Results and discussion. Each preparation was initially driven at a basic cycle length of 1000 msec for 30 min. If the preparations were found to show the typical shape of Purkinje action potentials[1], they were discarded. After being stabilized in the modified Tyrode solution, the tissue was perfused with K^+-free solution for 30 min without stimulation. If the preparations developed either spontaneous firings or depolarization of resting potentials to −60 mV or less, they were omitted from the further experiments since they might contain Purkinje fibres. After this period, the tissues were perfused with K^+-free,Ca^{++}-free solution for another 30 min and all the fibres which showed resting potentials more negative than −60 mV never developed spontaneous activity during this perfusion period. At the end of this perfusion, Ca^{++}-concentration in the medium were suddenly increased to 3.6–18 mM. The value of resting potentials at this stage showed −93.5±1.69 mV (mean±SE, n=21). When a train of impulses was applied to the preparation and suddenly stopped, low amplitude oscillatory afterpotentials of 2–5 in numbers appeared following the last driven action potentials, which were never seen in K^+-free,Ca^{++}-free solution (figure 1). These oscillatory after-potentials (OAPs) became larger with increasing the number of the driven action potentials. If they were large enough to reach the threshold, an extra-excitation triggered by the train ensued (figure 1, B, bottom).

This triggered-automaticity appeared not only as a single extrasystole but also more than 2, and occasionally as self-sustaining tachycardias. The average threshold potential of the triggered-automaticity induced in 10 preparations repeatedly was −70.1±3.17 mV (mean±SE, n=10). Once OAPs appeared, they could be recorded with every impalement of 6 to 12 different sites in each preparation. The amplitude of OAPs showed dependence not only on the numbers of the train but also on the basic cycle length (figure 2).

The present results were different from the findings reported by Müller in the working myocardium[6], since he observed automatic activity in K^+-free,Ca^{++}-free solution at decreased resting potentials, and no OAPs nor triggered-activity were described. OAPs and triggered-activity were mainly regarded as a characteristic feature of slow responses or observed in depolarized fibres[7,8], or activities limited to special regions of the heart[9–12]. The present experiments disclosed that ventricular muscle fibres could equally develop OAPs and triggered-automaticity at potentials more negative than −60 mV. Thus these results indicate that OAPs and triggered-automaticity are not unique features of the specialized tissues, but represent a general character inherent to all the cardiac muscles, and appear either at high or low resting potential levels. The mechanism of this OAP is not clear from the present experiments, but we assume that Ca^{++}-movement across the cell membrane under the decreased K^+-conductance may be operative as a basis of this OAP, since it could be induced only in K^+-free,high-Ca^{++} solutions.

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A simple method for observation of capillary nets in rat brain cortex

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Summary. By the authors' technic, the profiles of capillaries in rat brain cortex were clearly demonstrated. The capillaries formed complicated nets by sprouting of their finer branches and anastomosing with each other. Further, a kind of perivascular cells with yellow autofluorescent granules was distributed close to capillaries, arterioles or venules. They seemed to be a special form of macrophage in the brain cortex.

The distribution-pattern of capillaries in brain cortex had been studied with some technics using carbon particles or colouring matters[1]. Recently, some fluorescent substance was also employed for this purpose. However, it was difficult to follow a running course of capillaries by those methods, even if serial sections were employed.

On the other hand, according to de la Torr[2], endothelial cells specifically took up administered L-DOPA and emitted a green fluorescence. Further, by the authors' tentative observation, a kind of pericytes with yellow fluorescent granules was distributed close to capillaries in the cortex. Based on these findings, the authors tried to establish a simple method for a representation of a running course of capillaries and a distribution of fluorescent cells. However, it is difficult to elucidate a distribution of fluorescency emitted from L-DOPA by routine histological procedures, because L-DOPA was easy to diffuse into surrounding tissues.

After several trials, it was known that the following procedure was available for the authors' purpose. At 2 h after the administration of 10 mg of safrazine (monoamine oxidase inhibitor), 10 mg of L-DOPA was injected to Wistar rats weighing 230–250 g, s.c. After 1 h, the rats were anesthetized, and their brains were removed. Then the pieces excised from a cortex were placed on nonfluorescent glass slide and pressed by hand with the other opposite glass slide. And the slide was moved to a right side to get stretched specimens. The specimens were dried with electric fan for 20 min at a room temperature and placed in a desiccator for 20 min at 10^{-3} Torr. They were exposed to formaldehyde gas for 1 h at 80 °C for a detection of L-DOPA by Falck-Hillarp method[3] and examined under a

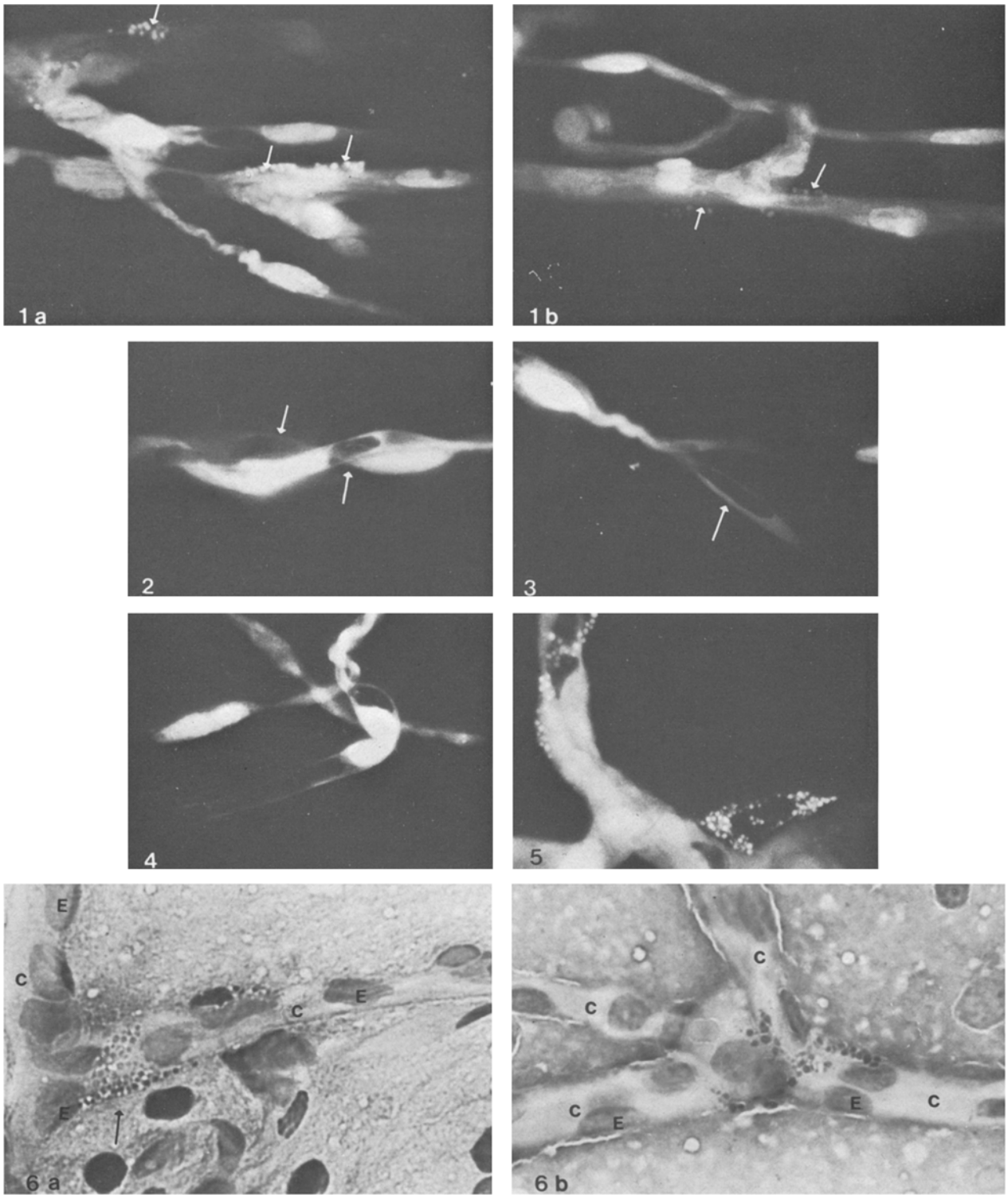

Fig. 1, a and b. In these photographs, the course of capillaries can be easily traced, and granules scattered along the capillary wall. Arrows indicate granules in the pericyte-like cells. Strong pale areas with slender or oval forms are endothelial cells. × 840.

Fig. 2. The capillaries twist and show a spiral pattern. Black shadows in these capillaries are red cells (arrows). They take various forms in capillaries. × 840.

Fig. 3 and 4. These photographs show a tangle of the finest branches of capillaries. At the lower part of figure 3, a flat red cell is seen in the capillary (arrow). The diameter of these fine branches looks to be smaller than the size of red cells. × 840.

Fig. 5. This photograph shows an arteriole or venule. Close to it, triangular pericyte like cells are seen. In their cytoplasm, a lot of yellow fluorescent granules are evenly distributed. × 840.

Fig. 6, a and b. These photographs were taken from the specimen stained with routine histological procedure. Figure 6, a, was stained with HE and figure 6, b, with PAS. In figure 6, a, 2 capillaries are depicted, and the nuclei of cells are stained strongly with hematoxyline. In bifurcating region, a cell having a lot of granules is seen (arrow). Figure 6, b, shows PAS positive granules in the pericyte like cells localizing in a bifurcating region of capillaries. Capillary (C), Endothelial cells (E). × 960.

reflected fluorescent microscope of Olympus, Tokyo (ext. 405 nm, em. 475 nm over). Adding to this, the specimens were stained with hematoxyline-eosine (HE) for a general view, periodic acid Schiff reaction and sudan black B, respectively, after a short exposure of formaldehyde gas for 5 min.

Figure 1, a and b, are examples of capillary nets revealed by the authors' method. Capillary lumina were filled with faint green fluorescence, while slender areas along the capillary wall were stained with a strong green fluorescence. These areas were identified as endothelial cells referring to the specimens stained with HE. Further, oval black shadows in capillary lumina were blood cells. With these observations, running courses of capillaries could be traced up to their periphery. Often, along the outer side of capillary wall, cells containing yellow fluorescent granules were observed. They appeared only in the perivascular areas and especially in bifurcating regions of capillaries, arterioles or venules. Occasionally, from capillaries, finer branches sprouted out (figures 3 and 4). The average diameter of capillaries in specimens was estimated as 2–8 μm (figures 1 and 2), but that of smallest branches as shown in figures 3 and 4 was only 1 μm. Here questions arose in mind; whether the blood cells could pass through those narrow portions or not, and whether the capillaries in the brain cortex were susceptible to the administration of L-DOPA or not.

Concerning a deformability of red cells, several reports had already been published from the stand point of rheology[4–6]. Further, in capillary nets a tangle of capillaries sometimes occurred. That is, in figures 2–4, 2 capillaries ran spirally and in figures 3 and 4, the finest capillaries were twined around each other.

At the first step of this investigation, the authors considered these findings as artifacts during a preparation of specimens. However, a twist or a tangle of capillaries could not be produced by the authors' procedure, because the stretching force for brain tissue was applied only from left to right as described afore. The fluorescent cells along the brain vessels appeared to embrace capillaries as depicted in figure 5. They contained a lot of autofluorescent granules of about similar size and often made a cluster of 2 to 3 cells close to bifurcating regions. The histochemical properties of intracellular granules were similar to type II of mast cells reported by Ibrahim[7]. That is, the granules were acidophile and stained with PAS reaction as shown in figure 6, a and b. They did not show a metachromasia with toluidine blue, but partially stained with sudan black B. Different from the report of Ibrahim, alcohol or acetone treatment could not induce a decrease of fluorescency. According to the authors' observation, the fluorescency of the granules was first detected at second week after birth (unpublished). They are assumed as one type of macrophage from the granular size and quality, and their function is a store house of some substances transported by a brain circulation. This speculation is being confirmed by the experimental studies in the author's laboratory.

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Experimental data on the neurotoxicity of methyl-ethyl-ketone (MEK)

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Summary. A severe potentiating effect of methyl-ethyl-ketone (MEK) on the peripheral and central neurotoxicity of n-hexane could be demonstrated in a chronic inhalation study in rats.

The hexacarbon compounds n-hexane, methyl-n-butyl-ketone (MBK) and 2,5-hexanedione have been identified as neurotoxins in several outbreaks of occupational neuropathies. There have been various reports of polyneuropathies in factory workers following exposure to n-hexane or MBK[2–4] and in 'sniffers'[5], i.e. juveniles who had used cements or solvents for their euphoric properties. In the latter cases, n-hexane was thought to be the major neurotoxic agent. N-hexane and MBK are metabolically related with 2,5-hexanedione and 2,5-hexanediol. Spencer and Schaumburg have demonstrated in extensive neurobiological studies that these compounds cause neuropathies of the 'dying-back' type in the form of central-peripheral distal axonopathies in rats and cats[6–8].

An outbreak of severe toxic neuropathies among Berlin solvent sniffers in 1975 was closely related to an alteration of the abused solvent mixture by denaturation with 11% methyl-ethyl-ketone (MEK), a substance previously considered as safe concerning neurotoxic properties[9]. The solvent was composed of 16% n-hexane, 26% benzine fraction, 29% toluene and 18% ethylacetate. The production of the MEK-containing thinner was subsequently officially stopped; 6 more cases occurred, however, in 1976 and 1977, after old, MEK-containing batches had been sold[10].

Material and methods. The following experiments were devised to investigate the effects of inhalation of MEK and n-hexane in rats. In plastic chambers with smooth floors 5 rats were exposed to 10,000 ppm n-hexane (99% purity; Merck Nr. 4367) for 15 weeks, 7 days per week, 8 h per day. 5 other rats were exposed to a mixture of 1100 ppm MEK and 8900 ppm n-hexane, another group of 5 animals to 6000 ppm MEK (99% purity; Merck Nr. 6014). The initial concentration of 10,000 ppm had to be decreased within a few days in the latter group because of severe irritation of the upper respiratory tract. 5 rats served as controls. In another set of experiments under the same conditions, 2 rats were sacrificed each week in each group in order to determine the date of earliest morphological alterations. At the time of sacrifice, animals were perfused in a slightly modified method described by Pease[11]. The peripheral nerves of the brachial plexus, sciatic nerve and its branches and several levels of the spinal cord and medulla were studied by light and electron microscopy. All representative specimens were embedded in araldite, hardened and ex-