Actions of medium conditioned by the secretion of endocrine glands (figure 1.) - Prothoracic glands (PG). PGs excised from 5- or 6-day-old 5th instar larvae induce a fast and intense development of cellular alterations, and a new epicuticle is deposited on a fibrillary procuticular material. The titre of ecdysteroids released into the medium never exceeds 50 ng/ml with 2 pairs of PGs[8]. However, 50 ng/ml of synthetic alpha ecdysone is not capable of inducing a new cuticular cycle. The whole process suggests another role for PGs involved in cuticular secretory mechanisms.
- Corpora allata (CA). A medium conditioned by CA from mature males or larvae[11] does not initiate secretory activity or cellular alterations. The result of the presence of corpora allata is an inhibition of all the events observed in a hormone free medium.
- Corpora allata and prothoracic glands. This association reduces the intensity of the cellular alterations induced with PGs alone. The secretory activity is not completely inhibited as it is with CA alone. A sparse fibrillary material is deposited after detachment of the old cuticle.
The epidermal cell development in vitro suggests that a defined hormonal context induces various secretory activities (with qualitative and quantitative variations) and various intensities of cellular alterations. The in vitro model appears to be a good tool for studying the mechanisms involved in molting processes. Alpha ecdysone and beta ecdysone have specific effects at physiological doses: - alpha ecdysone induces cell alterations, - beta ecdysone induces cuticular secretion.
PG which release alpha ecdysone[9,10] turns out to be as favorable as possible for total performance of preparatory alterations. Another role of PG is possible with regard to cuticular secretion in spite of low doses of alpha ecdysone released. Corpora allata which release C16 JH3[11] have inhibitory effects.
Preliminary results show that JH 1 and JH 3 have specific effects upon cuticular activity. They alter the threshold of response to alpha and beta ecdysone. With a low concentration of beta ecdysone, JH 1 reduces the threshold of secretion (0.050 µg/ml) and JH 3 enhances it (200–500 µg/ml). The numerous epicuticles induced by high doses of beta ecdysone are regulated by JH 3 so that the epidermis deposits only one perfect cuticle.

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PRO EXPERIMENTIS

FITC-dextrans as fluorescence and electron microscopic tracers in studies on capillary and cell permeability of the CNS

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Summary. FITC-dextrans can be detected at the ultrastructural level as round electron dense particles, with no sign of distortion or aggregation. Because this tracer is a stable, non-toxic polymer of biological origin obtainable in a wide range of molecular weights, it may be useful for further studies of tissue permeability.

Fluorescein isothiocyanate labelled dextrans (FITC-dextrans[2]) have been used for direct observation of vascular permeability in living animals. Svensjö et al.[3] examined the microvasculature in hamster cheek pouches and Nakamura and Wayland[4] studied the macromolecular transport in the cat mesentary.

Investigations of vascular permeability in the nervous system are usually carried out by injecting a tracer i.v., for instance fluorochrome labelled albumin[5]. For electron microscopic observations other tracers have been used, such as ferritin[6] (diameter ~ 100 Å) or peroxidase. The presence of the peroxidase can be detected by histochemical reactions and the deposition of an electron dense reaction product[7]. The variability in molecular size and chemical structure reduces the usefulness of these tracers.

Non-labelled dextrans were used in electron microscopic studies of the intestine and kidney[8-10]. Olsson et al.[11] and Tervo et al.[12] reported on light microscopic studies in the nervous system with FITC-labelled dextrans. FITC-dextrans are stable[12], non-toxic polymers obtainable in a wide range of molecular weights. It would be advantageous if this tracer could be utilized for both light microscopy and electron microscopy.

Material and methods. 10 adult, randomly-bred NMRI mice were used. A 10% FITC-dextran 150 (Pharmacia Fine Chemicals, Sweden) solution was applied under anaesthesia either into the tail vein (125 mg/kg) or by a stereotactic injection (David Kopf Instruments) into the left telencephalic ventricle (50 mg/kg), near the foramen interventriculare.
5 or 30 min after injection, the animals were sacrificed by a perfusion of formalin solution 1:9 for fluorescence microscopy (FM) or with phosphate-buffered 1% glutaraldehyde for electron microscopy (EM). The samples of brain for FM were excised immediately and frozen in liquid nitrogen, then sectioned in a cryostat. The 6-µm-thick sections were mounted in 50% glycerin in water and viewed under a Leitz fluorescence microscope with a high pressure mercury lamp (filters BG 12 and K 510).

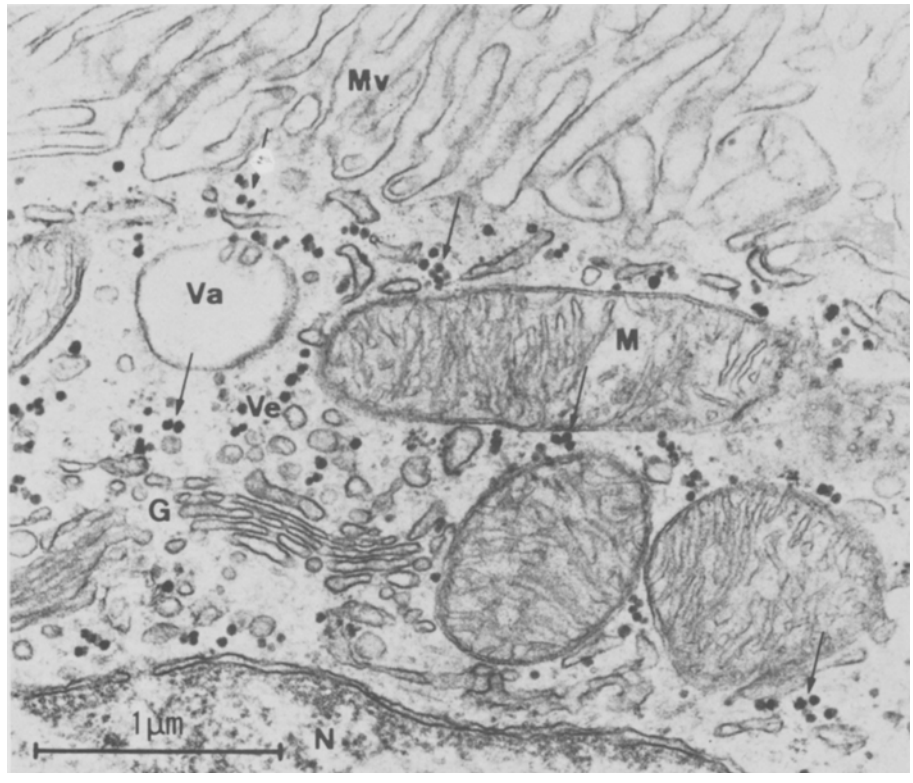

Fig. 1. Mouse plexus chorioideus in the telencephalic ventricle near the foramen interventriculare 30 min after the intraventricular injection of FITC-Dextran 150 showing the apical region of a plexus cell with tracer particles (arrows).

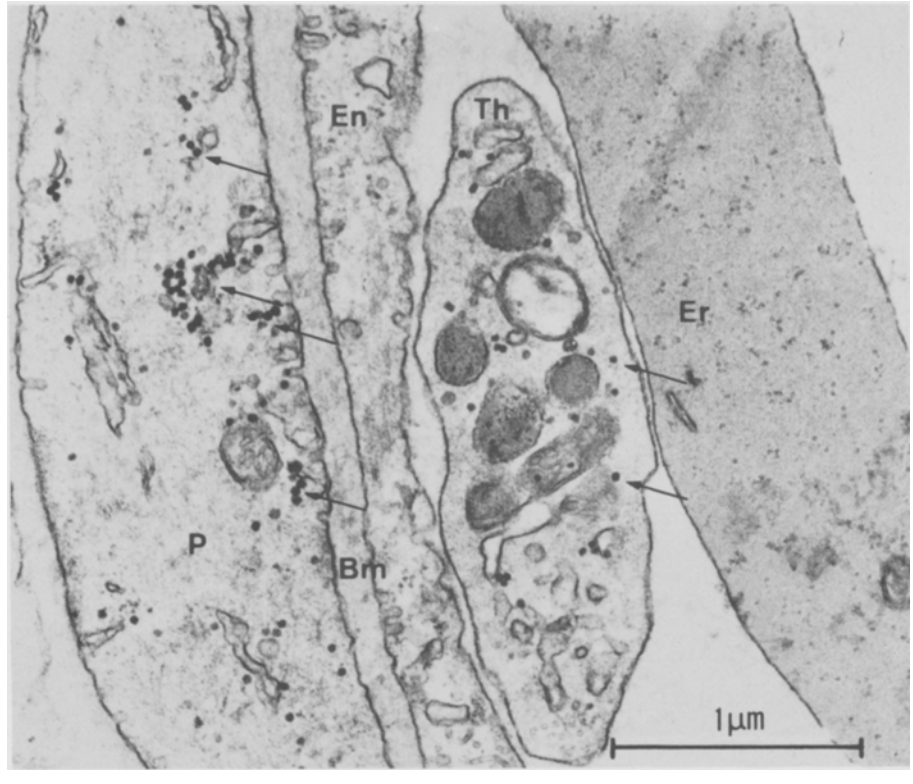

Fig. 2. Fenestrated blood capillary in a telencephalic plexus chorioideus 5 min after i. v. application of FITC-Dextran 150 (arrows). Abbreviations: Bm, basement membrane; En, endothelium; Er, erythrocyte; G, Golgi apparatus; M, mitochondria; Mv, microvilli; N, nucleus; P, pericyte; Th, thrombocyte; Va, vacuole; Ve, vesicle.

For EM the perfused brains were also quickly excized and brain halves immersed in Karnovsky's paraform-glutaraldehyde solution. The samples were then washed overnight in phosphate buffer (pH 7.2) and postfixed for 2 h in 1% aqueous osmium tetroxide with 1.5% potassium ferrocyanide[10], dehydrated in ethanol and embedded in Spurr. Thin sections were stained with uranyl acetate and lead citrate and examined with a TESLA BS 500.

Results. a) Intraventricular application route. NMRI mice of both sexes tolerated the injected FITC-dextrans with no signs of distress, even over a period of several days. In all animals that recieved the FITC-dextran injection in the telencephalic ventricle, the ependyma and the plexus epithelium of the ventricles I–IV fluoresced intensely after 15 and 30 min. With the EM, the FITC-dextran could be seen as non-aggregated, relatively round electron dense particles. Independently of the route of administration, nearly 80% were stained darkly and evenly. Inside the ependyma cells, a number of particles were enclosed in double-walled vacuoles. The underlying glia cells, with their processes, had particles in vacuoles and independently scattered in the cytoplasm. Particles could be distinguished easily from the ribosomes. The labelled dextran particles could be found in the epithelium of the plexus chorioideus of all brain ventricles (figure 1). The opaque tracer seemed to have no tendency to bind to any particular cell organelles and could be distinguished from vacuoles and vesicles of various sizes. b) Intravenous application route. FITC-dextran injected i.v. could also be detected easily in the blood vessels and in the underlying tissue after the passage of the fenestrated capillaries (figure 2). The thrombocytes showed an uptake of tracer particles. 5 min after injection, the endothelium and the basement membrane in the plexus chorioideus were free of dextrans. The particles had already passed into the pericytes and the plexus epithelium. Some of the FITC-dextran particles had also passed the blood-brain-barrier in the cortex and were gathered in the cytoplasm of the astroglia.

Discussion. The results show that FITC-dextrans can be visualized for use not only in FM, but also as a useful tracer for studying the physiological, as well as the ultrastructural aspects of capillary permeability. This tracer is particularly attractive compared with other tracers such as ferritin[6] or peroxidase[7], because it is a non-toxic, stable polymer[13], detectable individually and directly; it is of biological origin and can be obtained commercially in a wide range of molecular weights.

In our tissue specimens there was no indication that the labelling of dextrans with fluorescein isothiocyanate interferes with staining. The present results, unlike those reported by Simionescu et al.[9] for rat intestinal tissue, showed undistorted, unaggregated tracer. The varying electron opaqueness of the tracer was not due to passage through the endothelium or basement membrane. These discrepancies could be due to different tracer molecular weights, fixation procedure, and/or staining method. Ainsworth[10] did not observe aggregations of dextrans (mol.wts 40,000–250,000) in the kidneys of mice and rats.

The transcapillary transport of FITC-dextran 150 in the brain took place in the plexus chorioideus. Analogous results have been found in the fenestrated capillaries of hamster and rat brain[12]. So far as FM data[11,12] are concerned a blood-brain-barrier appears to exist in the other vascular walls of the brain. However, our EM specimens of the brain cortex clearly show single particles in the astroglia shortly after intravenous application which may have escaped detection by FM.

FITC-dextrans seem to be attractive tracers both for FM and EM studies of permeability, to elucidate the correlation of physiological data with structural findings at the ultrastructural level.

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A simple method for the verification of a successful cannulation of the rat cerebral ventricles[1]

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Summary. A simple and inexpensive method for the cannulation of the cerebral ventricles of the rat is described. The required tools consist of a stereotaxic instrument, a standard steel injection cannula and a polyethylene tube.

Intracerebroventricular administration of drugs is a commonly used method for studying the central nervous system. Usually the proper injection site is verified only after the experiment in the autopsy by leaving the cannula in situ or by injecting some dye such as methylene blue into the ventricle. However, in experiments which may last for several hours or even several days a considerable amount of work is spared if the correctness of the puncture is verified immediately after the insertion of the cannula, which enables unsuccessful punctures to be discarded at the very beginning of the experiment.

The applicability of stereotaxic coordinates to the ventricular puncture is lessened if the experimental animals differ in strain, sex or weight from those used for the derivation of the coordinates in the stereotaxic atlases. Furthermore, the brain topography may change considerably after the open-