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.

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Freezing and transplantation of brain tissue in rats

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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.

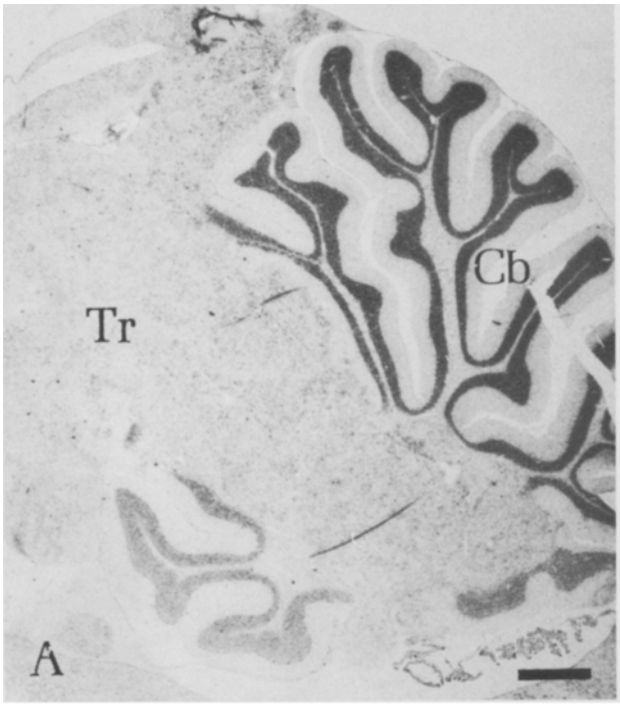
The neocortical transplants in the brains of all the host animals had survived, grown and differentiated. They had occupied about one-half of the host cerebellum (figure, A). Cytologically as well as cytoarchitecturally they could be readily distinguished from the surrounding cerebellum. The neurons 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

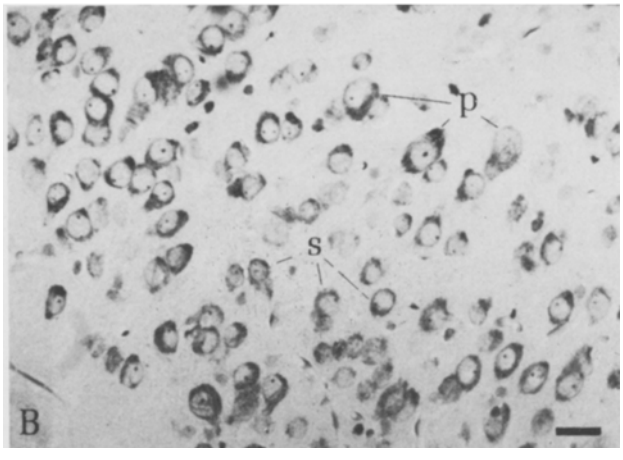
other experiments, where storage time of the tissue and the age of the host animals were varied, the neural transplants were seen to have survived and grown. They appeared very similar to those described above.

This material was compared with that where freshly obtained neural tissues were transplanted, and no differences were seen between the two[4,5,7,8]. This indicated that freezing of the embryonic neural tissues, storing and thawing them neither altered their viability nor hindered their transplantability. It is very likely that these tissues after transplantation may have progressed through the same developmental changes that a freshly obtained neural tissue does. Although there were no observable differences in the transplants frozen in 3 different media, it was more convenient to use Eagle's MEM and lactated Ringer's solution than the freshly obtained amniotic fluid.

Freezing of various tissues and storing them for different intervals prior to transplantation has been achieved by many investigators. In particular, the work on embryonic tissues indicates that tissues and organs such as pancreas[11,12], parathyroid glands[13] and heart[14] maintain their structural, as well as functional, viability after they are thawed and transplanted. Wittingham et al.[15] showed that early mouse embryos survived and grew when transferred into the foster mother even after freezing and storing at −196 °C for 8 days. In the present study we have demonstrated that embryonic neural tissues also can be frozen, stored, thawed and transplanted successfully in the brains of the laboratory mammals. These transplants not only showed normal cytological and cytoarchitectural differentiation, but also became anatomically integrated with the host brain. In light of other studies showing the presence of afferent and efferent fibres in the neural transplants, it is very likely that the transplants described in the present study may also have established reciprocal connections with the host cerebellum[16,17].

There are 2 main advantages of freezing embryonic neural tissues for research on transplantation. Firstly, it facilitates matching the ages of donors and host animals at one's convenience while using rats, mice, rabbits or guinea-pigs. Secondly, it makes research on higher mammals such as cats, dogs and monkeys feasible, where neural tissues obtained from embryos of specified developmental stages can be frozen, stored and shipped to other investigators when host animals of desired age and conditions become available. Before extending the findings on neural transplantation to the clinical field for man, it is imperative that the findings made on the rat be confirmed on 'higher' laboratory mammals. It is possible that freezing and storing of neural tissues for transplantation may prove to be an important step in the clinical application of this type of research.





A Embryonic day-16 neocortical transplant (Tr) which has grown to displace approximately one-half of the host cerebellum (Cb). Note that the transplant is anatomically integrated with the host cerebellum with no scar tissue or degeneration between them. Cresyl violet stain, bar 1000 µm.

B Microscopic view of normal appearing, fully differentiated neurons of the transplant. The primary cell types of the neocortex, pyramidal (p) and stellate (s) neurons, can be distinguished. Cresyl violet stain, bar 20 µm.

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