process of wound healing appeared to proceed normally in embryos in 300, 600 or 1000 µg/ml papaverine (figure 4). Thus, although papaverine had already inhibited 1 morphogenetic movement in these embryos, it did not prevent wound healing, and thus the control of wound closure is not identical to the control of neurulation.

Embryos in which neurulation has been blocked by papaverine can be induced to neurulate rapidly by treatment with the divalent cation ionophore A23187[16]. Thus papaverine might block the calcium fluxes which normally trigger neurulation, and the ionophore might overcome this block and allow neurulation to proceed. Calcium ions could also act as the trigger for the changes in cell shape which effect wound healing in *Xenopus* embroys[4]. One model for wound healing in this system consistent with the present results would be that the newly exposed lateral cell membranes at the cut edge of the wound have a greater ionic permeability than the normally exterior membranes. An influx of calcium through this newly-exposed membrane, resulting in a calcium-activated contraction of microfilaments localized in the margins of cells at the wound periphery could lead to the cell shape changes observed by scanning electron microscopy[4]. Further experiments are in progress to test this model.

1 Acknowledgments. We wish to thank Drs M.A. England and J. Wakely, whose friendly discussions first precipitated the idea for these experiments, Dr A.R. Cossins who read the manuscript, Mr F. Schaefer who prepared the semi-thin sections and Mrs J. Clumpus for technical assistance.

2 A.G. Jacobson and H. Gordon, J. exp. Zool. *197,* 191 (1976).
3 N. Nakatsuji, Devl Biol. *68,* 140 (1979).
4 M. Stanisstreet, J. Wakeley and M.A. England, J. Embryol. exp. Morph., in press.
5 L. Tilney, J. Cell Sci. *3,* 549 (1968).
6 T.E. Schroeder, J. Embryol. exp. Morph. *23,* 427 (1970).
7 T.E. Schroeder, Biol. Bull. *137,* 413 (1968).
8 P.C. Baker, J. Cell Biol. *24,* 95 (1965).
9 P.C. Baker and T.E. Schroeder, Devl Biol. *15,* 432 (1967).
10 D.J. Moran, J. exp. Zool. *198,* 409 (1976).
11 P.D. Nieuwkoop and J. Faber, A Normal Table of Xenopus laevis Daudin. North Holland Publ., Amsterdam 1956.
12 J. Cooke, J. Embryol. exp. Morph. *30,* 49 (1973).
13 M.J. Karnovsky, J. Cell Biol. *27,* 137A (1965).
14 M.H. maxwell, J. Microscopy *112,* 253 (1975).
15 J. Van Reempts and M. Borgers, Stain Technol. *50,* 19 (1975).
16 D. Moran and R.W. Rice, Nature *261,* 5560, 497 (1976).

Regression of granular pericytes in cerebral fine vessels of rats after administration of a vitamin E deficient diet[1]

M. Mato, S. Ookawara and K. Kurihara

Department of Anatomy, Jichi Medical School, Minamikawachi, Tochigi (Japan 329-04), and Nasu-Kogen Psychiatric Hospital, Nasu, Tochigi (Japan 325), 18 December 1979

Summary. Prolonged administration of a vitamin E deficient diet to Wistar rats resulted in regressive changes in granular pericytes distributed around fine vessels of the cerebral cortex. The regressive signs included expansion of endoplasmic reticula, swelling of mitochondria and increase of vesicular structures. Rupture of the limiting membranes of the intracellular granules often accompanied these changes. The finding seems important for an understanding of the physiology and nutriology of granular pericytes in the brain.

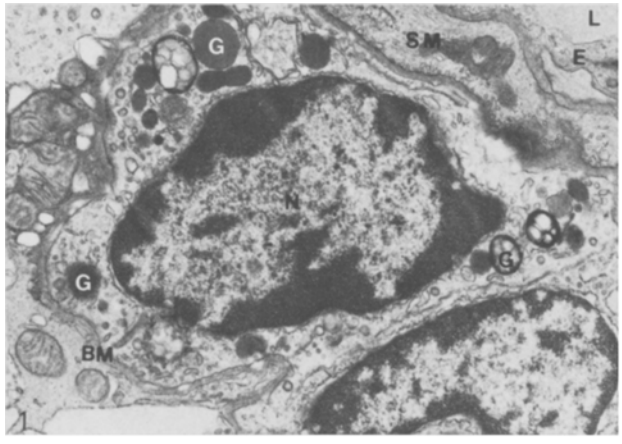
Granular pericytes localized in cerebral fine vessels were characterized by the presence of a lot of intracellular electron-opaque granules of various sizes. According to a previous study by the authors, the granular pericytes change in shape and content as the rats age. They possess a high concentration of acid phosphatase in intracellular granules[2].

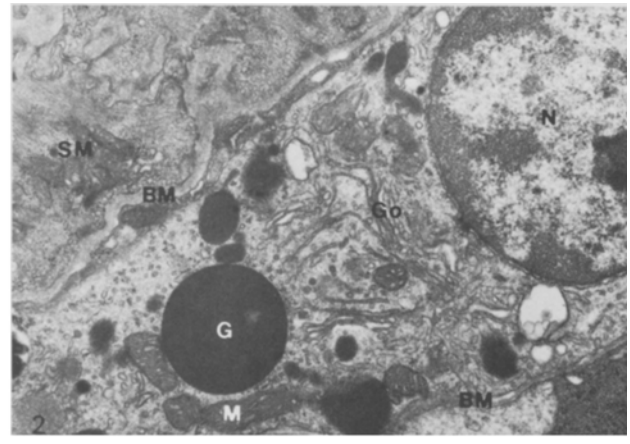
Recently, the authors had a chance to observe granular pericytes in the cerebral cortex after prolonged administration of a vitamin E deficient diet, and found regressive signs in them. These findings seemed to be important for an understanding of the physiology and nutriology of granular pericytes. This paper deals concisely with an ultrastructural change in granular pericytes under the conditions described below.

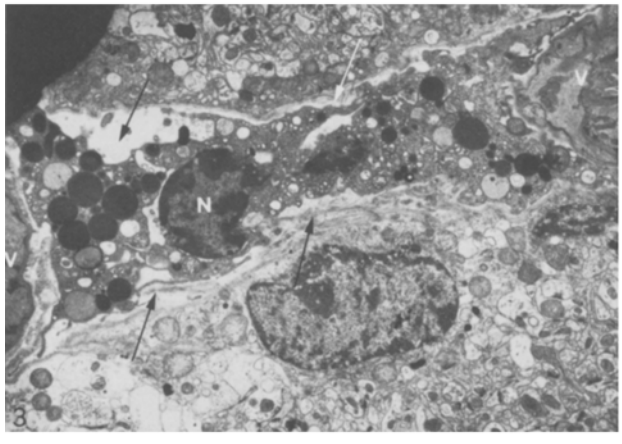
Material and methods. The vitamin E deficient diet contained 0.3 IU of vitamin E per 100 g of food and Kodak corn oil prepared with Oriental Co. (Tokyo), and supplied by courtesy of Eisai Co. (Tokyo). 10 Wistar male rats were fed with this diet for 10 months. The hair on their bodies became rough and they looked somewhat yellowish. The animals were killed by decapitation, and the cerebral cortex was sliced with a blade after removing pial tissue carefully in cold physiological saline, and then immersed in a mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate solution (pH: 7.4) for 2 h. The specimens were then postfixed with 1% osmium tetroxide buffered with the same phosphate solution for 2 h. The other procedures for embedding and cutting were the same as in a routine method.

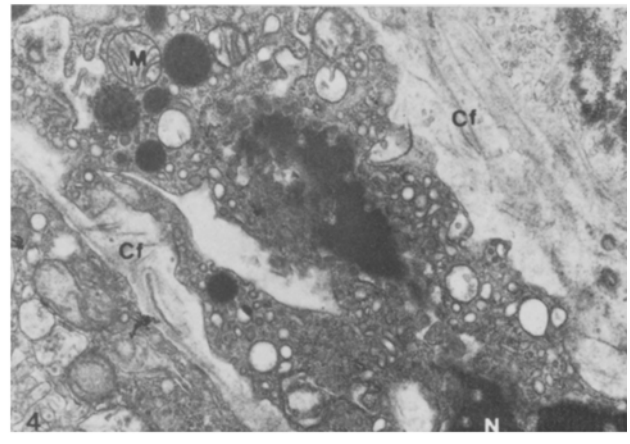
Observations. Figures 1 and 2 were obtained from controls and figures 3–6 from rats fed with the vitamin E deficient diet for 10 months. As seen in figures 1 and 2, the cytoplasmic organelles of the granular pericytes in control specimens were clearly defined, and electron-dense bodies with round or irregular shapes were distributed throughout the cytoplasm. Golgi lamellae and vesicles were evident in figure 2 and condensation of the perinuclear chromatin was obvious. Granular pericytes were delimited with a basal lamina. Surrounding them, smooth muscle cells and neuronal processes were depicted. However, the granular pericytes shown in figures 3–6 revealed dense matrices, and a lot of vesicles and vacuoles. Occasionally, a basal lamina became obscure and the interstices between the granular pericyte and the surroundings became wide (figures 3 and 4). In some parts, collagen fibers appeared (figure 4). Electron-dense bodies vacuolized and lost their limiting membrane (figure 5). Often mitochondria were swollen and the arrangement of their cristae became irregular (figures 4 and 6). As shown in figures 5 and 6, no signs of regression were observed in endothelium and smooth muscle cells adjacent to granular pericytes were observed in samples from the experimental group.

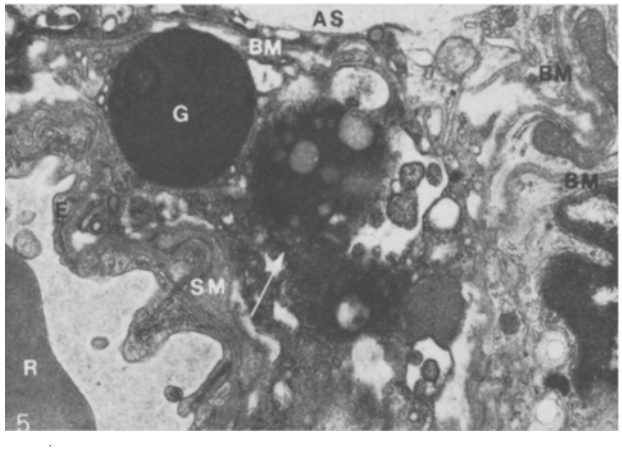
To summarize the findings mentioned above; a morphological difference in cytoplasmic organelles between control and experimental groups was marked in mitochondria,

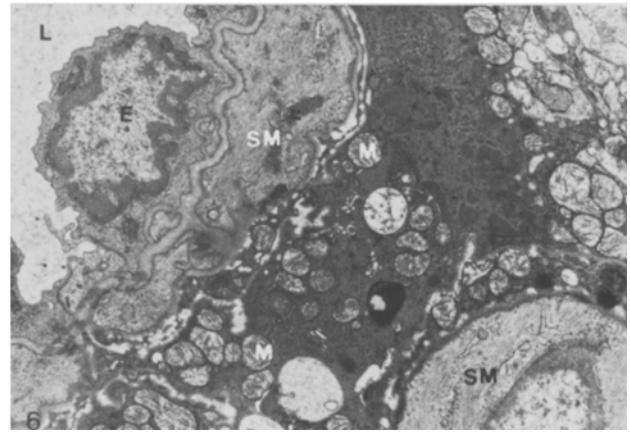












Figures 1 and 2 are granular pericytes in controls. Granular pericytes in these figures show oval or slender forms and their nuclei (N) are provided with perinuclear chromatin. They are surrounded clearly with basal lamina (BM). Intracellular electron dense granules (G) are various in size and distributed through the cytoplasm. In Fig. 2, mitochondria (M) and Golgi appearatus (Go) are evident. L: vascular lumen, SM: smooth muscle cell. Fig. 1, × 5600. Fig. 2, × 11,300.

Figures 3–6 are granular pericytes from the experimental group. Fig. 3. A granular pericyte located in the bifurcating region of fine vessels (V). The cytoplasm is stained dark and the nucleus (N) takes a peculiar form. Intercellular spaces are wide (arrows). × 2800. Fig. 4. High magnification of figure 3. A lot of vesicles and vacuoles can be seen in dark cytoplasm. Collagen fibres (Cf) appear in some parts of intercellular space. M: mitochondria, N: nucleus. × 11,300. Fig. 5. Granular pericyte is subjacent to thin endothelium (E) and smooth muscle cells (SM). Expanded endoplasmic reticula or vacuoles appear in cytoplasm. In the center of this figure, a disintegrated dense body is seen (arrow). R: red blood cell, BM: basal lamina, AS: astrocyte, G: granule. × 13,200. Fig. 6. The granular pericyte is subjacent to a smooth muscle cell (SM) and shows homogeneously dark cytoplasm. Mitochondria (M) are swollen and round in shape. Endothelium (E) and smooth muscle cells look healthy. L: vascular lumen. × 5600.

endoplasmic reticula and electron-opaque bodies, and regressive signs occurred in the specimens obtained from rats fed with a vitamin E deficient diet.

Discussion. From the observations, it was possible to say that vitamin E was essential for maintaining the morphology of granular pericytes and preventing collagen formation around fine vessels in the cerebral cortex. That is, in the case of deficiency of vitamin E in the diet, granular pericytes in the cerebral cortex tended to degenerate. Although the mechanism of this remains unclear, it could be postulated that vitamin E might contribute to the stability of the limiting membranes of electron-opaque bodies. As mentioned before, the intracellular dense bodies contain a large amount of acid phosphatase, and if the lysosomal membranes were ruptured as a result of a deficiency of vitamin E, cytoplasmic organelles in granular pericytes would have been damaged, and then the cells degenerated.

1 This study was supported in part by a grant in aid for Scientific Research from the Ministry of Education of Japan (No. 448085).

2 M. Mato, S. Ookawara, E. Aikawa, K. Kawasaki, Anat. Anz., in press (1980).

Freezing and transplantation of brain tissue in rats

J. D. Houlé and G. D. Das[1,2]

Department of Biological Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 20 December 1979

Summary. Neocortical tissue obtained from rat embryos was frozen and stored at −70 °C for 6 h prior to transplantation into the cerebellum of neonatal rats. Growth, differentiation, and integration of this tissue within the host brain was comparable to that obtained from freshly dissected and transplanted tissue. It is suggested that freezing to low temperatures does not adversely effect the viability or transplantability of the neural tissue.

In recent years it has been shown that embryonic brain tissue can be successfully transplanted in the brains of the laboratory mammals[3-6]. Such transplants not only survive, but also grow, differentiate, and become anatomically integrated with the host brain[7,8]. Tissues for such transplantations have always been freshly obtained from normal viable embryos and transplanted with minimal delay. It is not known if such tissues can be frozen and stored for a long period, and subsequently transplanted successfully. Our researches on this issue, employing various parameters, show that the embryonic neural tissue is capable of maintaining its viability after freezing and thawing, and that it can be successfully transplanted into the brains of rats.

Laboratory-bred Long-Evans hooded rats were used. Embryos of 16-day gestational stage provided the neural tissue. After dissecting them the cerebral cortex was removed, the 2 hemispheres separated, and placed in lactated Ringer's solution at room temperature. Each cerebral hemisphere was then transferred to a 1.5-ml Eppendorf polypropylene micro test tube containing 0.5 ml of one of the following media: Eagle's Minimal Essential Medium (MEM), lactated Ringer's solution, or amniotic fluid obtained from the embryonic sac at the time of dissection. To each of these media dimethyl sulfoxide (DMSO) had been added to a final concentration of 10%. The tubes were capped tightly and set in a wire rack placed on top of a bed of dry ice in a styrofoam chest. The capsules remained suspended in the dry ice vapors (−70 °C) permitting freezing of the tissue within 25–30 min (25 °C ambient temperature to 0 °C; at a rate of approximately 1 °C/min)[9,10]. The tissue remained at −70 °C for 6 h, and then it was thawed and prepared for transplantation. The tissue was thawed rapidly by placing the capsules in a 37 °C water bath. This took 35–40 sec. Following this it was washed with lactated Ringer's solution (room temperature) 6–8 times, effectively rinsing the tissue of DMSO. Finally the tissue was transplanted into the cerebellum of 10-day-old rats according to the technique described earlier[4,8]. Each host animal received only 1 transplant. 6 animals received transplants frozen in Eagle's MEM, 6 animals received tissues preserved in the lactated Ringer's solution, and 2 animals received tissues frozen in the amniotic fluid. The host animals were sacrificed 30 days after transplantation by transcardial perfusion with 10% neutral formalin. The brains were processed for histology, sectioned serially in a sagittal plane at 8 µm, and 1 out of 5 sections was saved. The material was stained with cresyl violet and luxol-fast blue.

In other experiments parameters of storage time of the tissue, and the age of the host animals were varied. The neocortical tissue after initial freezing to −70 °C was stored at −96 °C for 1–18 weeks. It was thawed and transplanted in the cerebellum of the host animals 10–20 days old. These host animals were sacrificed 30 days after transplantation, and their brains processed as described above.

The neocortical transplants in the brains of all the host animals had survived, grown and differentiated. They had occupied about one-half of the host cerebellum (figure, A). Cytologically as well as cytoarchitecturally they could be readily distinguished from the surrounding cerebellum. The neurons in these transplants appeared normal and fully differentiated. Pyramidal and stellate neurons of large and small size could be identified (figure, B). The transplanted neurons were not tightly clustered. They were spaced apart with neuropil in between. The neuroglial elements also were present in the transplants. These features indicated that the transplants were normal neural tissue. The transplants, although embedded within the parenchyma of the host brain, were surrounded by a band of neuropil. The neuropil between the transplants and the host brain tissue provided for anatomical continuity between the two. In the material stained with luxol-fast blue axonal processes were seen to course between the transplants and the host cerebellum. In no instance was there intervening pia mater separating the transplants from the host brain nor was any neuroglial scar formation observed. These anatomical observations indicated that the transplants had become integrated with the host brain. The remaining host cerebellum surrounding the transplants appeared normal and maintained its trilaminar cytoarchitecture. Neither transplants nor host brains showed any neoplastic characteristics. In