hydras was investigated. When TPA (100 ng/ml) solution was replaced by control medium at the end of 25 min, the contracted hydras did not re-extend. A 5-min exposure to TPA at this same concentration induced a contraction, with a latency of about 30 min; when the hydra were transferred to control solution such contractions were followed by incomplete relaxation. At a concentration of 10 ng/ml, however, the TPA effect was fully reversible in drug-free solution. Following a 1-week treatment with TPA at 1 ng/ml, the slightly contracted hydras re-extended upon transfer to control media. Thus the contraction induced by the lower concentrations of TPA was reversible. The contraction effect of lower concentrations of PDD and mezerein was also reversible.

Reversible contraction of hydras has also been observed with some lectins. Concanavalin agglutinin, *Lotus tetragonolobus* agglutinin, and *Ulex europaeus* agglutinin also induced a reversible contraction of hydra tentacles[13]. Methyl-α-D-glucoside inhibited the Con A-induced contraction, but did not inhibit the TPA-induced contraction. This indicates that TPA acts on hydra in a different manner from Con A.

With the limited number of compounds tested, there is a seemingly good positive correlation between tumor-promoting action and the contraction effect in hydras as described in the present communication. The relationship, if any, between tumor promotion and induction of contraction in hydras by the same substances is not clear. This investigation should be extended to include more tumor-promoting agents and their non-tumor-promoting derivatives at comparable concentrations.

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Ultrastructural observation on the tips of growing vascular cords in the rat cerebral cortex[1]

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Summary. The distal ends of the vascular cord in the cerebral cortex are investigated electron microscopically in rats at the 13th postnatal day. The tip of the vascular cord consists of central cuboidal cells (primitive endothelial cells) and surrounding flat cells (primitive pericytes), and has no lumen. The primitive endothelial cells (tip cells) possess several long tentacles which contain only fibrous structures and extend through the neuropile.

The growth of blood vessels in the developing cerebral cortex is based on sprouting of mesenchymal vascular cords[2–5]. The morphological property of the tip cells in the vascular cord and their tentacles has not been fully illustrated at the ultrastructural level. In this paper, the authors wish to present profiles of the vascular cord and surrounding tissues, and clarify the relationship between them.

For this purpose, Wistar rats at the 13th postnatal day were used. After cardiac perfusion with 2.5% glutaraldehyde (buffered with 0.1 M phosphate), parietal parts of the cerebral cortex were excised and postfixed in 1% osmic acid (buffered with 0.1 M phosphate) for 2 h at 0 °C. The observation was carried out from a proximal region of the vascular cord to a distal one.

In figure 1, a cross section of vascular cord is depicted. The cord measures about 5μm in diameter and consists of a pair of immature endothelial cells which are surrounded with pericytes. Irregular shaped narrow lumen appears between cuboidal endothelial cells and is filled with flocculent material. The cord is clearly lined with a basal lamina. Occasionally, attachment devices develop between endothelial cells (fig.1). In the cytoplasm of the cells, rough-surfaced endoplasmic reticulum, mitochondria, free and aggregated ribosomes and pinocytotic vesicles are distributed.

Distal to the region, at a distance of 5–7 μm from the patent portions of the vascular cord, one of the tip cells (primitive endothelial cells) with some tentacles appears (fig.2). The size of the solid cord reaches about 4 μm. The tip cell is surrounded with flat primitive pericytes which are partially encircled with ill defined basal lamina. In figure 2, 3 tentacles are sprouting together, and penetrate a basal lamina and extend into neuropiles. The other tip cell possessing tentacles is shown in figure 3. The tentacles are twisted, and one of them elongates and meets with the neuronal process. In all specimens, the tentacles are similar in size and measure about 0.15–0.25 μm in diameter. Their length attains to more than 10 μm and no branching is observed. The cytoplasmic organelles of the tip cell such as endoplasmic reticula and ribosomes diminish progressively, especially, in distal parts of the tentacles (fig.3). It is noteworthy that tentacles contain only fibrous structures (fig.4). As depicted in figure 2–4, tentacles run straight through the neuropile of the cerebral cortex. However,

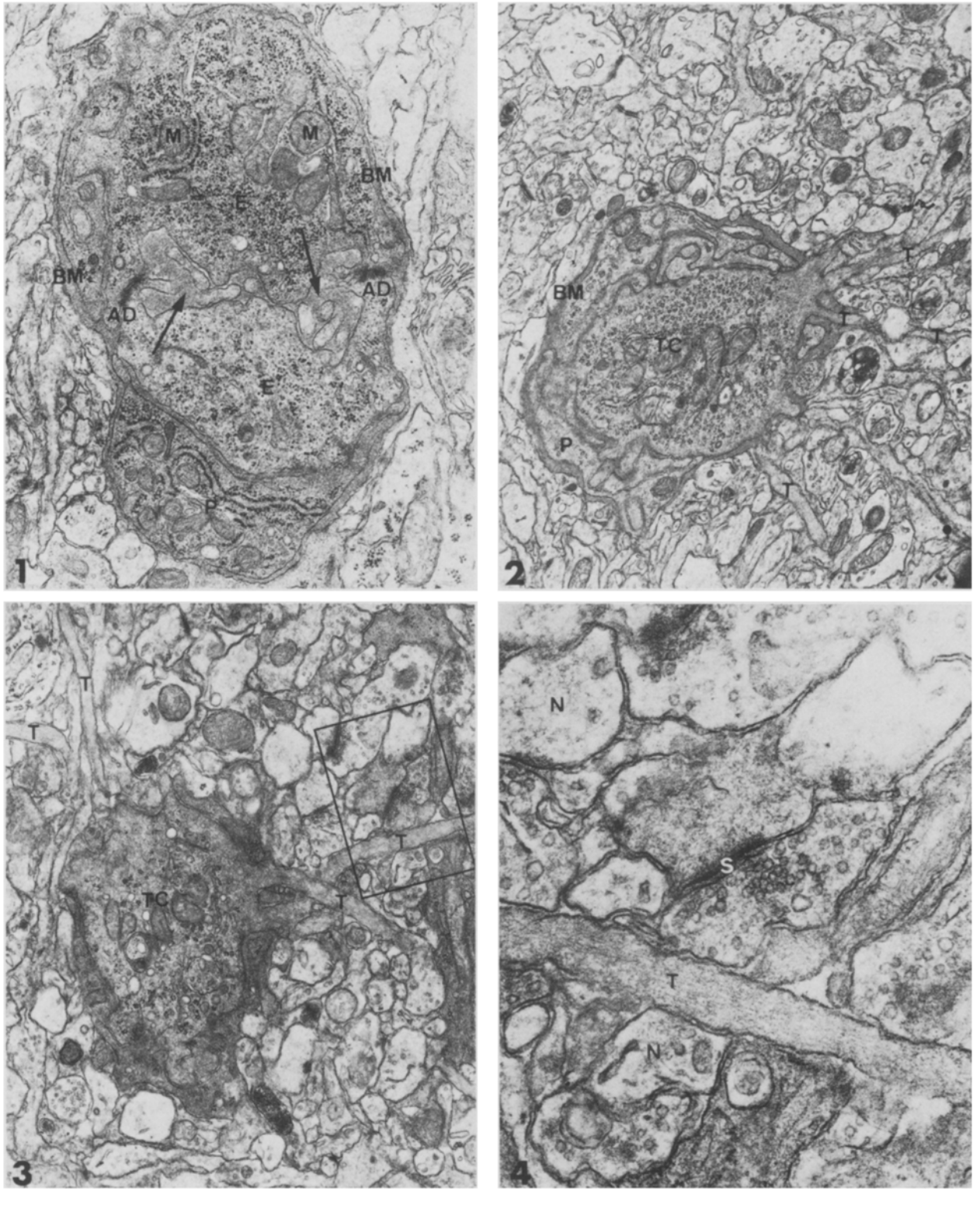

Figure 1. Immature endothelial cells (E) and flat pericytes (P) are seen in the vascular cord which contains the narrow vascular lumen (arrows). The endothelial cell at the upper side is rich in cytoplasmic organelles, while the lower one is poor. Between endothelial cells attachment devices (AD) develop. M, Mitochondria, BL, Basal lamina. × 19,200.

Figure 2. The luminae are not observed in this vascular cord. The centrally located tip cell (TC, primitive endothelium) protrudes cytoplasmic projections (tentacles) (T) into neuronal tissue. The tip cell interdigitates with the surrounding primitive pericyte (P). BL, Basal lamina. × 10,800.

Figure 3. The tip cell (TC) is provided with several tentacles (T). 2 tentacles are crossed. Each tentacle extends freely into the spaces between nerve and glial processes. × 10,800.

Figure 4. High magnification of the part indicated by frame in figure 3. In tentacle (T), fibrous structures are evident. Synapses (S) and neuronal processes (N) contact closely with the tentacle. No specialization of membranes of the tentacle and surrounding tissues is seen. × 54,200.

when they encounter neuronal processes and synapses, they change their course.

In summary, tip cells of the vascular cord may grow several tentacles and thereby contribute to the formation of the vascular network. At this developmental stage of the rat cerebral cortex, the widened intercellular spaces facilitate the vascularization, as recently reported by Caley[3] and Bär[4].

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Immunopotentiators and the protection they give against carbon tetrachloride hepatotoxicity

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Summary. Immunopotentiators such as BCG, levamisole, PS-K and OK-432 prevent carbon tetrachloride (CCl_4) hepatotoxicity, and in spite of exposure to CCl_4 the liver tissue levels of thiobarbituric acid (TBA) reactive substances were not increased in rats pretreated with such immunopotentiators.

It has been suggested that the liver injury caused by carbon tetrachloride (CCl_4) is due to lipid peroxidation in liver microsomes[1]. On the other hand, many immunopotentiators such as BCG, the anthelmintic compound levamisole, a protein polysaccharide from myceria Coriolus vesicolor PS-K[2] and a streptococcal preparation OK-432[3] are now in clinical use in Japan. Levamisole was shown to be an antioxidant in rat liver microsomes in vitro, and also inhibited lipid peroxidation induced by X-irradiation[4]. The present study was undertaken in order to determine the preventive effect of such immunopotentiators against liver damage induced by CCl_4, and its inhibitory effect on lipid peroxidation due to CCl_4. These immunopotentiators were compared with the well known antioxidant α-tocopheryl acetate (vitamin E) for their ability to prevent CCl_4 hepatotoxicity.

Materials and methods. Wistar strain female rats, weighing 250 mg, were treated with BCG; 2.5, 10.0, 25.0 mg/kg, i.p.: levamisole; 10.0, 25.0, 50.0 mg/kg, p.o.: OK-432; 2.5, 3.8, 5.0 mg/kg, i.p.: PS-K; 250, 500, 1000 mg/kg, perorally, and α-tocopheryl acetate; 20.0, 100.0, 200.0 mg/kg, i.p. for 7 successive days. At the end of the treatment period, CCl_4 was injected once in a dose of 0.5 ml/kg, i.p. 24 h after injection of CCl_4, all rats were killed. The liver was perfused with 0.9% NaCl via the portal vein before homogenization. After washing with 0.9% NaCl, tissue homogenates were prepared at a ratio of 1.0 g of wet tissue to 9.0 ml of 1.15% KCl, using a Teflon Potter-Elvehjem homogenizer. Lipid peroxide levels in the liver homogenates were determined by the thiobarbituric acid (TBA) method according to Ohkawa et al.[5]. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured according to Reitman and Frankel[6].

Results. Serum GOT and GPT of rats treated with immunopotentiators or vitamin E were not elevated after the administration of CCl_4. The protective effect of immunopotentiators against CCl_4 hepatotoxicity was dose-dependent (table). Histological examination also indicated that,

Effects of immunopotentiators or vitamin E on serum transaminases and liver TBA reacting materials in the rats treated with CCl_4

Pretreatment dosage (mg/kg)	Treatment (0.5 ml/kg)	GOT (Karmen U)	GPT (Karmen U)	TBA reactants[b] (nmoles/100 mg wet wt)
BCG (i.p.)	+ CCl_4			
2.5		968.2 ± 425.1[a,c]	137.3 ± 74.2[c]	54.9 ± 12.5[c]
10.0		561.5 ± 219.7[c]	73.0 ± 40.5[c]	36.0 ± 6.2[c]
25.0		338.2 ± 104.3[c]	34.5 ± 6.2[c]	35.3 ± 5.7[c]
Levamisole (p.o.)	+ CCl_4			
10.0		3137.2 ± 1134.6	984.9 ± 310.6	95.0 ± 33.5[c]
25.0		1051.2 ± 437.4[c]	299.2 ± 104.5[c]	56.9 ± 27.7[c]
50.0		897.9 ± 467.8[c]	165.7 ± 93.4[c]	47.6 ± 8.5[c]
OK-432 (i.p.)	+ CCl_4			
2.5		1779.0 ± 723.9	363.0 ± 129.1[c]	151.6 ± 51.8
3.8		1279.6 ± 511.5[c]	235.5 ± 78.2[c]	92.5 ± 41.5[c]
5.0		522.0 ± 118.1[c]	49.8 ± 16.7[c]	31.5 ± 4.2[c]
PS-K (p.o.)	+ CCl_4			
250.0		1860.3 ± 697.3	749.2 ± 299.6	84.8 ± 29.5[c]
500.0		911.8 ± 379.2[c]	360.8 ± 84.6[c]	67.3 ± 29.3[c]
1000.0		291.7 ± 82.9[c]	58.0 ± 30.4[c]	38.1 ± 9.8[c]
α-Tocopheryl acetate (i.p.)	+ CCl_4			
20.0		1141.7 ± 301.6[c]	204.9 ± 50.5[c]	37.2 ± 5.2[c]
100.0		628.2 ± 292.1[c]	144.0 ± 67.3[c]	36.6 ± 4.4[c]
200.0		196.4 ± 35.4[c]	19.0 ± 5.1[c]	33.0 ± 6.4[c]
Saline (control) (i.p.)	+ CCl_4	2986.5 ± 816.1	925.6 ± 258.5	177.6 ± 36.7
Saline (i.p.)		203.1 ± 41.3[c]	22.4 ± 6.3[c]	34.1 ± 6.3[c]

[a] Data represent mean ± SD of 10 rats in each group. [b] Measured as nmoles of malondialdehyde per 100 mg wet wt. [c] $p < 0.001$ for difference from controls by Student's t-test.