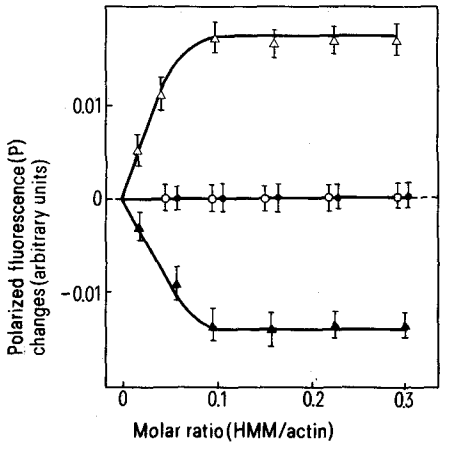
performed in a solution containing 100 mM KCl, 2 mM $MgCl_2$, 67 mM phosphate buffer, pH 7.0.

*Results and discussion.* It has already been shown that after extraction of myosin, troponin and tropomyosin from a ghost single fiber there remain tryptophane residues of F-actin, their dipoles of emission being oriented preferably perpendicular to the fiber axis[1-3], i.e. anisotropically. On the other hand, tryptophane residues of proteins other than F-actin are arranged practically isotropically. Furthermore, their contribution to the general emission of a fiber is much lower than that of F-actin, therefore $P_\perp > P_\parallel$[1,3]. $P_\perp$ and $P_\parallel$ depend on the optical properties of F-actin tryptophane residues, the latter being defined by the structural parameters of the actin helix in the thin filaments. Changes in $P_\perp$ and $P_\parallel$ are therefore sensitive to the conformational changes of the protein[1].

HMM binding to F-actin decreases the anisotropism of tryptophane fluorescence of a ghost single fiber, resulting in $P_\perp$ decrease and $P_\parallel$ increase (fig.). Since the anisotropism is not sensitive to orientation of myosin heads in fibers[6], the changes of $P_\perp$ and $P_\parallel$ indicate conformational changes of F-actin induced by HMM binding.




Dependence of changes of polarized fluorescence (P) on the molar ratio of HMM to actin monomers (HMM/actin) of ghost single fibers (△—△, ▲—▲) and fibers treated with glutaraldehyde (○—○, ●—●) after addition of 5 mg of HMM/ml. △—△, ○—○; ▲—▲, ●—● – fibers at parallel and perpendicular orientation to the plane of the exciting light, respectively. Measurements were carried out in a solution containing 100 mM KCl, 2 mM $MgCl_2$, 67 mM phophate buffer, pH 7.0. Each point is the average of 50 measurements obtained in 10 fibers. Vertical bars show SD.

It should be noted that the changes of fluorescence anisotropism are only observed if F-actin is capable of changing its conformation. Thus, glutaraldehyde-treated F-actin binds HMM, the interaction being functionally effective as measured by ATPase activities, but, the conformation of F-actin remains unchanged due to fixation[7], therefore $P_\perp$ and $P_\parallel$ are constant (fig.).

HMM binding to F-actin induces an increase of fluorescence intensity. Since after decoration $I_m$ increases due to HMM tryptophane residues, it is possible to estimate the molar ratio of HMM to actin. For example, an increase of $I_m$ by 25% corresponds approximately to a molar ratio of 0.1, since according to the data of Yanagida and Oosawa[3] an increase of fluorescence intensity by 80% reflects a molar ratio of 0.3. The decrease of tryptophane fluorescence anisotropism was not directly proportional to the amount of bound HMM (fig.). The binding of 1 single myosin head to 10 actin protomers is enough to induce maximal changes of tryptophane fluorescence anisotropism of F-actin in thin filaments. This phenomenon suggests a conformational change in neighboring actin molecules which is induced co-operatively by the conformational change in the actin molecule on the binding of HMM.

From the use of polarized fluorescence analysis[3,8], and having made a mathematical model of the data obtained, it can be assumed that HMM binding induces cooperative changes of the orientation of protomers in F-actin as well as an increase in the flexibility of the thin filament. The results show a certain amount of agreement with those which have been obtained by other methods[3,9,10]. Such structural changes of F-actin might be an important factor in the interaction of actin with myosin during muscular contraction.

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## Intra-aortic prostaglandin $E_1$ infusion in maturation of neuroblastoma

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*Summary.* A $7^2/_3$-year-old boy with unresectable abdominal neuroblastoma received an intra-aortic prostaglandin $E_1$ infusion (0.4–0.5 ng/kg/min) over a total period of 6 months, as well as systemic papaverine and multiagent chemotherapy. At second-look surgery 9 months later, tumors grossly appearing to be ganglioneuromatous were subtotally resected. Histology revealed the evidence of neuroblastoma maturation.

The prognosis of disseminated neuroblastoma remains poor[1]. In vitro and in vivo studies demonstrate that neuroblastoma differentiates and maturates into ganglion-like cells under the effect of adenosine-3′,5′-monophosphate (cAMP)[2-4]. Clinical application of these findings is worth attempting in order to induce tumor maturation and im-

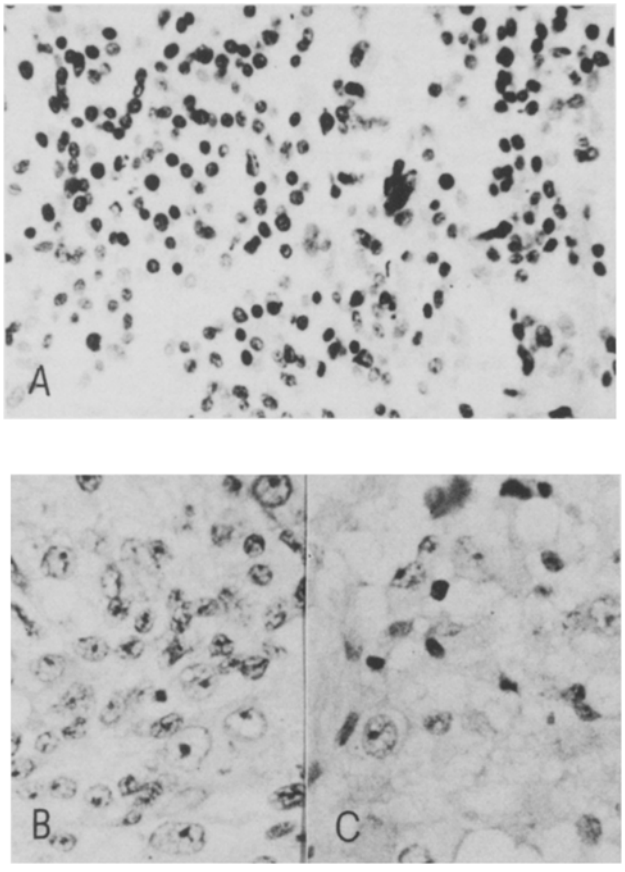
prove therapeutic strategy in neuroblastoma. In patients with neuroblastoma, intratumor cAMP may be increased by systemic administration of adenylate cyclase stimulants and/or cAMP-phosphodiesterase inhibitors. High dose papaverine given by Helson[5], combined with other chemotherapeutic agents, achieved some success but was not satisfactory in patients with stage IV neuroblastoma.

Previously, we found that prostaglandin $E_1$ given simultaneously with the conventional dosage of papaverine was superior to papaverine alone in increasing cAMP. However, the use of i.v. prostaglandin $E_1$ (50–100 ng/kg/min) is limited because of fever, nausea, abdominal pain etc.[6]. We therefore decided to infuse a small amount of prostaglandin $E_1$ through the aorta proximal to the feeding arteries to the tumor, combined with systemic administration of papaverine and multiagent chemotherapy.

A 7-year-8-month-old boy was admitted to our clinic, because of an abdominal mass and fever on January 8, 1981. CAT scan, ultrasound and aortography revealed a large retroperitoneal mass in the left supra-renal area, which displaced the left kidney downward and laterally. In addition, the tumor extended paravertebrally from $Th_9$ to $L_5$ and crossed the midline involving major arteries. Calcification was noted in the center of the main supra-renal tumor. Bone survey and bone marrow were negative. Urinary excretion of vanillylmandelic acid and homovanillic acid was abnormally high (VMA 19.1 µg/mg creatinine and HVA 26.7 µg/mg creatinine).

Laparotomy on January 21, 1981 found the tumor unresectable and a biopsied specimen demonstrated immature neuroblastoma. A chronofuzer tube (i.d. 0.5 mm) was inserted through the *A. gastroduodenalis* via the *A. hepatica communis* into the aorta at the $Th_8$ level and connected with a portable infusion pump (PIP-21, Sharp). Prostaglandin $E_1$ was started at 0.26 ng/kg/min and maintained at 0.43 ng/kg/min over a period of 3 months. Papaverine (120 mg/day, p.o.) was started simultaneously. During this period, no side effect was noted and 7 cycles of cyclophosphamide (150 mg/m$^2$, i.v., day 1–7) and adriamycin (35 mg/m$^2$, i.v., day 8)[7] were given. 3 cycles of cis-platinum (90 mg/m$^2$, drip, day 1) and VM26 (4′-demethyl-epipodophyllotoxin-β-D-thenylidene glucosie (NSC 122819)). (100 mg/m$^2$, drip, day 3) followed, together with papaverine, for 2 more months. Re-evaluation of the tumor status in August, 1981 indicated a technical difficulty in resecting the total tumor masses. Therefore, as a 2nd course, a tube was inserted through the *A. femoralis profunda* via the *A. iliaca externa* and into the aorta at $Th_8$ level. Prostaglandin $E_1$ was infused at 1.0 ng/kg/min, reduced 25 days later to 0.5 ng/kg/min because of fever and positive CRP (C-reactive protein) and maintained at the lower level without any further trouble for another 2 months. During this period, 5 cycles of cyclophosphamide-adriamycin and papaverine were administered. Subsequently, the tumors became smaller being reduced to approximately less than half their original size with intensified calcifications and VMA and HVA returned to normal. On October 28, 1981, tumors grossly appearing to be ganglioneuromatous were subtotally resected and a thorough histological examination disclosed extensively degenerated tumor tissue with marked fibrosis. Remaining tumor cells mostly resembled atypical ganglion cells with some small round cells seen focally (fig.).

The authors believe that prostaglandin $E_1$ and papaverine combined with multiagent chemotherapy significantly reduced tumor size and promoted maturation of neuroblastoma in this case. Especially, an intra-aortic infusion of prostaglandin $E_1$ proximal to the feeding arteries seems to be an innovative and effective procedure without noticeable side effect, which made it possible to administer this compound for as long as a total of 6 months. Intraoperative tumor appearances at the 1st and 2nd surgeries did not support the possibility that the tumor had originally been a ganglioneuroma which remained after the biopsy-proven neuroblastoma had disappeared. However, one may still argue that conventional chemotherapy alone may produce maturation of neuroblastoma, or that this was a natural course of neuroblastoma unrelated to the drugs used. These questions should be answered by future therapeutic approaches of this kind.




*A* Neuroblastoma biopsied at first laparotomy (×280). *B* and *C* Ganglioneuromatous tumors resected at second-look surgery (×280).

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