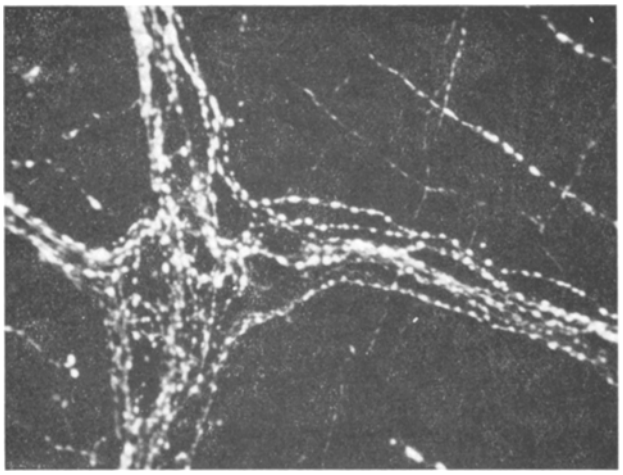

Fig. 1. Rabbit small intestine. Strip preparation of the Auerbach's plexus. A large ganglion and several meshes richly supplied with adrenergic fibres are apparent. × 220.

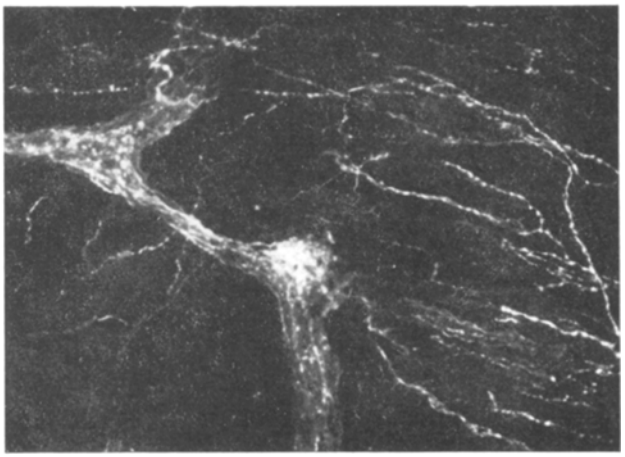

Fig. 2. Guinea-pig cardia. Section through the muscle layers. Many adrenergic fibres are observed in the Auerbach's plexus ganglia, and in the outer (left) and inner (right) muscle layers. × 150.

cular adrenergic fibres, besides being indicated by the lack of adrenergic cells within the intestinal wall, is further borne out by their complete disappearance after experimental denervation of the intestinal loops (experiments of denervation by means of criocautery of the mesenteric nerves at the intestinal hilum[12]). Lastly, the existence of adrenergic fibres pertaining to the muscle layers has been substantiated by electron microscope studies in the rat[13]. In the circular muscle layer of the small intestine, in fact, intermingled with smooth muscle cells, nerve fibres are observable which exhibit varicosities rich in vesicles ranging from 300–700 Å in diameter and containing a highly osmiophilic granule. These vesicles are regarded as specific of adrenergic terminals (RICHARDSON[14]).

In our opinion, there is therefore reason to believe that the presence of extrinsic adrenergic fibres in very close proximity to muscle effectors demonstrates the existence of an alternative pathway to the neuron chain envisaged by NORBERG.

In summary, adrenergic varicose nerve fibres are observed both around intramural nerve cells and in mienteric and submucous plexuses, and in close proximity to muscle and glandular effectors. The possibility thus exists of a direct action of adrenergic fibres on the effectors and an indirect one through the intramural nerve cells[15].

Riassunto. Le ricerche istochimiche effettuate sull'innervazione adrenergica dell'intestino hanno dimostrato che fibre adrenergiche sono presenti non solo attorno ai neuroni e nelle maglie dei plessi mienterico e sottomucoso, ma anche nello spessore stesso dello strato muscolare interno e in intimo rapporto con elementi ghiandolari della tonaca mucosa. Vi è quindi la possibilità che sugli effettori ghiandolari e muscolari le fibre adrenergiche esercitino sia un'azione diretta sia un'azione indiretta attraverso i neuroni intramurali.

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Structure of Nuclear Membranes Isolated from Brain Cells

Despite of the increasing number of studies dealing with the mechanisms of the nucleocytoplasmic interaction, our present knowledge of the structural basis of these processes is still insufficient. Particularly, the question of the structure of the nuclear pore complexes, which are thought of as the most important pathways for the controlled nucleocytoplasmic exchange of macromolecules, remained unsolved (references to this problem e.g. in [1] and [2]). Since a generally applicable method for isolating nuclear membranes opens the possibility for comparing the structure of nuclear envelopes of various cells[3], one of the main intentions in our laboratory is to collect the structural data of the nuclear membranes of different plant and animal tissues[3,4] as well as those of the same cell type in different physiological and cytological states[5,6]. The present study is concerned with the structural details of the isolated nuclear envelopes of neuronal and glial nuclei from the rat brain.

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Rats of both sexes were sacrificed by decapitation, and the brains were removed, cleaned from the meninges and from the blood under permanent rinsing in cold 1% NaCl solution. Portions of the brain were then immediately incubated in the cold isolation medium (for details see[3,4]; in a few experiments Mg^{2+}-ions in a concentration of 2 m*M* were added) for 6–12 h. The nuclei were isolated according to the method of KUEHL as described earlier[3]. The nuclear fraction was free of any contaminants and consisted of the intact nuclei of both neuronal and glial cells (Figure 1). Only some remnants of ER-cisternae were occasionally observed adhering to the nuclear envelope. Capillaries and endothelial nuclei, which often can be found to contaminate nuclear fractions from brain material[7–11], were almost totally eliminated by the discontinuous gradient centrifugation step. The isolated nuclei were then fractionated and pieces of nuclear envelopes were isolated according to the procedure previously described[3,4]. The isolated nuclear membranes were fixed with 1% OsO_4 buffered to a pH of 7.0 with cacodylate, then negatively stained with phosphotungstic acid, adjusted to pH 7.0, and examined with a Siemens electron microscope Elmiskop IA.

With respect to the disruption of the nuclei by the hypotonic treatment and the sonication described by FRANKE[3] brain cell nuclei, in comparison e.g. with liver nuclei, were more fragile (see also[7,8,10]), even though they exhibited a remarkable rigidity. As a result of these properties, the envelope pieces obtained were, in general, small and irregular in shape as can be seen in Figures 2 and 3. Frequently, the nuclear envelope is fragmented around the pore complexes so as to produce envelope remnants which do not contain pores in addition to others which contain pores (Figure 3). This outline of fragmentation indicates that the pore complex is a site of increased stability as can also be seen in comparable micrographs presented by Yoo and BAYLEY for nuclei from pea seedlings[12] and by MONROE et al. for nuclei of lymphoma cells[13]. The surface of the envelope fragments was mostly covered by an unidentified fibrillar material, probably nucleoprotein, which adsorbed a relatively great amount of the staining phosphotungstate so that the structure of the pore complexes was somewhat obscured.

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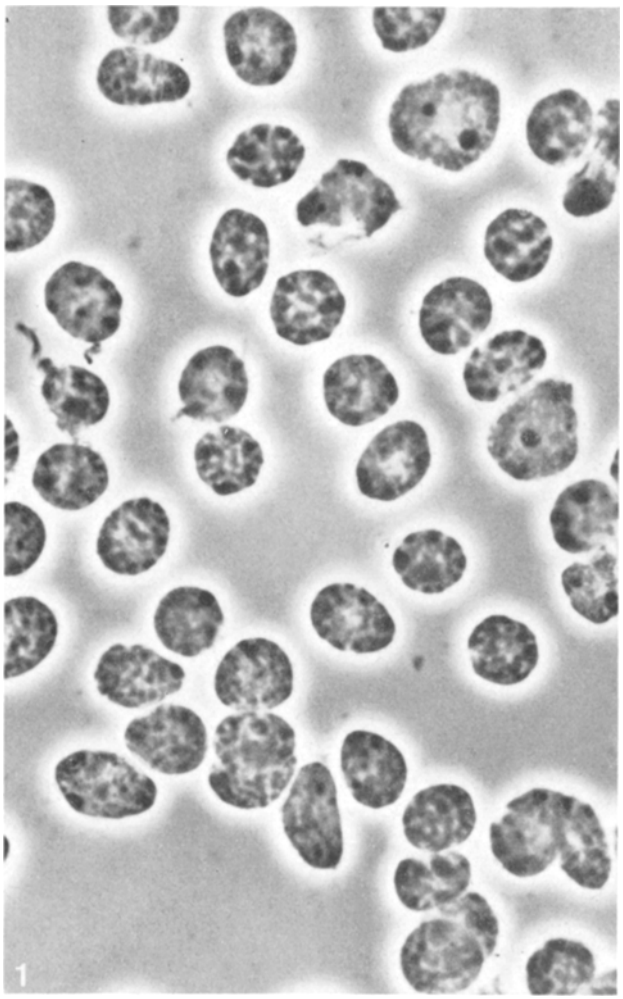

Fig. 1. Phase contrast micrograph of the fraction of nuclei from brain cells. Glial as well as the larger neuronal nuclei are present. × 1400.

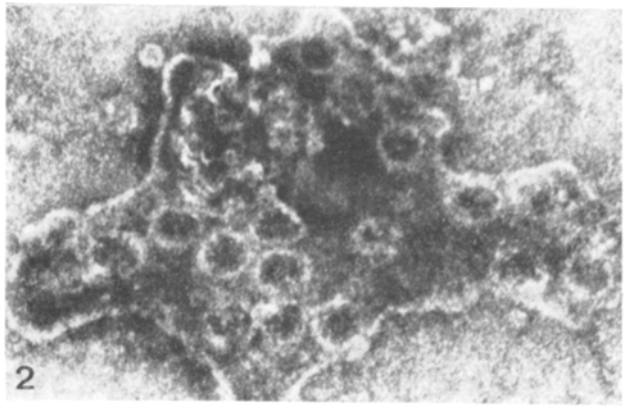

Fig. 2. Electron micrograph of a typical piece of nuclear envelope isolated from brain cells. × 70,000.

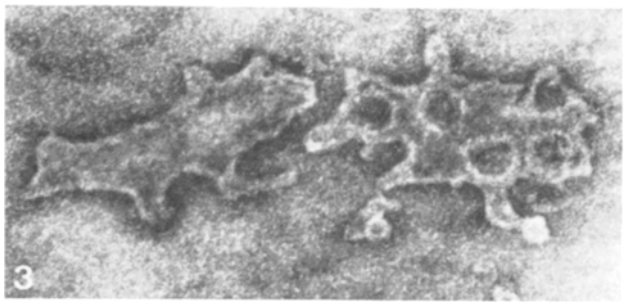

Fig. 3. Electron micrograph of two nuclear envelope fragments. The fragment in the right contains pores while the left one does not. × 70,000.

The negatively stained nuclear pore complexes revealed a thin, 'white' delineated pore perimeter (Figures 2, 3 and 4b) associated with more or less prominent annular globules (Figures 4b, c and 5) and the centrally located dot (Figures 4a and c). The diameters of the globules are from 8–17 nm while the diameter of the central dot is from 10–22 nm. In those pores in which the perimeter could clearly be recognized, the 'true diameter' of the lumen of the pore complex was measured and found to be in the range of 68–80 nm. This value is apparently high when compared with the pore diameters of the nuclei of other animal cells, provided that a comparable adequate type of preparation has been used.

So, for instance, the pore diameter in rat liver nuclei is 65 nm[4], in amphibian oocytes 66 nm[14] and in macronuclei of the ciliate Tetrahymena 46–65 nm[5,6]. It is interesting to note that the pore diameter of the brain cell nuclei rather is of the same magnitude as of lymphoma cells as reported by MONROE et al.[13]. In those pore complexes in which the perimeter of the pores is hidden by annular material (e.g. Figure 4), an outer annulus diameter of about 90–110 nm and an inner annulus diameter of 50–70 nm is distinguished (terminology sensu GALL[1]). In a great many pores some centripetal projections could be detected originating from the annulus into the lumen of the pore (Figure 4d) as described from other material, too[3,4,6,12].

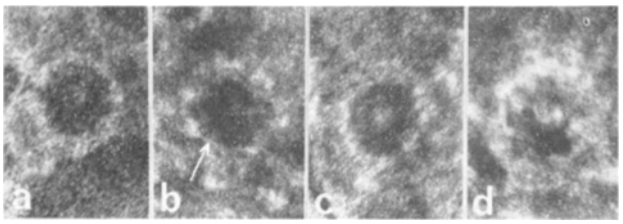

Fig. 4a–d. Structural details of the pore complexes. Fig. 4a shows a typical pore complex at the margin of an envelope fragment with a central dot and annular material. In Fig. 4b the annular subunits can be seen lying at the sharply outlined perimeter (arrow). Fig. 4c presents the central dot. In Fig. 4d the tips of more diffuse material can be recognized as projecting from the annulus towards the central dot. All × 150,000.

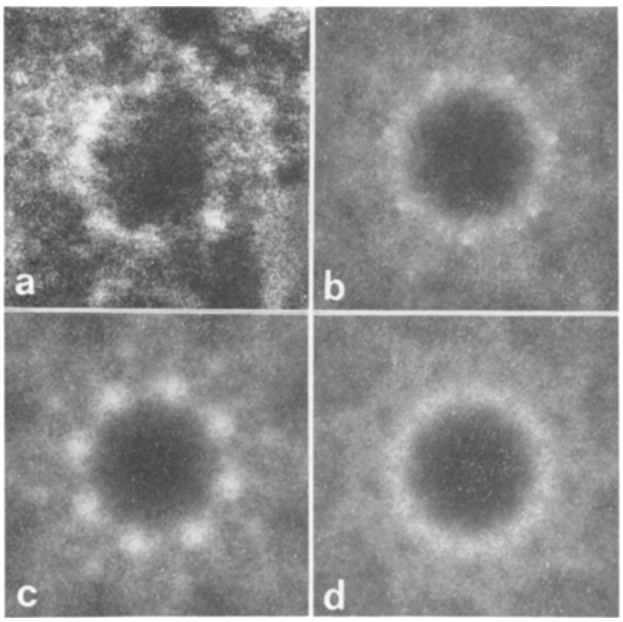

Fig. 5a–d. Markham analysis of an electron micrograph of a nuclear pore complex from rat brain cells (a). The radiary pattern of the globular subunits of the annulus is markedly reinforced for n = 8 (c), but not for n = 7 (b) and n = 9 (d). × 250,000.

The pore frequency, i.e. the number of pores per square micron of the nuclear surface, was found to be very low. The majority of the envelope pieces contained only 30–45 pores/μ^2. This means that no more than 13–20% of the nuclear surface is pore area (for comparative data see[3,4,6,12,15]). When the distribution of the annular granules was tested with MARKHAM's rotation technique[16] a pronounced enhancement of radial structure was generally found for n = 8 (Figure 5). This demonstrates that in the case of the brain cell nuclei the globular subunits of the annulus are arranged in a precise eightfold symmetry, a pattern which is known to exist in nuclei of other cells also (e.g.[3,4,13,15]). This widespread occurrence leads one to the generalization that a symmetrical arrangement of 8, or in some cases 9[4], subunits is a general feature of the nuclear pore complex of all cells.

The nuclear fraction as used in this study was heterogeneous in that neuronal as well as glial nuclei were present. Thus, some slight differences between different envelope pieces could not be correlated to either neurones or glia cells. Such differences in nuclear envelope structures, i.e. structures which might be involved in the mechanisms of RNA and protein synthesis, would be of particular interest in connection with the findings and hypotheses of HYDEN and his co-workers (e.g.[17]) on the functional metabolism of neurons and glia cells. Thus, a fractionation into neuronal and glial nuclei, as outlined by the work of KATO and KUROKAWA[11], preceding the isolation of the nuclear membranes, would be especially worthwhile for the further investigations on these problems.

Zusammenfassung. Von einer reinen Fraktion von Zellkernen aus Rattenhirn wurden Stücke der Kernmembran isoliert und nach Negativkontrastierung elektronenmikroskopisch untersucht. Dabei wurden sowohl Neuronen- als auch Gliazellkerne erfasst. Die Porenhäufigkeit betrug 30–45 Poren/μ^2, d. i. etwa 13–20% der Kernoberfläche. Die Porendurchmesser lagen zwischen 68 bis 80 nm. Die auf dem Porenrand liegenden Untereinheiten des Annulus sind in einer achtstrahligen Radiärsymmetrie angeordnet. Die Ergebnisse werden in Zusammenhang mit den entsprechenden Strukturdaten aus anderen Zellen vergleichend diskutiert.

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