and/or delays in neuro-ontogeny (i.e., lactation group), however, may disturb the temporal and spatial sequence of brain development, thereby affecting such factors as dendritic orientation and synaptic connectivity. Ultimately, these changes may be of significance in neurobiological functioning. Some evidence of neurally related disorders has in fact been reported in rats perinatally exposed to methadone[8-11]. Finally, the clinical ramifications of the present results need to be considered, since methadone maintenance is commonly employed for the treatment of narcotic-addicted pregnant women[2,3,5]. Within the context of evidence gathered in our study, and given differences in routes of drug administration and/or pharmacokinetics between exposure in humans and laboratory animals, it appears that offspring subjected to methadone in early life may be compromized in terms of their physiological integrity and intellectual achievement. In fact, some functional problems in children of narcotic-addicted mothers have already been documented[5,6].

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Rodlet-cells: Gland cell or protozoon?

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Summary. In the dense core of the inclusions of the rodlet cells in fish, DNA is indicated by the method of Bernhard and by DNase digestion. The inclusions, the rodlets, seem to be transport forms of genetical material. This finding supports the theory of the protozoan nature of these cells.

In 1892, 90 years ago, Thélohan[1] discovered a peculiar cell in various organs of teleosts and described it as sporozoan parasite (*Rhabdospora thélohani*, Laguesse 1906[2]). This cell is characterized by a fibrillar capsule and conspicious inclusions, the sporozoites. Usually it is located within an epithelium (e.g. intestinum, kidney tubules, gallbladder, endothelium, gill epithelium). Its apex is always oriented towards the surface of the epithelium. Occasionally it is seen free in the bloodstream but also in the connective tissue. Developmental stages have been reported[3-6]. At last, the inclusions are released and the rest of the cell degenerates.

Only few years after Thélohan, the same cell was interpreted as baffling, but normal fish cell ('Stäbchendrüsenzelle' or 'rodlet cell'), presumably with a secretory function[7]. The inclusions, the rodlets, are seen to be secretory material.

The rodlet cells have been considered as regulatory elements associated with special functions of the epithelia such as ion transport, osmoregulation etc.[3,8], and even as blood cells (granulocytes)[9-12]. (For reviews, see[6,8,13,14]).

To date, this problem is still undecided. Morphological features (e.g. capsule, desmosomes), as well as the varying distribution of these cells in some organs of fish, may be taken as support for either theory. An endogenous nature is suggested by the absence of a pathological tissue reaction and the well developed desmosomes between epithelial cells and rodlet cells, but junctional complexes also occur between parasitic protozoa and e.g. erythrocyte membranes[15]. The theory of parasitic origin is supported by the capsule and by histochemical investigations. Light microscope tests have shown that the cortex of the rodlets consists of proteins and non-acid polysaccharides[16,17]. The dense core is Feulgen negative[16,17], but gives a positive reaction for RNA[6,17].

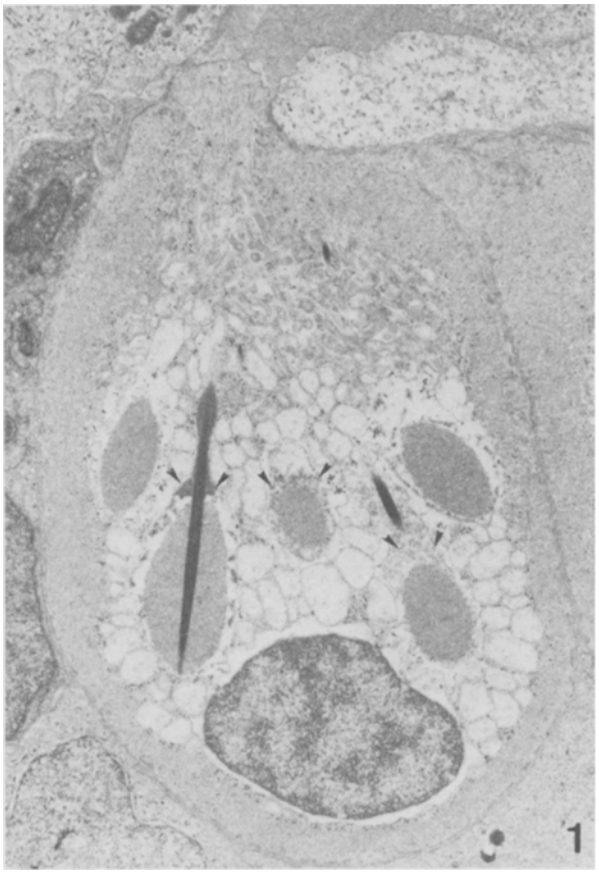
In the present paper an attempt is made to clarify whether the dense core consists of genetic (DNA, RNA) or secretory material by the use of electron microscopical routine, histochemical and enzymehistochemical methods.

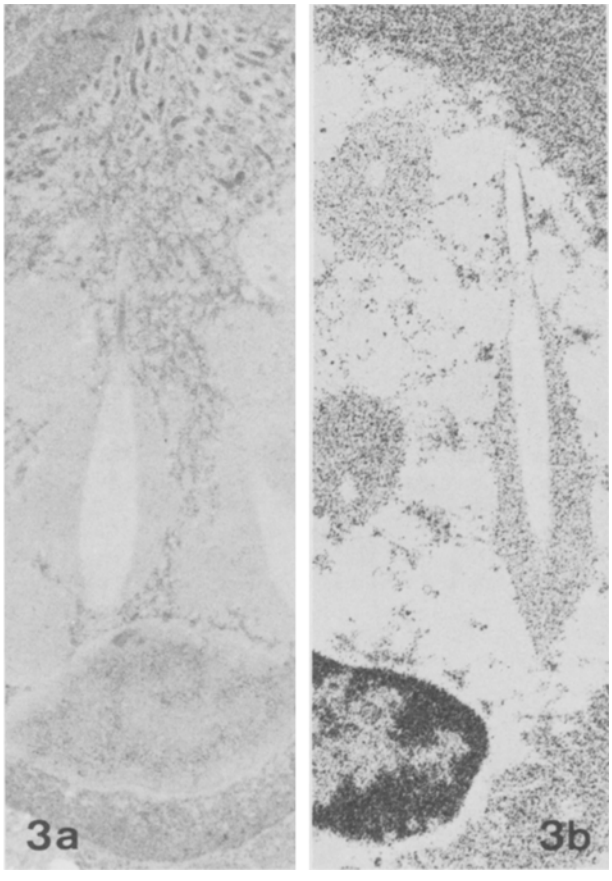
Material and methods. Routine preparations were made from kidney tissue of *Cyprinus carpio* L., fixed in phosphate-buffered glutaraldehyde (2%, 0.1 M, pH 7.4) with or without postfixation in Palade-buffered OsO_4, dehydrated with ethanol and embedded in Epon 812.

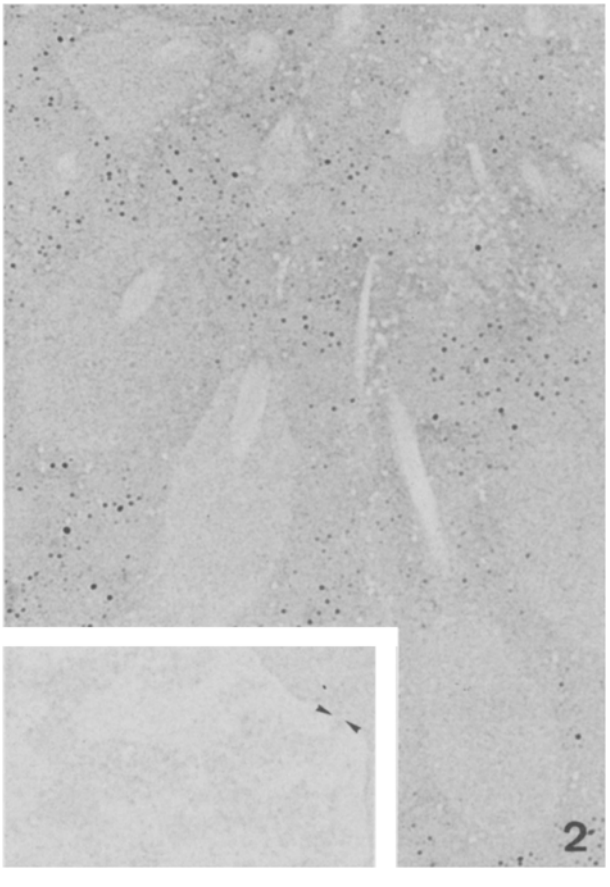
Reactions on DNA. For the EDTA-method after Bernhard[18], blocks were fixed only in glutaraldehyde. Sections (grey to silver) were stained with 5% aqueous uranyl acetate for 5 min, then floated on 0.2 M ethylene diamine tetraacetic acid (EDTA) at pH 7.0, for 30-60 min and poststained with Reynold's lead citrate for 5 min.

DNase digestion was done on ultrathin sections, floating on 0.01% DNase I, type I from bovine pancreas, approximately 2000 Kunitz units per mg protein, substantially free of RNase, Mg-ion concentration: 4.2 mM (Sigma), in 0.05 M Tris-HCl buffer pH 7.6 for 30 min-6 h at 37 °C. After washing in Aqua destillata and mounting on grids, the sections were stained with uranyl acetate and lead citrate.

For the Feulgen-silver methenamine method after Peters and Giese[19], ultrathin sections of only glutaraldehyde-fixed







and Epon-embedded material were hydrolyzed free floating on 5, 1, and 0.1 N HCl for 5–60 min at 4, 22, and 60 °C. After intensive washing with distilled water, the sections were stained with silver methenamine solution (10 ml double distilled water, 10 ml freshly prepared 3% methenamine, 0.5 ml 5% silver nitrate, 1.2 ml 5% sodium tetraborate – all in double distilled water) at 60 °C for 1–2 hours in darkness until the sections appeared brown. Then they were washed with distilled water, 5% sodium thiosulphate and again with water before they were mounted on grids.

Reactions on RNA. RNase digestion was undertaken with sections floating on 0.1% RNase-A (from bovine pancreas, protease free, type X-A, pH 6.4, Sigma) at 37 °C for 2–6 h. Afterwards they were stained with uranyl acetate and lead citrate.

Figure 1. Longitudinal section of a rodlet cell. The rodlets show their typical electron dense central cores. Dense, but flocculent material is also seen at the outer border of the cortex (arrowheads). Conventional staining, × 7350.

Figure 2. Rodlets after treatment with EDTA. The former electron dense cores are bleached now, which strongly indicates DNA. The intensity of the reaction can be proved in comparison with an adjacent nucleus (inset: nucleus of a lymphocyte; arrowheads: nucleoporus). × 22,400, inset × 16,800.

Figure 3. *a* Part of a rodlet cell after DNase digestion for 6 h. The condensed chromatin of the nucleus as well as the cores of the rodlets are digested. As after EDTA-reaction, in the axis of the cores a light shadow, possibly a proteinaceous scaffold, can be seen. × 7500. *b* Feulgen stained (hydrolysis: 5 N HCl for 10 min at 22 °C; silver methenamine: 2 h) rodlet cell. The cores of the rodlets are negative, the chromatin of the nucleus shows a distinct reaction. × 15,700.

Results and discussion. With conventional staining (uranyl acetate/lead citrate) the rodlet cells show their typical inclusions. The latter display a cortex of varying density, sometimes with a flocculent and more condensed zone at the outer border. The electron dense core runs the whole length of the rodlet and ends in an apical protrusion (fig. 1). The EDTA-method is known to result in the bleaching of chromatin, while RNA containing structures retain their contrast. The investigated rodlets show in their dense cores a heavy loss of contrast (fig. 2), similar to blocks of condensed chromatin (fig. 2, inset: nucleus of an adjacent lymphocyte). Only a light shadow in the axis, possibly a proteinaceous scaffold, still exists. Because certain secretory substances also react with EDTA, DNase digestion was performed as a 2nd test. The loss of electron density in the cores after DNase treatment also supports the presence of DNA (fig. 3a). In accordance with earlier light microscopical Feulgen stainings[16,17] the dense cores of the rodlets and also the rodlets themselve, show no positive reaction by electron microscopic Feulgen technique too, although the DNA of the nuclei is well stained (fig. 3b). This seems to be contradictory to our own EDTA- and DNase-results, but Feulgen negative DNA is known in organisms from Amoeba to insects to flowering plants[20]. However, the DNA of the rodlets can be assumed to be in a totally different configuration than the chromatin of the nucleus of the rodlet cell.

In sections treated with protease-free RNase of type X-A the cores remain dense, whereas the electron density of the nucleoli is markedly diminished. This contradicts the findings of Barber et al.[6]. As these authors give no specifications of the RNase used, although the quality of the enzyme is an essential factor, their results might be caused by proteases acting on certain protein components of the inclusions (e.g. the above mentioned proteinaceous scaffold).

These results strongly indicate, that at least the core of the rodlets is constituted of condensed chromatin with extraordinary configurations. This presents the essential argument that the rodlet cells have no secretory function, either as special exocrine cell or as granulocyte type. The rodlets are seen to be transport units for genetical material. Therefore, the original presumption of Thélohan[1] seems still the most probable proposition, although the life cycle and the systematical classification of the 'rodlet cell' is open to further investigations and discussions.

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Anti-noradrenergic drugs do not interfere with the development of callosal connections in the rat

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Summary. When noradrenergic transmission was suppressed by 6-OHDA, propranolol or phentolamine callosal fibers developed the same innervation pattern as in normal rats and the density of callosal connections did not increase.

After lesions in the entorhinal cortex commissural connections of the fascia dentata expand their terminal territory by reinnervating the vacated apical dendrites (for review see Lynch et al.[2]). If neonatal rats have been treated with 6-OHDA, the commissural afferents expand farther into the outer molecular layer than in the presence of noradrenaline (NA)[3]. In fascia dentata, this reactive synaptogenesis of commissural fibers is cytologically not distinct from synaptogenesis during normal ontogenesis[4]. There is physiological evidence that NA also interferes with neuroplasticity in the neocortex[5]. Hence, it is conceivable that NA influences synaptogenetic processes in the neocortex also during normal development. In order to test this hypothesis, the effect of anti-NA-ergic drugs on the normal development of commissural connections was studied in albino rats.

25 Sprague-Dawley rats of both sexes were used in the present study. 3 types of experiments were performed: 1. 6-hydroxy-dopamine was administered s.c. on days 5, 6 and 7 after birth at a dose of 100 mg/kg, which has been shown to reduce the NA-ergic innervation predominately in the forebrain[6]. Callosotomy followed at 6 weeks of age. 2. Propranolol, a centrally acting β-receptor blocker, was applied during the 3rd postnatal week, i.e. when the callosal connections develop the characteristic distribution pattern[7] (daily injections between 14 and 20 days p.n., 0.5 mg/kg i.p.[8]). 3. During the same phase of development as in 2. phentolamine, a centrally acting α-receptor antagonist, was given at a dose of 10 mg/kg[8] per day. In the latter cases the callosotomy was performed 1 day after the last injections of drugs. The callosotomies were performed as elongated parasagittal lesions in the right hemisphere under