Evolution of the Extracellular Space in Immature Nervous Tissue

Most electron microscopists assign a low value, about 5% of the total, to the volume of the extracellular space in the central nervous system (CNS)[1-3]. Many physiologists, however, claim that this value should be much higher, perhaps as much as 30%[4,5]. This electron microscopical study on the maturing cerebellum shows a progressive reduction of the extracellular space as the age of the animal increases.

More than 200 albino rats ranging in age from 1 day to 8 months were used. In previous work[6,7] the present authors have given an account of the electron microscopical techniques. Therefore, only the fixation procedures will be described. The fixatives used were: 4% formalin, 2.5% and 6% glutaraldehyde, a mixture of 5% formalin and 1% glutaraldehyde, and a combination of formalin and picric acid[8]. Fixation was accomplished by immersion or perfusion, and the tissues were postfixed in 1 or 2% osmium tetroxide. Phosphate and cacodylate buffers of different concentrations served as fixative vehicles. Cerebella from 20 animals were directly fixed by immersion in osmium. Temperature of the aldehyde solutions was either 4 °C, room temperature (approximately 22 °C), or 60 °C to vary the diffusion rates. In-block staining was accomplished with uranium, the tissues embedded in Epon, and ultrathin sections stained with lead. A Siemens I/A electron microscope with modified objective was used at 80 kV.

The cerebella of animals 1–4 days old showed the largest extracellular spaces. Figure 1 shows the molecular layer neuropil adjacent to the external granular layer in a 2-day-old rat. Irregular gaps of extracellular space in places more than 1000 Å wide exist between the cell processes. At the few points where 2 processes become adjacent a minimal cleft 150 Å wide is found. The synaptic cleft has about the same width as that in the mature animal.

By the ninth to twelfth postnatal day, a reduction of the extracellular spaces has occurred. Figure 2 shows the neuropil below the external granular layer (molecular layer) in a 10-day animal. The processes are generally separated from each other and from the cell bodies by a 150 Å space. At points where more than 2 processes meet values several times higher can be found. By the seventeenth to twenty-first postnatal day only a few gaps larger than 150–200 Å can be found.

Within the limits of the present experiments the osmolarity of the fixative, its chemical nature, temperature, or method of fixation caused no important variation in the extent of the extracellular spaces in the youngest animals. These were much smaller in the external granular layer than in the internal layer. During the second and third week cell migration and proliferation of neuronal and astroglial processes progressively reduce the extra-

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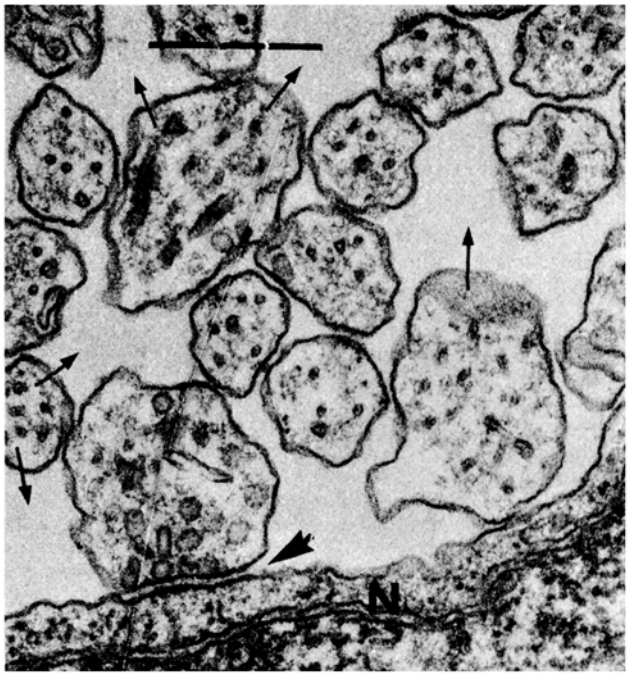

Fig. 1. Molecular layer in a 2-day-old rat. Gaps of extracellular space, some more than 1000 Å wide (small arrows), exist between cell processes. Large arrow points to a synaptic cleft of the same width as in the adult. Neuron (N). Calibration mark 0.5 μ.

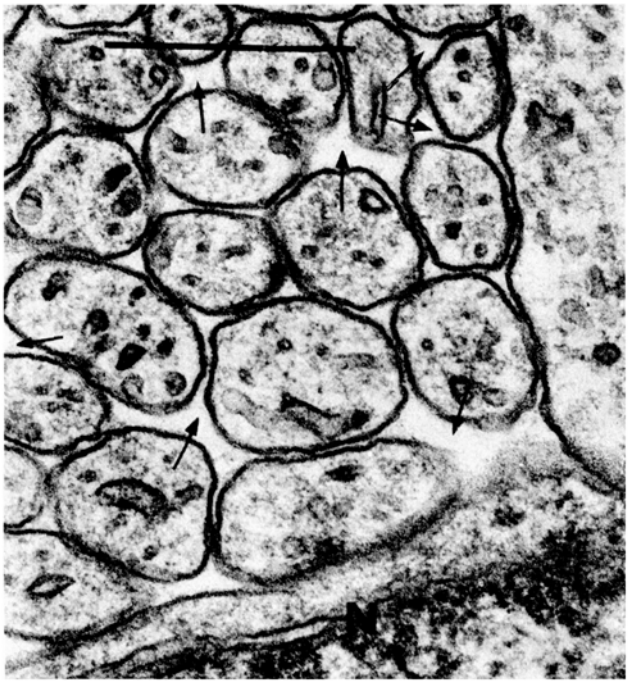

Fig. 2. Molecular layer in a 10-day-old rat. Gaps approximately 150 Å wide separate cell processes from each other. At points where more than 2 processes meet the extracellular space is larger (arrows). Neuron (N). Calibration mark 0.5 μ.

cellular space until it reaches the adult size about the end of the fourth week. Comparable results have been reported by other workers[9] on the basis of an entirely different approach.

The consistent way in which these changes occur even when a variety of fixation procedures are used tends to support the thesis that the smaller value for the extracellular spaces in the CNS is not artificial as implied by others[10–12].

Resumen. En el cerebelo de rata recién nacida existen espacios extracelulares mayores que 1000 Å los cuales progresivamente se reducen hasta que en la tercera semana, como en el adulto, las celulas y fibras se hallan separadas por endiduras de 150–200 Å. Estas observaciones indican que el reducido espacio usualmente hallado en el adulto no es un artificio tecnico como ha sido sugerido por algunos autores.

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Über kollagenhaltige perivaskuläre Räume an «Kapillaren» in der Medulla oblongata des Rhesusaffen

Nach den bisherigen elektronenmikroskopischen Befunden besitzen Hirnkapillaren in normalen erwachsenen Gehirnen verschiedener Tiere und des Menschen keine perivaskulären Räume (PVR) in den Gebieten, in denen eine Blut-Hirn-Schranke nachweisbar ist[1–3]. Lediglich an venösen Gefässen[4] und unter pathologischen Bedingungen auch an Kapillaren[5] trennen sich die endothelialen und gliösen Basalmembranlamellen stellenweise und umfassen echte PVR. Nur vereinzelt wurde erwähnt, dass auch an «normalen» Kapillaren oder postkapillären Gefässen des ZNS einzelne Kollagenfibrillen oder kleine Bündel zwischen die gespaltenen Basalmembranen eingelagert sein können[5–7].

Im Verlauf von elektronenmikroskopischen Untersuchungen an verschiedenen Gebieten der Medulla oblongata von erwachsenen Rhesusaffen (Nucl. cuneat. extern., gracilis, orig. N. XII, intercalat., tract. solitar., dors. N. X) wurden neben typischen Hirnkapillaren regelmässig terminale Gefässe mit verschiedenem Durchmesser beobachtet, die keine glatten Muskelzellen aufweisen, aber ganz oder teilweise von PVR umgeben sind. Diese Gefässe kommen im Gegensatz zu früheren Beobachtungen[7] nicht nur oberflächennahe, sondern auch tief im Parenchym vor. Die PVR können verschieden weit sein und unterschiedliche Mengen von Kollagen und sogar mesenchymale Zellen enthalten, die keine Basalmembran tragen (Figur). Durchmesser und Wandstruktur zeigen, dass es sich dabei nicht nur um Venolen und kleine Venen, an denen dieses Verhalten bekannt ist[2,3,6], sondern auch oft um typische Kapillaren handelt (Figur).

Da Kapillaren mit einem PVR von den meisten Autoren für pathologisch oder «schrankenlos» gedeutet werden, schien uns dieser Befund der Mitteilung wert. Zukünftige Studien müssen ergeben, ob es sich dabei um eine Arteigenschaft des Affen oder um eine typische Kapillarform des Hirnstammes handelt. Sicher kommen sie hier häufiger vor als in anderen Gebieten des ZNS. Der Befund ist auch für das Verständnis des morphologischen Substrates der Blut-Hirn-Schranke wichtig. Das Vorhandensein von perikapillärem Kollagen darf jedenfalls nicht ohne weiteres als pathologisch gewertet werden[8].

Summary. Electron-microscopic observations in various parts of the Rhesus monkey's brain stem revealed true pericapillary spaces containing collagen fibers and partly even mesenchymal cells.

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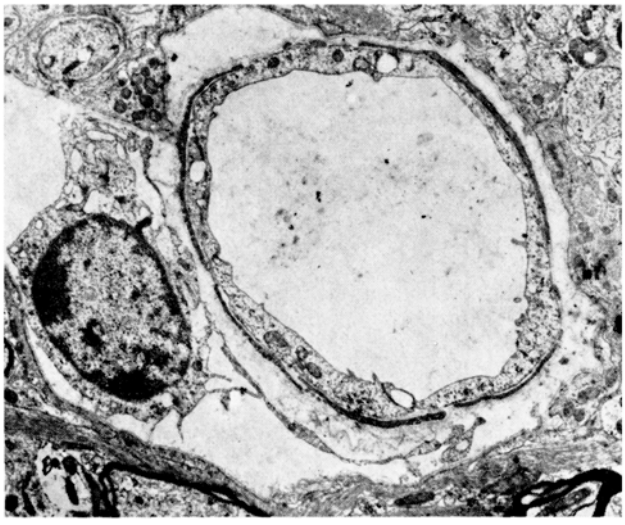

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