neurons[16,17,18]. Ben-Ari et al.[9] and Krnjevic et al.[10] proposed that part of the effect of acetylcholine in the hippocampus is mediated by interneurons. Ashwood et al.[19] have described several putative interneurons of the rat hippocampus and discussed the possibility that they mediate a feed-forward inhibition. Afferent excitation of area dentata interneurons has been described by Buzsáki and Eidelberg[20]; electrophysiological evidence has been found that the septum may be one source of such excitation[21,22]. The results presented here support these findings, as well as the observation of Chandler and Crutcher[2] who found by anterograde tracing of horseradish peroxidase transport after injections in the septum, that HRP-reactive terminals made synapses with granule cells and putative pyramidal basket cells. Hence, the results offer an anatomical basis for direct excitation of granule cells as well as for feed-forward inhibition, mediated by interneurons, from an *extrahippocampal* structure. Anatomical evidence for *intrahippocampal* feed-forward inhibition of area dentata interneurons by contralateral hilar neurons has been described by Seress and Ribak[23]. Feed-forward inhibition is not only a rather economical mode of influencing large populations of principal neurons by exciting a relatively small number of interneurons. A parallel afferent projection to both interneurons and granular cells would allow selective excitation of some of the latter, while the other part is inhibited. This would be optimal, in terms of information theory, for increasing the 'signal-to-noise ratio'; feed-back inhibition may be too slow for this purpose[24]. It is possible that other hippocampal afferents will be described in the future which allow the generation of feed-forward *and* feed-back inhibition; moreover, the coexistence of both effects may be a widespread principle in brain organization.

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A comparative study of the innervation of the choroid plexus in amphibia

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Summary. The aminergic and cholinergic innervation of choroid plexuses in three species of amphibia was investigated. Plexuses of the Japanese toad and the bullfrog had poor innervation by adrenergic nerves of sympathetic origin, but in the clawed toad, these plexuses were heavily innervated by adrenergic axons from ganglion cells located in the plexus stroma. Nerve fibers positive for acetylcholinesterase were not found in the plexuses, except for a few fibers with very weak enzyme activity in the clawed toad.
Key words. Adrenergic nerves; cholinergic nerves; ganglion cells containing (nor)-adrenaline; choroid plexus; amphibia.

In the mammalian choroid plexuses, dual innervation by sympathetic adrenergic nerves that arise exclusively from the superior cervical ganglia and by parasympathetic cholinergic ones of unknown origin has been found histochemically and ultrastructurally[2-9]. Pharmacological experiments[2,5,9-14] showed that sympathetic nerves affect the two major functions of choroid plexuses; inhibition of the production of cerebrospinal fluid (CSF) by the epithelial cells, and regulation of transport exchange between the CSF compartment and the blood. For submammals, little is known about the innervation of these plexuses. Here, we report the distribution pattern of aminergic and cholinergic nerves in the choroid plexuses of three amphibia.

Materials and methods. 30 clawed toads *(Xenopus laevis)*, 20 Japanese toads *(Bufo bufo)*, and 30 bullfrogs *(Rana catesbeiana)* were used. The animals were anesthetized with ethyl ether, perfused through the aorta with Ringer solution and decapitated. For whole-mount preparations, choroid plexuses and cerebral arteries were carefully dissected out. Choroid plexuses are very undeveloped and sometimes absent in the lateral ventricles of clawed and Japanese toads, but are well-developed in the bullfrog. The materials were either stretched over nonfluorescent glass slides and transferred to a desiccator to be dried in vacuo over P_2O_5 for 1 h, or else fixed with 4% buffered formaldehyde (pH 7.2) for 30 min at 4 °C. Small blocks of the brains containing choroid plexuses were quickly frozen in isopentane chilled with dry ice. Then, they were freeze-dried[15], or else 15 µm sections were cut with a cryostat, mounted on glass slides, and fixed with 4% cold formaldehyde for 30 min.

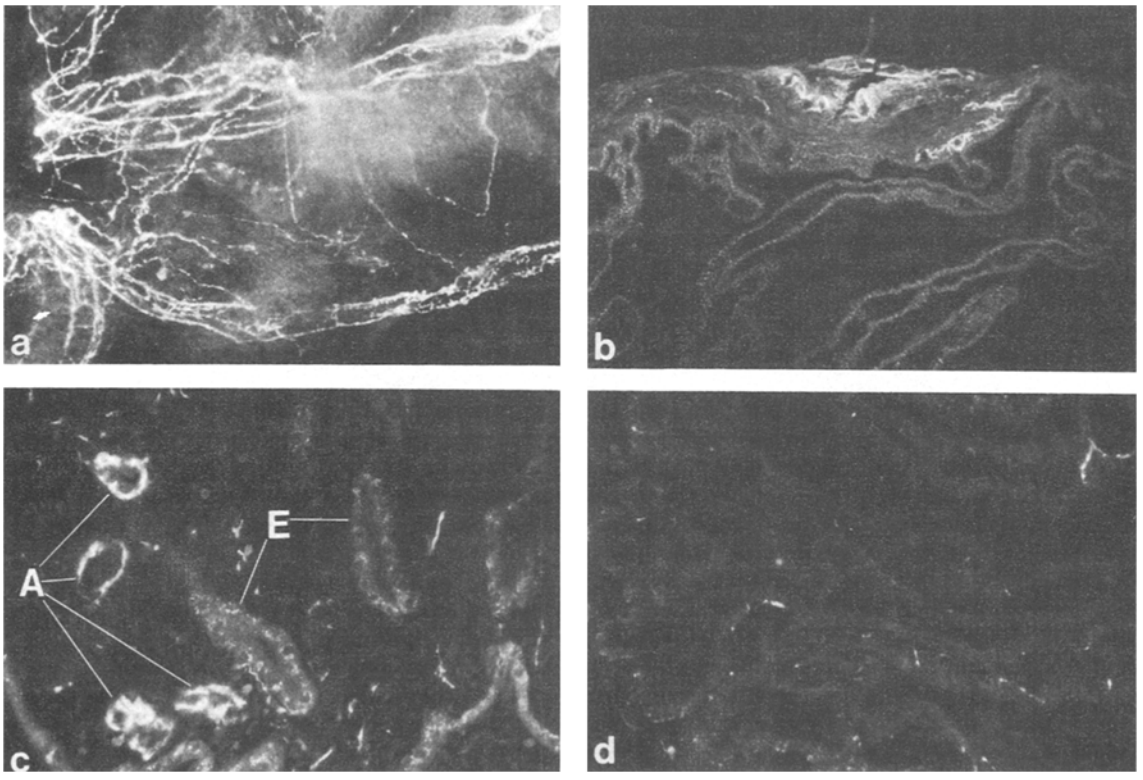

Figure 1. Aminergic innervation in whole-mount *(a)* and sections *(b–d)* of choroid plexuses from the Japanese toad *(a, b)* and bullfrog *(c, d)*. *a* and *b*) choroid plexus IV. *a*: ×160; *b*: ×80. *c)* choroid plexus III. A, arteriole; E, epithelium. ×160. Note lipofuscin autofluorescence around nuclei of epithelial cells (see *c, d*).

For demonstration of aminergic nerves, air-dried materials and freeze-dried blocks were treated with formaldehyde vapor from paraformaldehyde for 1 h at 80 °C[16]. The blocks were then infiltrated with paraffin and cut 15 µm thick. Amines were identified using microspectrofluorimetry with the Nikon SPM-RFL system[17]. To differentiate between dopamine and (nor)-adrenaline, HCl vapor treatment was done[18]. Small whole-mount pieces on quartz slides were exposed to the vapor of a fresh sample of concentrated HCl solution at room temperature for 1 to 10 min in a closed Petri dish. They were mounted in glycerol under quartz coverslips. For cholinergic nerves, whole-mount preparations fixed in formaldehyde and cryostat sections were maintained in Karnovsky's medium[19] without acetylcholine iodide for 30 min at 4 °C and then incubated in the complete medium, containing 2×10^{-4} M tetraisopropyl pyrophosphoramide as an inhibitor of nonspecific cholinesterase, for 1–5 h at 20 °C.

Results. In the Japanese toad and bullfrog, as reported previously[20,21], sympathetic adrenergic nerves that enter the cranial cavity next to the internal carotid and vertebral arteries were in well-developed plexuses surrounding the major cerebral arteries. Arteries of various sizes within the choroid plexuses from the lateral, third and fourth ventricles of these two species had rather dense and longitudinally-oriented networks of adrenergic axons issuing from these perivascular nerves (fig. 1). The veins, capillaries, and epithelium had a scanty nerve supply. Fluorescent ganglion cells were not found in the choroid plexuses or in the pial arteries.

In the clawed toad, we did not observe any fluorescent nerves on the pial blood vessels (fig. 2a, b). However, there were very dense, meshed plexuses of aminergic nerves in the choroid plexus of the fourth ventricle (P-IV) (fig. 2c, d). The nerve supply was poor along the major intrachoroidal artery and vein, but abundant throughout their small branches, capillaries, and epithelium. The plexus of the third ventricle (P-III) also had rich aminergic innervation (fig. 2h). The distribution of nerves here was similar to that in P-IV. In the stroma of both choroid plexuses, a number of ganglion cells emitting intense greenish-yellow fluorescence were seen alone or in small groups with aminergic nerves (fig. 2c, e, f, g, h). They were more numerous in P-IV than in P-III. In the microspectrofluorimetric analysis (fig. 3), these ganglion cells and nerves had a peak at 415 nm in the excitation spectrum, with a low shoulder at 330 nm, and a peak at 480 nm in the emission spectrum. After short exposure to HCl vapor, the highest peak of the excitation spectrum shifted to 370–400 nm. Longer treatment raised the peak of the excitation spectrum at 330 nm and lowered the peak at 370–400 nm; fluorescence also faded. This is a typical pattern for noradrenaline and adrenaline[18].

In the choroid plexuses of three amphibia, nerve fibers positive for acetylcholinesterase (AChE) were detected only in the clawed toad (fig. 4a). These fibers were few and had low AChE activity even after 5 h of incubation; their profiles could not be seen in cross-section (fig. 4b). Ganglion cells with demonstrable AChE activity were not found. In the clawed and Japanese toads, a sparse nerve plexus positive for AChE, composed of both well-stained fiber bundles and poorly stained thin fibers, was found along the major cerebral arteries of the internal carotid system after 1 h of incubation (fig. 4c). Such nerve fibers were absent from the major arteries of the vertebro-basilar system and the small pial and choroidal arteries (fig. 4d).

Discussion. By fluorescence histochemistry we found that the choroid plexuses of the clawed toad, Japanese toad, and bullfrog have adrenergic nerves, but the origin, density, and regional distribution of nerves within the plexuses differ. The plexuses of the Japanese toad and bullfrog, as is the case for mammals[2,4,5,7], are innervated by sympathetic adrenergic nerves surrounding the pial arteries. In the clawed toad, however, there were no fluorescent nerves in the pial blood vessels, while numerous

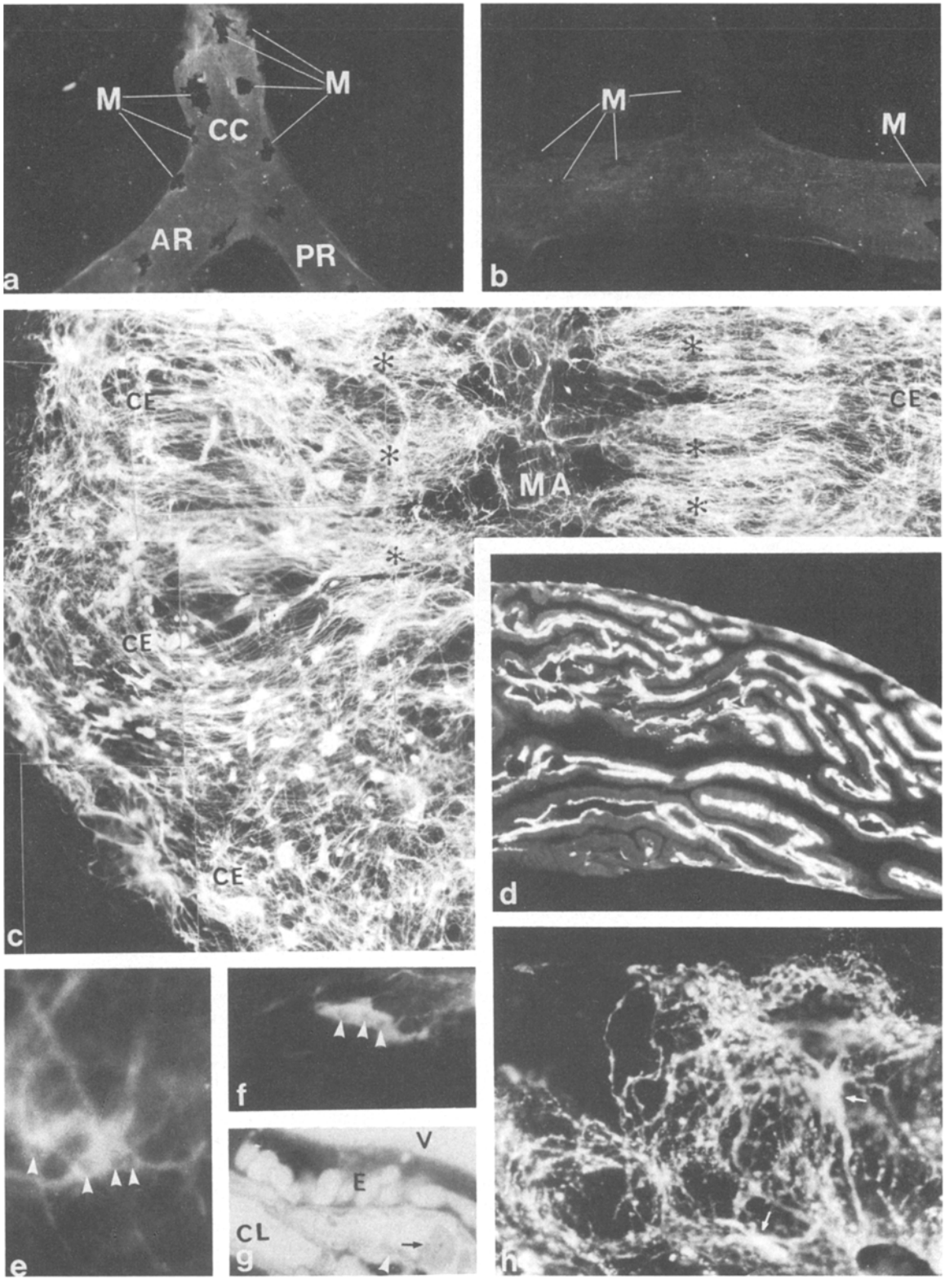

Figure 2. Aminergic innervation in whole-mounts *(a–c, e, h)* and sections *(d, f, g)* of major cerebral arteries and choroid plexuses from the clawed toad. *a)* cerebral carotid artery (CC) and anterior and posterior rami (AR, PR). M, melanocyte. ×80. *b)* basilar artery. M, melanocyte, ×80. *c)* distribution of aminergic nerves in choroid plexus IV. MA, major intrachoroidal artery; asterisks, small arterial and venous branches; CE, capillary-epithelium regions. Note that numerous fluorescing ganglion cells are distributed mainly over the capillary-epithelium regions. ×50. *d)* capillary-epithelium complex of choroid plexus IV. ×140. *e* and *f)* fluorescent ganglion cells in choroid plexus IV. Arrowheads, nucleus. Note the three contiguous cells. ×250. *g)* single ganglion cell in the stroma of the choroid plexus IV. Arrow, cell body; arrowhead, axon; E, epithelium; CL, capillary lumen; V, ventricle. Epon section (2 µm thick; toluidine staining). ×500. *h)* choroid plexus III. Arrows, fluorescent ganglion cells. ×160.

ganglion cells containing (nor)-adrenaline were in the stroma of the choroid plexuses. This indicates that the adrenergic nerves innervating the plexuses of this toad are not of extrinsic origin, but originate in the axons from these ganglion cells. In mammals, as a whole, the adrenergic nerve supply is richest in P-III, and poorest in the P-IV[4,7]; in amphibia, plexus innervation with adrenergic nerves is different. P-IV of the clawed toad was most richly supplied. The density of nerves in the plexuses of all four ventricles of the Japanese toad and bullfrog was about the same, much less than in the clawed toad. The innervation in the clawed toad is mostly in the microvascular system and epithelium; in the other two species, as in most mammals[4,7], it is mainly restricted to the artery system. The role of adrenergic nerves in amphibian choroid plexuses is not known. However, based on pharmacological evidence of sympathetic influence on small blood vessels and epithelial cells in mammalian choroid plexuses[2,5,9-14], the adrenergic innervation of the plexuses in the clawed toad must relate to some function in the regulation of microcirculation, CSF secretion, and transport action in that vascular-epithelial structure.

The rich innervation of mammalian choroid plexuses by parasympathetic cholinergic nerves via the pial arteries was found by AChE staining after sympathectomy[3,6]. In the amphibia we studied, a few weakly stained nerve fibers were found only in the clawed toad. These nerve fibers had no direct connection with the cholinergic nerves that travel with the major cerebral arteries. Nerve cells with much AChE activity, such as those in the parasympathetic ganglia, were not found in the choroid plexuses. This enzyme often has low activity in sensory and adrenergic neurons[22-27]. We think that the AChE positive nerve fibers within the plexuses of the clawed toad are sensory or adrenergic or both in nature.

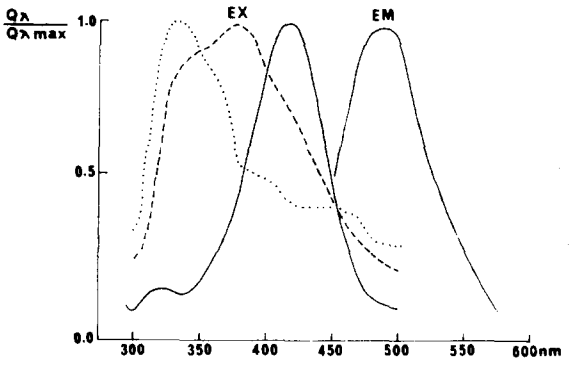

Figure 3. Excitation (EX) and emission (EM) spectra obtained from fluorescent ganglion cell bodies in choroid plexus IV of the clawed toad. ——, after formaldehyde treatment only; – – –, after formaldehyde treatment followed by treatment with HCl vapor for 1–2 min; · · · · · ·, after formaldehyde treatment and treatment with HCl vapor for 5 min or more. All spectra were corrected and expressed as relative quanta.

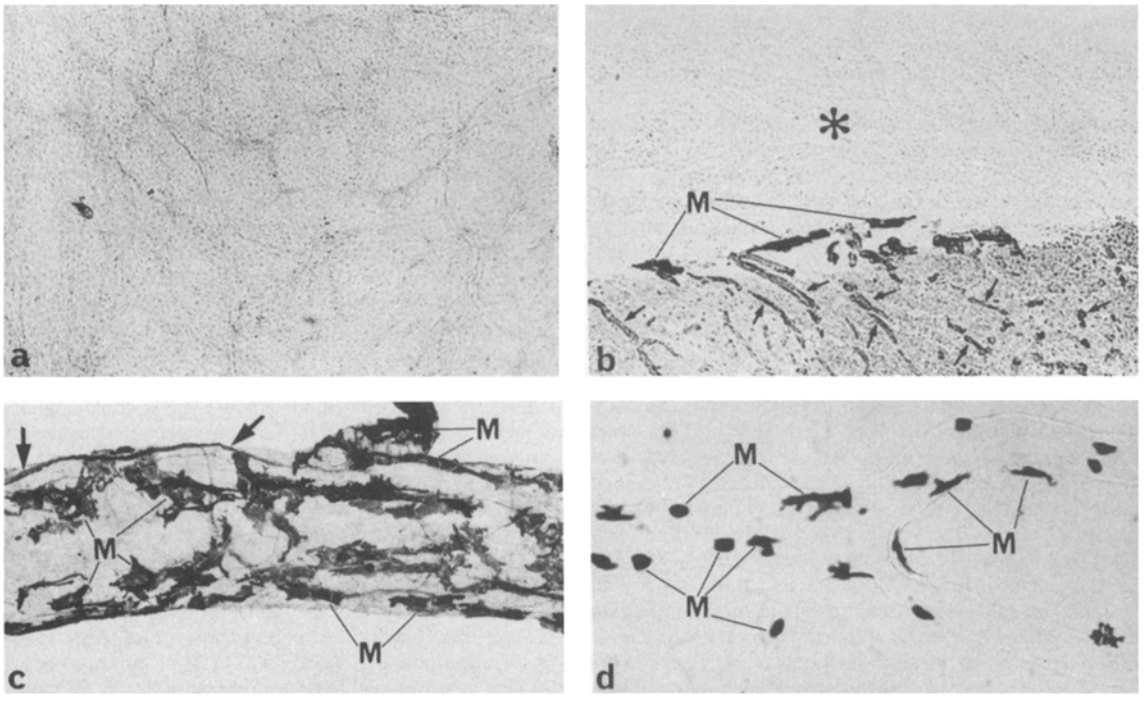

Figure 4. AChE staining in whole-mounts *(a, c, d)* and a section *(b)* of the choroid plexuses and pial arteries from the clawed toad. *a* and *b)* choroid plexus IV. M, melanocyte; asterisk, choroid plexus; arrow, capillaries with heavy non-nervous AChE activity in the medulla oblongata. ×80. *c)* anterior ramus. M, melanocyte; arrows, fiber bundle with strong AChE activity. ×80. *d)* choroidal artery supplying choroid plexus IV. M, melanocyte. ×80.

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Effect of glucose and phloretin-2′-β-D-glucose (phloridzin) on in vitro fertilization of mouse ova

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Summary. The fertilization ratio of mouse ova in vitro decreased when glucose concentration in the medium was lowered. However, the addition of phloretin-2′-β-D-glucose (phloridzin), known as a glucose uptake inhibitor, restored the fertilization ratio back to the control level. The glucose moiety of the phloridzin seemed to be responsible for this effect.
Key words. In vitro fertilization; mouse ova; capacitation; glucose; phloretin-2′-β-D-glucose (phloridzin).

The physiological nature of sperm in vitro was greatly influenced by the energy source which was added to the medium[2–4]. In rat and mouse, in vitro fertilization was achieved only in glucose (or mannose)-containing media but not in fructose or other hexoses[5,6]. It could therefore be predicted that glucose uptake inhibitors would prevent the fertilization of mouse ova in vitro. Phloretin-2′-β-D-glucose (phloridzin; known as a glucose uptake inhibitor in intestine as well as in kidney cells), however, did not decrease the fertilization ratio in vitro. Rather, it actually improved the fertilization ratio in a low glucose concentration medium.

Materials and methods. In vitro fertilization of mouse ova. Media used were modified Krebs-Ringer bicarbonate buffer supplemented with glucose[7] (glucose-KRB) or fructose (fructose-KRB). The sperm were obtained from the cauda epididymis of mature ddy mice (weighing 35–40 g) as previously reported[7]. 4–5-week-old ddy mice were injected with 5 IU of PMSG (Teikoku Zoki) 48 h before the administration of 5 IU hCG (Teikoku Zoki) i.p. 14–16 h after the hCG injection, the ova in cumulus clots were collected by puncturing the ampullar portion of the oviduct with a needle. Ova collected from 10–20 female mice were mixed by pipetting and were divided and placed in 0.4 ml of glucose-KRB in test tubes. More than 25 ova were put into each tube. The right epididymis from each of two male mice was chopped in glucose- or fructose-KRB, while the procedure was repeated in fructose-KRB for the left half. Sperm were preincubated for 40 min in these media at a concentration of approximately $1.0–1.5 \times 10^6$ sperm/ml. 40 µl of the sperm suspension was introduced to the ova placed in 0.4 ml of glucose-KRB. Sperm were diluted to an appropriate concentration for in vitro fertilization and exposed to glucose following this procedure. After various intervals of sperm addition, hyaluronidase was added to the ova and the penetration of sperm through the zona pellucida layer of the ova was observed under a phase contrast microscope. Chemicals used were phloretin-2′-β-D-glucose (phloridzin), phloretine (guaranteed reagent grade; Nakarai Chem.) and 3-O-methyl-D-glucose and 2-deoxy-D-glucose (Sigma Chem.). The reagents were dissolved or suspended in distilled water and were stored at −20 °C. Stock solutions were diluted with fructose-KRB and used for the experiment.

For the statistical analysis, Student's t-test was employed to assess the significance of differences between the mean values for the control group and the sample added group.

Results and discussion. Epididymal sperm need time before penetrating the zona pellucida. Therefore, to investigate the capacitation stage, the sperm preincubated in fructose-KRB were introduced to the ova in glucose-KRB, and the time required for the zona penetration was observed. As shown in table 1, none of the sperm preincubated in fructose-KRB could penetrate the zona pellucida within 20 min, while capacitated sperm (preincubated in glucose-KRB) penetrated without a time lag. The addition of fructose instead of glucose to the medium preserved sperm motility, indicating that fructose may have been utilized as an energy source for motility. However, it was obvious that sperm were not capacitated during incubation in the fructose-containing medium (table 1).

The decrease of glucose concentration (fructose remained present in all media at a concentration of 6 mM) lowered the fertilization ratio. This effect was most pronounced between 1.2 and 0.6 mM of the glucose concentration (table 2). Considering the fact that fructose and glucose share almost the same metabolic pathway, the authors assumed the possibility that glucose serves as a triggering signal for capacitation apart from func-

Table 1. Effect of sperm preincubation on the time required for the penetration of zona pellucida

Preincubation	10 min	20 min	30 min	40 min	50 min
0 min in glucose-KRB	0	0	25 ± 8	57 ± 9	63 ± 4
40 min in glucose-KRB	56 ± 11	63 ± 7	65 ± 9	ND	ND
40 min in fructose-KRB	0	0	42 ± 12	52 ± 6	69 ± 10

Values are mean ± SE of four independent experiments. ND: not done.