In all ganglia studied, ^{3}H-leucine had infiltrated the neurons. Outlines of cell bodies could easily be seen due to dense concentrations of the silver grains (fig. 1). This heavy labeling could readily be traced from the cell bodies into the nerves leaving the ganglia (fig. 2). These large nerves branched repeatedly forming a plexus of smaller labeled nerves which accompanied small arteries (fig. 3). This network of small nerves and blood vessels could be followed to the organs being studied. In addition small labeled nerves in close proximity to blood vessels were found within the stomach, spleen and pancreas (fig. 4). No apparent differences were observed between 48 and 98 h postinjection survival periods.

This study demonstrates autoradiographically that nerve cell bodies in the celiac ganglion have postganglionic sympathetic fibers which innervate the spleen, stomach, pancreas and liver.

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Tumor-promoting phorbol esters and mezerein induce contraction in hydra

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Summary. 12-*O*-tetradecanoyl phorbol-13-acetate (TPA, 1–100 ng/ml) induced a reversible contraction in the hydra, *Hydra japonica*. Another tumor-promoting phorbol ester, phorbol-12,13-didecanoate (PDD), and mezerein also induced contraction, but non-tumor-promoting derivatives such as 4*a*-PDD and phorbol did not.

Tumor-promoting phorbol esters, as represented by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), are known to produce various biological and biochemical effects when administered to cultured cells[2]. They enhance cell transformation[3,4], modulate cell differentiation[5–7], enhance cellular biochemical activities[2,6], and alter membrane properties[2,6]. These compounds exert their effects on cells of different tissue or species origin, as well as on normal and tumorgenic cells. More recent studies suggest that they also interfere with early development in lower animals such as sea urchins[8] and nematodes[9]. Although interaction with cell surface membranes is thought to be essential for their action[2,6], the mechanism of the interaction leading to these pleiotropic effects remains ill-defined.

In this study, we have examined the effects of these tumor-promoting phorbol esters on another lower animal form, the coelenterate, *Hydra japonica*. Interestingly, these agents induce a marked and sustained contraction in the hydra, whereas non-tumor-promoting derivatives do not.

Materials and methods. *Hydra japonica*[10] was cultured in a medium containing 1.0 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.1 mM KCl and 2.0 mM $NaHCO_3$ (pH 7.8)[11]. The hydras were fed daily with *Moina macropopa* and were starved 1 or 2 days before experiments. 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), phorbol-12,13-didecanoate (PDD), 4*a*-PDD, phorbol, and mezerein were kindly donated by Dr H. Yamasaki of International Agency for Research on Cancer. The compounds were dissolved in dimethyl sulfoxide to a concentration of 0.1%.

Results and discussion. In control experiments, hydras were sucked up into pipettes and dropped into 2 ml of culture medium. Irritation from pipetting caused the hydras to contract, but the contracted hydras extended immediately and remained so in the absence of mechanical or light stimulation. When a contracted hydra was dropped into a TPA-containing solution, it extended first and then contracted markedly, forming a tightly-contracted ball. The tentacles contracted more rapidly than the body column. At a low concentration of TPA (1 ng/ml), numerous contraction-extension movements were observed before the final prolonged contraction. Therefore, the TPA-induced contraction was scored as positive only when a contracted hydra remained like a ball with markedly contracted tentacles for at least 30 min. The table shows the average time required by various phorbol esters to induce the contraction. In the presence of 10 ng/ml TPA, the tentacles, but not the body column, of a contracted hydra were partially dissociated after 24 h. In the presence of 1 ng/ml TPA, a slight extension of the body column was observed after marked contraction, and the contracted hydra gradually extended during the following 12 h. The hydras remained alive during the 2 weeks of treatment with 1 ng/ml TPA, and feeding responses were observed.

Another tumor-promoting phorbol ester, PDD, and mezerein (both at 100 ng/ml) also induced marked contraction, whereas the non-tumor promoting phorbol derivatives, phorbol and 4*a*-PDD, produced no contraction, even at 100 ng/ml.

Detached tentacles with hypostome contracted in TPA (100 ng/ml) with a delay of 14.3 min (n = 14), whereas isolated body columns had a delay of 34.9 min (n = 15). Thus TPA acts on the tentacles more rapidly than on the body column. A preferential effect on tentacles has also been observed with glutathione[12].

Since biological effects of TPA are reversible both in vivo and in vitro[2,5,7], the reversibility of the TPA effect on the

Average time required for contraction induction in hydra

Compound		Time (min) required for contraction after addition of test compounds
TPA	100 ng/ml	9.5 ± 4.2* (n = 22)
	10 ng/ml	28.5 ± 18.5 (n = 20)
	1 ng/ml	160.4 ± 43.3 (n = 16)
Mezerein	100 ng/ml	8.9 ± 2.5 (n = 21)
PDD	100 ng/ml	111.3 ± 48.8 (n = 19)
4*a*-PDD	100 ng/ml	> 720** (n = 16)
Phorbol	100 ng/ml	> 720** (n = 20)

* Mean ± SD. ** No contraction within 12 h. See text for further details.

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,