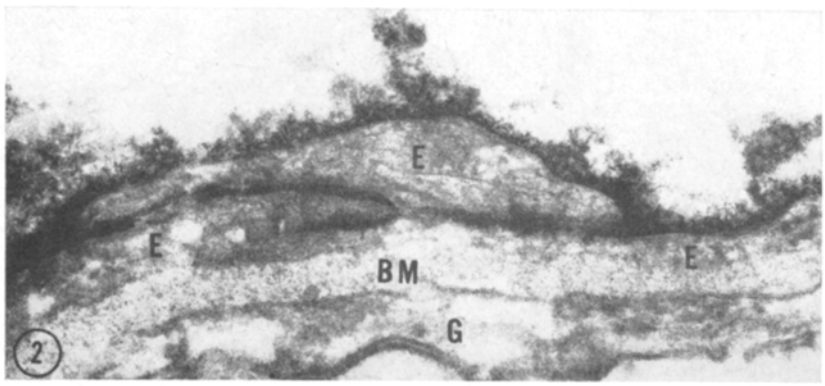

Fig. 2. Cerebral capillary. Again deposits are visible in the lumen, a junction, and external to the endothelial cells (E) amongst the basement membrane material (BM). A glial cell (G) is also shown. Lead stained. × 70,000.

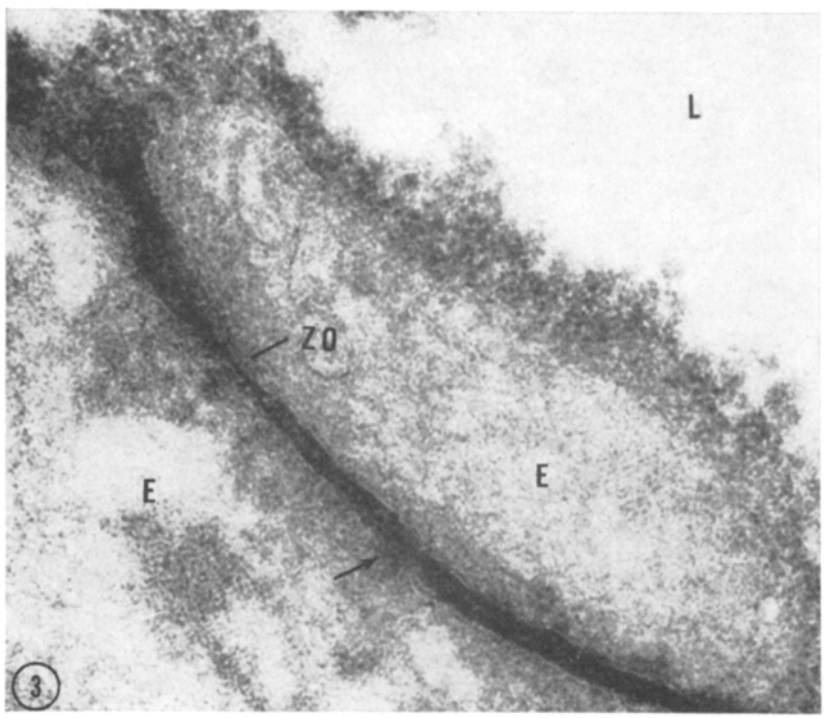

Fig. 3. Cerebral capillary. A portion of a junction is shown between 2 endothelial cells (E); the lumen is shown (L). The junction has one obvious narrowing, indicating a zonula occludens (ZO). A second narrowing (arrow) may also indicate one. The deposit of Prussian blue is continuous through both of these regions. In some places the staining of the membranes by the reaction may be seen. Unstained. × 250,000.

dissecting microscope was helpful in selecting the best blocks for further study. These were sometimes post-fixed in 2% Osmium tetroxide for 1 h, before being embedded in araldite by the usual techniques. Lead citrate[23] was used to stain some of the sections, but they were usually examined unstained. This made it harder to make out the details of the cells, but meant that the deposits of precipitated ions were more easily visible, and prevented confusion with staining artefacts.

Results. The normal fine structure of the cerebral and retinal capillaries has been described[1-3,5,6,24]. These findings were confirmed; in particular it was noted that all the junctions which were sufficiently perpendicular for the unit-membranes to be visible had quintuple-layered portions. (This was still observed when any one of the ions was used.) Thus it is very likely that the endothelial cells are surrounded by complete zonulae occludentes. The marked paucity of the small vesicles was also evident. The general preservation of the tissues was quite good, in both the control and experimental material, but in the latter the absence of the normal electron-stains and the presence of precipitates rendered some of the structures invisible.

The light microscope showed numerous vessels in both the brain and the retina with the Prussian blue reaction. The electron microscope (Figures 1–3) showed large deposits in the vessels' lumens, precipitated on their plasma membranes, in the intercellular junctions, occasionally in small vesicles attached to both plasma membranes and in the centre of the cells, in the basement membranes, in the general extracellular species between the glial, neural and retinal cells, and contained in 'synaptic' vesicles at nerve endings. The distribution of the 2 ions were the same, but the ferri-ferrocyanide precipitates were more dense and, as is known[18,25] were also present in the adjacent cellular membranes. The fact that this staining extended the length of the junctions shows that the ions responsible – Barium[20] and ferrocyanide[25] – were able to penetrate between the cells. The amounts of precipitates in the different sites varied, no doubt depending on local concentrations, diffusion rates and elapsed times, etc.

The most significant finding was the presence of deposits in many endothelial intercellular junctions; in fact, if there were deposits in the vicinity of a vessel, they were almost invariably found in all the junctions. It is especially important that the deposits appeared to be continuous right through the zonulae occludentes (Figure 3). Also, as all the junctions appeared to possess these zonulae, and as the deposits frequently continued the length of the junctions, it was evident that ions were present in the zonulae.

While the occasional small vesicles sometimes contained the reaction product, it is likely that vesicular transport is far too slow to account for the movements of small molecules across endothelium elsewhere[12,15,17]. In the cerebral and retinal vessels, with their paucity of vesicles, it is even less likely.

Discussion. It is unlikely that these results are artefactual. The first of the pairs of ions were non-injurious and isotonic with plasma[18,20]; the tissues in general appeared normal. Also intravitreous and intraventricular injections of peroxidase and ferritin produce no morphological changes in the retinal or cerebral vessels, which remain impermeable to them[3,5]; in particular, the retinal endothelial junctions, unlike the iridic ones, appear not to be disrupted by histamine or ocular paracentesis[24].

It is evident that cerebral and retinal ones must have passages through them large enough for ions to pass, but too small for peroxidase[6]. Even in regions where peroxidase can pass through some parts of the junctions, other parts are impermeable[7,8]. By contrast, almost all junctions appear to be permeable to ions[18,20]. It is known that the degree of tightness can vary from one part of the cell to the next, and from site to site in the body[4,6-8,11,19]. A number of recent studies indicate that zonulae occludentes may consist of meshes of fibrils immersed in mucopolysaccharide. Variations in permeability could result from slight variations in the numbers and orientations of these fibrils and in the degree of polymerization.

It is concluded, therefore, that the junctions between the endothelial cells of cerebral and retinal capillaries are permeable to ions and presumably to other small molecules. There is no need to postulate that small molecules have to pass through the endothelial cells, which would imply various specializations in their functions[26].

Résumé. Des injections intra-thécales et intra-vitreuses d'ions de ferrocyanure et de sulfate dans les rats étudiés au microscope électronique, permettant de constater que les jonctions endothéliales des vaisseaux cérébraux et rétinaux sont perméable aux petites molécules.

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1 T. S. BODENHEIMER and M. W. BRIGHTMAN, Am. J. Anat. *122*, 249 (1968).
2 H. S. BENNETT, J. H. LUFT and J. C. HAMPTON, Am. J. Physiol. *196*, 381 (1959).
3 M. W. BRIGHTMAN, in *Progress Brain Research 29* (Ed. A. LAJTHA and D. H. FORD, Elsevier, Amsterdam 1967), p. 19.
4 J. R. CASLEY-SMITH, *Lymphology 1*, 77 (1968).
5 A. LASANSKY, J. Cell Biol. *34*, 617 (1967).
6 T. S. REESE and M. J. KARNOVSKY, J. Cell Biol. *34*, 207 (1967).
7 R. S. COTRAN and M. J. KARNOVSKY, J. Cell Biol. *37*, 123 (1968).
8 M. J. KARNOVSKY, J. Cell Biol. *35*, 213 (1967).
9 R. R. BRUNS and G. E. PALADE, J. Cell Biol. *37*, 277 (1968).
10 J. R. CASLEY-SMITH, Q. Jl exp. Physiol. *49*, 365 (1964).
11 J. R. CASLEY-SMITH, Br. J. exp. Path. *46*, 35 (1965).
12 J. R. CASLEY-SMITH, J. Microscopy, submitted for publication (1969).
13 M. A. JENNINGS and H. W. FLOREY, Proc. R. Soc., B *167*, 39 (1967).
14 M. A. JENNINGS, V. T. MARCHESI and H. W. FLOREY, Proc. R. Soc. B *156*, 14 (1962).
15 S. M. SHEA and M. J. KARNOVSKY, Nature *212*, 353 (1966).
16 S. W. KUFFLER and J. G. NICHOLLS, Ergeb. Physiol. *57*, 1 (1966).
17 E. M. RENKIN, Physiologist Wash. *7*, 13 (1964).
18 J. R. CASLEY-SMITH, Q. J. exp. Physiol. *52*, 105 (1967).
19 J. R. CASLEY-SMITH, Experientia *25*, 374 (1969).
20 R. S. COTRAN and G. MAJNO, Protoplasma *43*, 45 (1967).
21 S. ROSE and P. H. GLOW, Aust. J. exp. Biol. med. Sci. *43*, 737 (1965).
22 J. B. CAULFIELD, J. biophys. biochem. Cytol. *3*, 827 (1957).
23 E. S. REYNOLDS, J. Cell Biol. *17*, 208 (1963).
24 M. SHAKIB and J. G. CUNHA-VAZ, Expl Eye Res. *5*, 229 (1966).
25 J. R. CASLEY-SMITH, Jl R. microsc. Soc. *87*, 463 (1967).
26 This work was performed with the aid of an Australian Research Council Grant. The assistance of Mrs. V. D. GARROW is gratefully acknowledged.