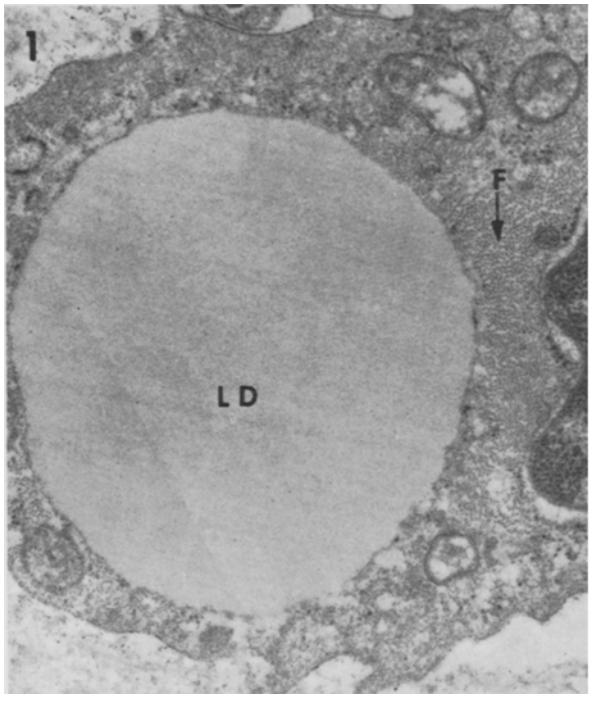

Fig. 1. Part of a peripheral chondrocyte which contains a medium-sized lipid droplet (LD). Cytoplasmic filamnets (F) are visible predominantly in transverse section. ×26,500.

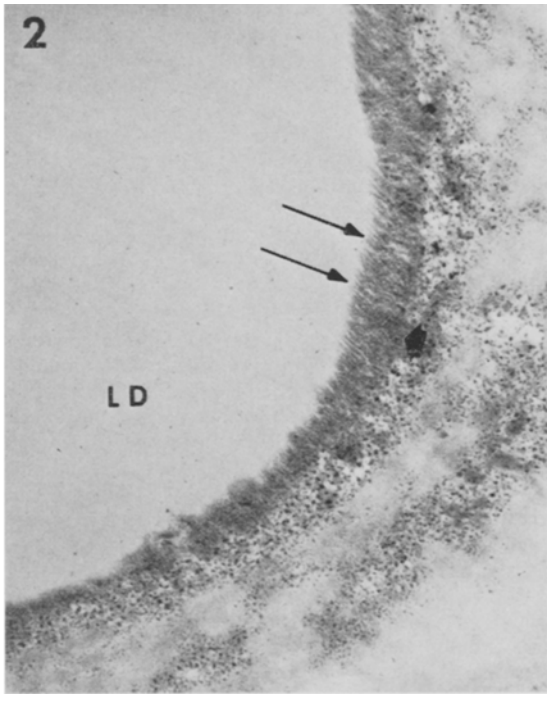

Fig. 2. Periphery of a large, univacuolar central chondrocyte. The large lipid droplet (LD) is surrounded by densely packed parallel filaments (thin arrows). The short thick arrow points to the outer cell membrane. Right and below: intercellular material. ×28,000.

parallel arrangement (Figure 1). They are distributed all around the nucleus, between the nucleus and the lipid droplet, and around the lipid droplet itself. The close relationship of filaments to the lipid droplet can best be discerned in mature univacuolar chondrocytes, which make up the central part of the cartilaginous plate. The thin rim of cytoplasm, which surrounds the lipid droplet, is largely occupied by densely packed filaments. Their uniform, parallel arrangement is clearly visible in areas in which the periphery of the weakly osmiophilic lipid droplet is sectioned tangentially (Figure 2).

The findings described are similar to observations of LUCKENBILL and COHEN[3] and WOOD[1] in chick subsynovial and developing bone marrow adipose cells respectively, with the exception that the orthogonal pattern of distribution of filaments was not observed in our material.

The significance of the close association of cytoplasmic filaments with the intracytoplasmic lipid droplets remains obscure. This relationship is sometimes described as an almost common feature in adipose cells[5], but it must be pointed out that this statement is based only on sporadical observations on differently located adipose cells, predominantly in the chick[1-3]. The abundance of cytoplasmic filaments in the cytoplasm of rat auricular chondrocytes has been described, but their relationship to the lipid droplet was not mentioned[6]. Further systematic investigation is needed in order to cast more light on this problem. The observations described above on the rat auricular chondrocytes suggest that the presence of abundant cytoplasmic filaments is principally related to the mechanical role of the tissue rather than to the presence of lipid droplets within the cytoplasm.

Zusammenfassung. Im Ohrknorpel der Ratte werden feine, intracytoplasmatische, 60–70 Å dicke Filamente beobachtet, welche, parallel zueinander angeordnet, die Oberfläche der in den Knorpelzellen enthaltenen Fetttropfen überziehen.

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cAMP-Mediated Regulation of the Permeability in the Brain Capillaries

In view of the powerful impact exerted by some substances upon the functioning of the central nervous system, the recognition of those cellular mechanisms which regulate the transport in capillaries from blood circulation towards the brain would be of utmost importance. Recent results, obtained by studying the distribution of exogenous peroxydase[1], and the capillary ultrastructure after adenosine triphosphatase inhibition[2],

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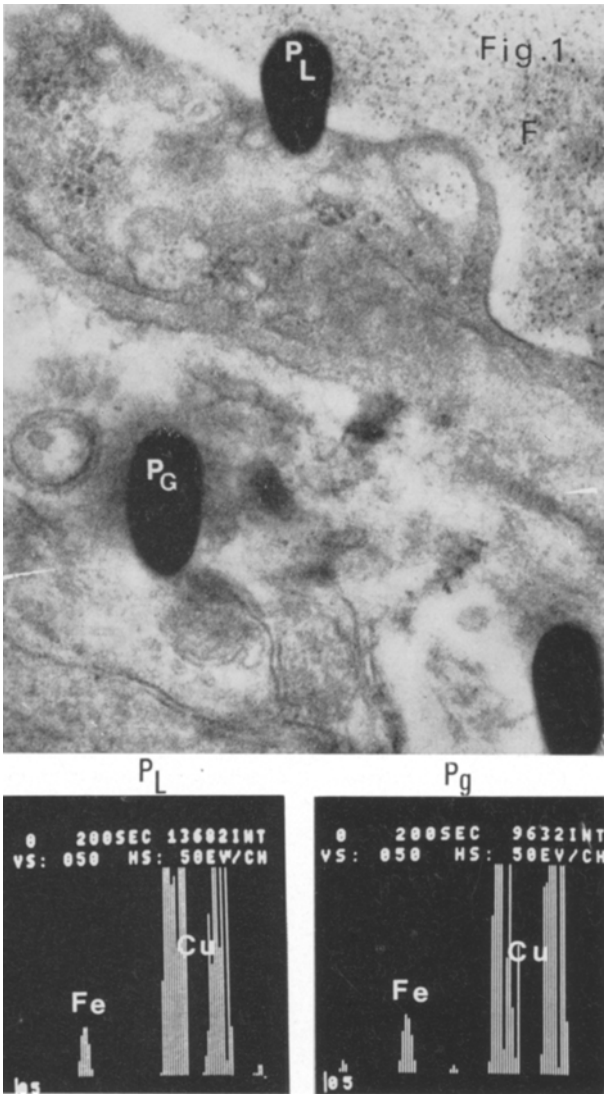

Fig. 1. Electron microscopic appearance of a capillary and a glial end foot after X-ray microanalysis. Ferritin (F) was injected together with dibu-cAMP 30 min prior to fixation. Sites of probes are larger than analyzed areas because of contamination. P_L, probe in the lumen; P_G, probe in the glial end foot. ×66,000. Iron deriving from Ferritin was detected by energy dispersive X-ray microanalysis after background substraction in the lumen (P_L) and in the glial end foot (P_G). Cu, copper from the grid.

have drawn attention to the significance of the capillary endothelium and, in particular, to certain enzymes being confined histochemically to the capillary wall in the maintenance of the blood-brain barrier. Though an increase in the pinocytotic activity was observed after dibutyryl cyclic adenosine monophosphate (dibu-cAMP) administration[3], it was not proved whether 1. the increased pinocytosis is accompanied with enhanced permeability and 2. adenylate cyclase is confined to the capillary wall.

In our experiments, 40–50-day-old female Wistar rats were used. To establish changes in permeability, 0.3 ml Cd-free Ferritin (Serva Entwicklungslabor, Heidelberg) was injected i.v. to 5 rats and together with 10 mg/kg dibu-cAMP to another 5 rats under light ether anaesthesia. Small cubes of parietal cortex were removed 30 min after treatment and fixed in aldehyde fixative of high osmolarity[4], then post-fixed in Millonig's buffered osmic acid[5] solution. Araldite sections of gold interference colours were viewed in a JEOL 100B transmission electron microscope equipped with a scanning attachment (JEM-ASID) and EDAX energy dispersive spectrometer. This analytical system allowed viewing of the image and localization of the beam for excitation of a spot approximately 200 Å in diameter. Figure 1 shows a detail of the capillary and the surrounding astrocytic process which had been analyzed. In the dibu-cAMP treated cases, significant peaks characteristic of iron ($K_{\alpha 1,2}$ at 6.4 keV and $K_{\beta 1}$ at 7.0 keV) could be detected not only in the capillary lumen but also in the swollen glial end feet. By contrast, in control sections, iron peaks were revealed in the capillary lumen only.

In other series of experiments, brain capillaries were isolated from the cerebral cortex by a procedure elaborated earlier[6]. Animals (10 rats in 1 experiment, 70 rats altogether) were perfused prior to homogenization with 0.9% sodium chloride through the aorta under ether anaesthesia in order to get rid of the expected undesirable presence of blood corpuscules during enzyme determinations. Adenylate cyclase activity was determined by the method of Drummond and Duncan[7]. The basal activity of the homogenate was 1387 ± 331 pM/mg protein/5 min (mean ± SE, $n = 4$); whereas those of the capillary-rich fraction was 10,245 ± 994 pM/mg protein/5 min (mean ± SE, $n = 4$). Activation of adenylate cyclase in the capillary-rich fraction was found with histamine but not with 10^{-5} M noradrenaline. Receptor properties of histamine-activated capillary adenylate cyclase was evidenced by specific inhibitors[8,9]. Figure 2 shows the cAMP production in the presence of various concentrations of histamine and its receptor inhibitors chloropyramine and burimamide. These results indicate that, in the capillary-rich fraction, both H_1 and H_2 receptors were present.

As the permeability of brain capillaries was increased by dibu-cAMP treatment, the possibility of cAMP mediation during physiological functioning can be raised. High adenylate cyclase activity of capillaries suggests that cAMP can be produced in vivo locally in certain membranes of the capillary wall. The fact that capillary adenylate cyclase could be activated by histamine receptors reflects an important phenomenon of pathological interest. Increased pinocytosis accompanied by enhanced macromolecular permeation has long been observed in several damages of the blood-brain barrier[10,11]. Histamine was also found to produce severe increase of permeability in brain capillaries[12]. It is proposed that, in pathological cases, effective pinocytosis giving rise to an increase in certain transport processes of brain capillaries could derive from the enhanced cAMP production occurring due

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Fig.2.

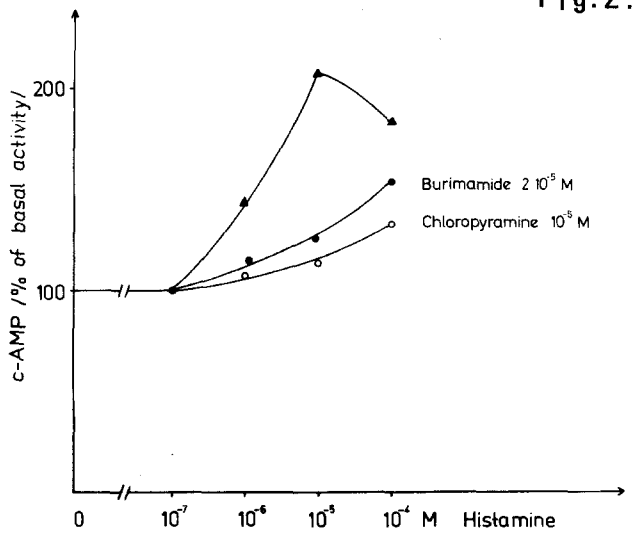

Fig. 2. Histamine activation and inhibition of brain capillary adenylate cyclase. Adenylate cyclase assay contained: 2 m*M* $MgCl_2$, 2 m*M* ATP (0.2 μCi ^{14}C ATP), 50 μg bovine serum albumin, 2 m*M* cAMP, 1 m*M* tetrahydroperparine, 100 μg protein kinase, 2 m*M* phosphoenolpyruvate, 50 m*M* *Tris*-HCl pH 7.4 buffer, 100 μl brain capillary fraction (1 mg/ml protein) in a total volume of 300 μl. The capillary fraction was homogenized with 2.0 ml 0.25 *M* sucrose at 0 °C in a Potter apparatus. The incubation was carried out for 5 min at 32 °C. The values represent the means of 3 experiments. The basal activity (control) was expressed as 100%.

to the activation of capillary adenylate cyclase. As to the origin of histamine, the physiological activator of the reticulo-endothelial system[13], it is thought that, as a consequence of change of environment, histamine is released from its natural sources, that is from leukocytes and/or mast cells[14].

cAMP mediation was found in the permeability-modifying effect of neurophypophyseal hormones[15]. On the basis of our results, the cAMP mediation in the permeability regulation of brain capillaries can be hypothesized.

Zusammenfassung. In den mit cAMP behandelten Kapillaren des Rattengehirns wurde mit Ferritin eine erhöhte Permeabilität gefunden. Die Adenylatzyklase-Aktivität wurde in den in Gehirnkapillaren angereicherten subzellulären Fraktionen bestimmt und mit Histamin aktiviert. Da sowohl H_1- als auch H_2-Rezeptoren in den Fraktionen vorhanden waren, wird angenommen, dass das cAMP in der Permeabilitätsregulation der Gehirnkapillaren eine Rolle als Mediator spielt.

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At what Stage of Development does the Somitic Mesoblast Invaginate Into the Primitive Streak of Chick Embryo?

After the careful work of Pasteels[1], it was generally accepted that the presumptive somitic mesoblast was already present in the postnodal area of the fully grown primitive streak. However, Wolff[2] had argued that at this stage of development, the presumptive somitic tissue is still contained in the upper layer, in two areas contiguous to the anterior part of the primitive streak. Recently, Nicolet[3], on the basis of autoradiographic study, was drawn to the same conclusion. At present, we are trying to test these 2 hypotheses.

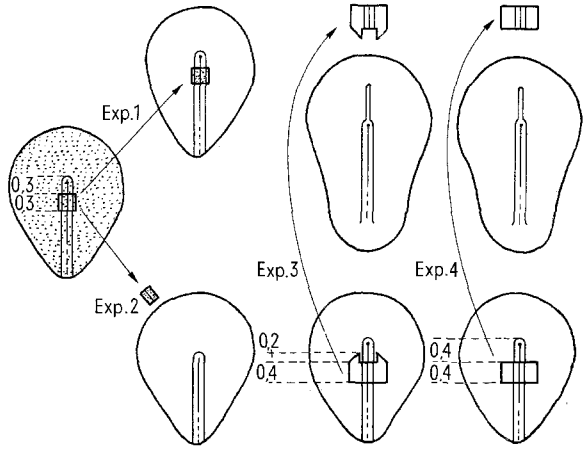

Fig. 1. Diagram showing the experimental procedures. Stippling indicates the lebelling.

The experiments were performed on chick blastoderms cultured in vitro (Gallera and Nicolet[4]). They were distributed in 4 series (see Figure 1). Our experiments consisted either in exchanging the postnodal length (0.3 mm) of the fully grown streak with the same piece, but taken from blastoderms, labelled with tritiated thymidine (Figure 1; Exp. 1), or in explanting the postnodal area of the streak, excised with or without contiguous ectoblast, into the area opaca of the host blastoderm (Figure 1; Exp. 2, 3 and 4). The donors were always at the fully grown streak stage, the hosts were of different ages, namely: the fully grown streak stage (series 1), the long streak stage (series 2), or the head process stage (series 3 and 4). At the latter stage the ectoblast is not more competent (Gallera and Ivanov[5]) so that without labelling, it was easy to distinguish the graft's structures from those of the host. The ventral side of the graft was applied against the ventral side of the host's ectoblast.

The host blastoderms were fixed about 20 h after the grafting and analyzed on serial sections. The blastoderms

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