Neural Transplantation:  
A Report on the IVth International Symposium

William J. Freed and Jeffrey M. Rosenstein

NIMH Neuroscience Center at St. Elizabeths, 2700 Martin Luther King Ave, Washington, D.C. 20032
and Department of Anatomy, The George Washington University, Washington, D.C., USA

The IVth International Symposium on Neural Transplantation consisted of a total of 330 papers, including 236 submitted papers. Of the submitted papers, 27 were selected for oral presentation and 209 were chosen for poster presentation. In addition, there were 94 invited oral presentations. The total number of papers in the symposium series continues to increase, from 215 papers presented in Rochester in 1987 and the 233 papers presented in Cambridge in 1989, representing a remarkable continued increase in participation and expansion of the field. The overall quality of the material presented was outstanding, and there was a substantial participation of scientists from various fields related to neural transplantation, including studies of neurotrophins and other growth factors, CNS immunology, and brain development.

The following report represents a selective discussion of papers which were presented at the meeting. The amount of material presented was vast, and no attempt has been made to be comprehensive. Thus it should be emphasized that many outstanding papers are not included in the present report. Apologies are extended to the numerous participants whose papers are not described herein. Citations are given to the abstracts (Restorative Neurology and Neuroscience, Volume 4, number 3, July, 1992) and extended abstracts (Journal of Neural Transplantation and Plasticity, Volume 3, number 4, Oct.-Dec., 1992), and to other primary sources in some cases. Five abstracts of papers presented at the symposium, which arrived too late for publication, were printed in the meeting program. This summary report covers selected aspects of basic studies, CNS plasticity and development, growth factors, immunology, cell lines, glial cells, studies related to Parkinson's disease and catecholaminergic cells, Huntington's disease, cortical function, new models, and pain. Four additional brief summary reports which discuss the sessions on biomaterials and encapsulation, the visual system, new animal models, and spinal cord follow.

HUNTINGTON'S DISEASE

A session on Huntington's disease included papers on the properties and connectivity of striatal tissue grafts in rodent models, as well as papers on mechanisms through which striatal grafts produce functional effects, behavioral models, and studies in a primate model.

Graybiel and associates /52/ described a well-known excitotoxic model of Huntington's disease and its use for studies of transplantation. Differentiation of striatal tissue was studied following transplantation into the brain, using immunocytochemical markers to characterize the grafts. The striatal phenotype was found to be expressed, but only in parts or regions of the grafts. Several striatal markers are expressed in patches of striatal-like tissue, in a "matrix" or "surround" of non-striatal tissue.

In normal striatum, c-fos expression (an early marker of cell activation) in striatal cells is turned on by cocaine, but only in those cells that express the protein DARPP-32. Effects of cocaine on DARPP-32-positive cells in striatal grafts were also
examined: In grafted cells, cocaine also induced c-fos expression only in DARPP-32-positive cells, just as in the normal striatum. This suggests the use of manipulation of c-fos expression with cocaine to test for normal functioning of striatal tissue grafts. Although these grafts develop similarly to normal striatum, they are not identical. In particular, 6-hydroxydopamine (6OHDA) causes increased expression of enkephalin in the host brain, but expression of enkephalin is hyper-increased in grafts.

Wictorin and coworkers /153/ reported on studies of the connectivity of striatal tissue grafts. These grafts were found to have both efferent and afferent connections with most of the brain regions which normally project to the striatum. Afferent connections include projections from cortex, thalamus, dopaminergic projections from host substantia nigra (SN), and serotonergic projections from host raphe nucleus. The major efferent projection of striatal grafts appears to be to the globus pallidus.

An important development in the potential use of transplantation in Huntington’s disease is the availability of a primate model. Isacson et al. /67/ described a baboon model of Huntington’s disease, involving unilateral excitotoxic lesions of the striatum induced by ibotenic acid or quinolinic acid. Animals with these lesions do not show spontaneous abnormalities, but following administration of apomorphine abnormal movements, including chorea, orofacial dyskinesias, and dystonia, are seen. Effects of transplantation were examined in this model using fetal rat donor tissue, in host animals immunosuppressed with cyclosporin A. The specificity of the grafts in inducing behavioral recovery was illustrated by showing reversal of the improvement after withdrawal of the immunosuppression, and by implanting control animals with brain stem tissue. This model will be of considerable value in the future for development of any procedure with potential applicability to human patients.

Norman /100/ discussed and reviewed general factors related to behavioral efficacy, and techniques for intrastratial implantation of grafts. It was noted that striatal grafts can be behaviorally effective when transplanted as solid tissue fragments or as dissociated cells. Transplanted astrocytes, however, produce considerably smaller effects and by certain measures are not effective. The grafts are effective only when placed into the striatal parenchyma; striatal tissue grafts in the lateral ventricle or the globus pallidus are not effective. Some studies have also suggested that striatal tissue grafts, and possibly grafts of other cell types, may offer a partial protective effect against subsequent excitotoxic lesions of the striatum.

Most of the behavioral studies of striatal tissue grafts have employed hyperactivity induced by excitotoxic lesions of the striatum as a behavioral measure. Striatal grafts reverse this hyperactivity over the course of 3 - 9 weeks after transplantation, and also can reverse amphetamine-induced hyperactivity. More complex skilled behavioral tasks which are impaired following excitotoxic lesions of the striatum, such as paw reaching and grasping of food pellets, are also improved following striatal tissue grafts. Another model which has been employed to assess the effects of striatal tissue transplants is the use of rotational behavior in response to apomorphine. One pitfall in the use of this technique is that the rotational behavior appears to vary with the specific lesion location: More posterior lesions tend to produce ipsilateral rotation, while more anterior lesions produce more contralateral rotation. Sensitization caused by repeated apomorphine injections must also be taken into account when using rotational behavior to assess the effects of grafts. Also, changes in the form of rotation can occur: Animals may pivot in one spot, or they may walk around in circles using all four paws. The pivotal form of rotation is seen as being the more severe. Norman /100/ suggested that striatal tissue grafts may change the form of rotation from pivotal to walking /also cf. 122/.

The final oral presentation on Huntington’s disease models was given by Sirinathsinghi and associates /129a,130/ on the regulation of cellular activity within striatal tissue grafts and in target areas by afferent input. GABA release in the globus pallidus was decreased 95% by striatal lesions; striatal grafts restored pallidal GABA release to
34% of normal levels. Both baseline GABA release and amphetamine-stimulated GABA release were similarly affected. Electron microscopic studies have shown that tyrosine hydroxylase (TH)-immunoreactive boutons synapse with dendrites within the grafts, presumably indicating ingrowth of dopaminergic axons. Expression of mRNA for cholecystokinin, neuropeptide Y, dynorphin, and pro-enkephalin in striatal grafts was generally higher than in control striatum. Evidence was presented suggesting that the dopaminergic afferentation of striatal grafts regulates mRNA expression; this regulation was similar to normal striatum in terms of both amount and direction of the changes induced.

A particularly interesting finding was that GAP-43 expression, a marker for elongating neurites, was highly expressed in grafts for the first 30 days after transplantation, and decreased to baseline levels by 90 days after transplantation. This may indicate that synaptic growth and restructuring within striatal tissue grafts is completed between 30 and 90 days after transplantation. This time course resembles the time required for normal development of striatal circuits. It was concluded that there is very good growth of fibers into striatal tissue grafts, even in mature host animals.

GROWTH FACTORS AND AXONAL GUIDANCE

Schwab and Schnell described studies which shed light on the mechanisms which control and limit CNS regeneration. Regeneration of lesioned fibers involves sprouting, elongation, and target recognition. Experiments by Aguayo and others (e.g. /11/) in which peripheral nerve segments were transplanted to bridge CNS lesions, were described. Similar experiments were conducted in vitro with sympathetic or sensory neurons. Fibers grow into sciatic nerve but not into optic nerve. Even if NGF is added to the medium in large amounts, fibers do not grow into the optic nerve, although general fiber growth is greatly increased everywhere else. Thus, an inhibitory factor must be present in optic nerve tissue.

This factor was found to be in the oligodendrocyte membranes. Three molecules in CNS myelin with molecular weights of 35, 60, and 250 kDa were implicated. Antibodies to each cross-react, and the 35 kDa molecule is a subunit. The active sites are found in the protein parts of the molecule, not the carbohydrate. It is specific to CNS myelin, and tightly bound to the membranes. The protein is effective in very low concentrations, equivalent to fractions of a ng per cm² of dish. The mode of action occurs through contact between growth cones and CNS membranes. Following such contact, the growth cones collapse. Antibodies block the inhibitory effect on fiber growth, as well as the inhibitory effect observed in growth cones. Calcium in growth cones increases 30-fold following contact with membranes, preceding the growth cone arrest.

This information was employed to design in vivo studies in the partially transected spinal cord. The method used involved implantation of living hybridoma cells, which produce a monoclonal antibody to the inhibitory factor from oligodendrocyte membranes. The hybridoma cells were contained within a millipore filter matrix. Distances to which sprouting fibers penetrated past the spinal cord lesion were measured. In control animals, these distances were rarely more than 1 mm, and were usually in the range of 0.1 - 0.5 mm. With the neutralizing antibody-producing cell implants, the distances to which these fibers penetrated was increased markedly, usually to 4 - 7 mm, and sometimes as long as 11 mm. In one animal, sprouting fibers penetrated 11 mm in 16 days.

What about the possibility of actually removing oligodendrocytes? This can be accomplished by X-irradiating the spinal cord at birth, as these are the last cells to differentiate. In these irradiated animals, distances of sprouting are also greatly increased, usually to distances of between 4 and 8 mm, and sometimes more. These fibers can, however, only grow through intact spinal cord, and cannot cross a gap. Several materials were tried in attempts to bridge the gap. None of these worked, even though some of them work in the brain. Thus, the spinal cord is an especially difficult tissue for achieving CNS repair. Undoubtedly, future studies will concentrate on the development of methods to apply this strategy to repairing completely transected spinal cord, such as closing the gap via spondylectomy. Ultimately, this information will
also be useful for studies of transplantation in models of spinal cord injury (cf. /109/).

The growing family of substances with neurotrophic activity and their receptors, as well as possible therapeutic applications, was the subject of several papers /35,56,81,108,146/. The neurotrophins including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4) received particular emphasis. Oral session number 24 was devoted to papers on growth factors, with a particular emphasis on studies aimed at stimulating CNS plasticity. Ebendal /35/ described the use of a mouse cell line, clone 3E, which contains several hundred copies of the gene for rat NGF and contains large amounts of NGF mRNA. These cells were able to decrease the death of septal cholinergic cells, from a loss of 27.5 to 8% of the total cells, following implantation into a lesion cavity in the fimbria-fornix. Another area of study described by Ebendal /35/ involved the development of spontaneously immortalized cells from several fetal rat tissues, including muscle and brain, which was achieved by long-term growth and repetitive replating at low density. This is similar to the methods which have previously been used, for example, to develop the immortalized NIH-3T3 mouse fibroblast cell line. Human NGF, BDNF, NT3, and ciliary neurotrophic factor (CNTF) constructs were expressed in immortalized rat muscle cells. These cells were transplanted into several brain areas, including the striatum and hippocampus, using a total of 800,000 cells in two 4 µl injections. These cell grafts expand and damage the host brain, thus removing some target cells. Effects of the cell grafts on expression of various mRNAs, such as those for the trk neurotrophin receptors, the low-affinity NGF receptor, and choline acetyltransferase, were examined.

Persson /108/ described the target-derived model for neurotrophins, according to which neurotrophic molecules produced by target cells play a role in the survival of neurons which provide afferents to that system. The model described involved the use of the whisker fields as target, and trigeminal ganglion cells as afferents. When these trigeminal neurons are cultured, NGF, NT3, and NT4 promote survival very effectively, while BDNF has a smaller effect. Areas of the brain which were labelled for neurotrophins and their receptors were also described. BDNF expression was greatest in the substantia nigra, and NT-3 labelling was greatest in the spinal cord (also cf. /5/). The trk receptors, which confer the specificity of neurotrophin interactions, also showed specific regional distributions, with trk-B labelled most strongly in the cortex and cranial nerve ganglia, and trk-C most strongly labeled in cortex.

Carvey (/16/; also cf. /17/) discussed the distinction between target-derived factors with trophic activity, autoconditioning factors, and neuroinhibitory factors, as factors of each of these different types may influence the development and function of dopaminergic neurons. The growth-promoting activity of striatal extracts on primary cultures of dopaminergic neurons was described. Factors influencing this growth-promoting activity were examined. It was found that activity was decreased by kainic acid lesions of the striatum and chronic administration of dopamine agonists, and increased by chronic administration of dopamine antagonists. Carvey /16/ also described a factor of less than 10 kDa molecular weight which is a neuroinhibitory factor for TH-positive mesencephalic cells.

A detailed description of the known effects of BDNF on dopaminergic and cholinergic neurons was presented by Hefti /56/. For cholinergic neurons, BDNF in a concentration of about 1 ng per ml, is as effective as NGF. NGF, however, is effective at approximately 100-fold lower concentrations. In vivo, BNDW was evaluated for a protective effect on septal cholinergic neurons following knife-cut lesions of the fimbria-fornix. Using this in vivo model, BDNF was considerably less effective than NGF in preventing neuronal death. There is also evidence that cholinergic activity regulates BDNF expression.

In mesencephalic cell culture, BDNF produces an approximately two-fold maximal increase in dopamine uptake. The maximal stimulation of dopamine uptake by basic fibroblast growth factor (bFGF) is about 1.5-fold. Des-IGF1 also has a slight effect. Interestingly, neither BDNF nor bFGF increases total neurite length or the number of
branching points. Mainly, BDNF increases only the number of primary neurites. BDNF does not increase TH expression, and it has a very modest effect on neuron survival. BDNF does, however, significantly protect against MPTP-induced toxicity (also cf. /7/). Thus, it does not appear that BDNF fulfills all of the criteria which would be expected for a dopamine neuron neurotrophic factor. In an axonal transection model, which induces a 70% loss of SN dopaminergic neurons, BDNF was not effective in preventing cell death, whether administered into the ventricle, lesion site, or directly to the cell bodies. It is possible, however, that BDNF works in concert with other factors to regulate growth and differentiation, and that no single substance regulates trophic effects on dopaminergic neurons.

TRANSPLANTS FOR THE STUDY OF DEVELOPMENT

Fekete and co-workers /36a/ discussed the use of gene transfer techniques to study cell lineage in the developing brain. Three histochemical markers, β-galactosidase, alkaline phosphatase, and an antibody to the viral protein GAG, can be used to identify marked cells. Viral vectors insert their genes into dividing cells only when the cells are dividing. Early in development, labelling a cell will mark several cell types and a variety of clones, while cell progenitors marked later in development produce fewer clones. One technique that was employed to increase the percentage of cells that were labelled for transplantation was the use of cells producing replication-competent virus, rather than disabled virus, as is usually employed. Using this technique, essentially all donor cells were labelled. The problem of subsequent viral replication was controlled through the use of hosts which are resistant because they lack the receptors for viral proteins which are required for infection.

Sotelo and Avarado-Mallart /134/ described studies of expression of the protein Rat 401, using immunohistochemical studies. This protein is a 200 kDa glial intermediate filament, not an adhesion molecule. It is normally present in glial cells only when subserving Purkinje cell migration. The presence of immature transplanted Purkinje cells induces expression of Rat 401, suggesting that immature Purkinje cells interact with glia to induce expression of Rat 401. Even though it is not an adhesion molecule, adhesion events could very well induce expression. Also, it was pointed out that Rat 401 re-expression can be induced by other methods in other brain regions.

O'Leary and Schlaggar /101/ considered processes of cortical differentiation. Cortical areas can be sharply distinguished by either cytoarchitecture or input-output projections. In general, fetal cortical regions can assume the attributes of other cortical regions when transplanted. For example, barrel fields can develop in foreign transplanted cortical regions. At relatively later stages of cortical development, clonally-related cells become widely distributed to cortical areas.

An interesting study by Krum /77/ employed the glial toxin 6-aminonicotinamide to examine the role of glial factors in blood-brain barrier development. Brain-derived endothelial cells develop blood-brain barrier properties only if co-cultured with astrocytes. Krum /77/ administered 6-aminonicotinamide to neonatal animals, giving four systemic injections of 10 mg/kg during the first weeks of life. This dosage causes damage of both astrocytes and oligodendrocytes, and in the experimental animals widespread spongy degeneration in grey and white matter and swelling of astroglial processes were seen. When administered to adult animals, 6-aminonicotinamide caused glial degeneration only in specific regions of spinal cord, medulla, cerebellum, and thalamus. In neither neonatal nor adult animals were the tight endothelial cell junctions damaged, although there was evidence of protein leakage through venous hemorrhages in the adult animals. This method may nevertheless be of interest for other purposes related to studies on the role of glia in CNS development and plasticity; for example, studies akin to those by Schwab (see section on Growth and Trophic factors, above; /125/), who showed that removal of oligodendrocytes by X-irradiation promoted neurite growth in the injured spinal cord.

Another interesting technique was described by Macklis /88/, for producing a highly selective
model of neuronal degeneration. Macklis and Madison /89/ described the use of laser illumination of brain tissue, using long wavelengths (650 - 850 nm), which are able to penetrate brain to a depth of several mm. A non-toxic dye, chlorin e6, was incorporated into latex nanospheres, and these nanospheres can in turn be incorporated into cells, such as neuroblastoma cells. This compound, when illuminated by sufficiently intense light in the near-infrared range (660-670 nm), generates singlet oxygen (102), which is toxic to cells. Chlorin e6 continues to generate singlet oxygen for extended periods without bleaching. Normal tissue is thus unaffected by the laser light, while grafted neuroblastoma cells incorporating the chlorin e6-latex nanospheres are destroyed by the laser illumination /89/.

Macklis /88/ discussed the use of this method to produce highly selective lesions of cortical pyramidal cell populations, through retrograde transport of such chromolytic dyes. When neuronal populations which have accumulated these dyes are illuminated by laser light, the neuronal population is destroyed without damage to intermixed neurons, glia, or blood vessels. Transplantation of fetal cortical neurons into these areas of neuronal depopulation revealed that: (i) the transplanted neurons migrated for up to 1 mm and were integrated specifically into the zones of neuronal depopulation; (ii) control grafts of other types of neurons into kainic acid-lesioned hosts showed minimal spread of the transplanted neurons without preference for the lesioned lamina II/III; (iii) transplanted neurons within the lesioned zones tended to assume a pyramidal shape, while transplanted neurons found in other areas were not pyramidal. This technique allows for investigation of graft-host neuronal integration with minimal interference from factors such as gliosis and crude disruption of the brain parenchyma, as compared to other models and more crude lesioning techniques.

Doucet and coworkers /27/ studied the ability of neonatal (P7 and P14) and adult serotonergic neurites to grow into transplanted fetal (E14) mesencephalic tissue. They found that the fetal mesencephalic grafts received a profuse serotonergic afferentation when transplanted into P7 hosts, but not when transplanted into P14 or adult hosts. This study is of interest because even adult serotonergic neurites are able to grow profusely into other types of fetal CNS grafts. Thus, there is a target tissue-specific loss of growth potential of serotonergic fibers, which takes place between postnatal days 7 and 14, and is specific for the target tissue, rather than for the afferent system. It was suggested that serotonergic axons bear tissue-specific receptors which control their growth with a different developmental sequence in different brain regions.

ALZHEIMER'S DISEASE AND MEMORY MODELS

A number of interesting models were described, in which grafts of acetylcholine-rich tissues (septum or basal forebrain), raphe neurons, or hippocampus were transplanted into the hippocampus of animals with hippocampal injury or deafferentation. Hodges et al. /62/ discussed experiments involving hippocampal or basal forebrain tissue grafts in animals with hippocampal injury induced by ischemia, excitotoxic lesions, or colchicine. Nilsson et al. /99/ described studies of acetylcholine release from septal grafts in the hippocampus. Factors which influence recovery of function following grafts of cholinergic neurons in animals with septohippocampal injury were discussed by Will and coworkers /155/.

Kolb /74/ presented studies involving lesions of the medial prefrontal cortex. Such lesions induce deficits in learning and memory, particularly on tasks which involve a delay, and deficits in skilled forepaw movements. Unilateral lesions produce no lasting deficits, so that studies of these cortical lesions require the use of bilateral lesions. The effects showed a strong dependence on age.

The task selected was the Morris water maze, in which animals are required to swim in a tank of water (made opaque with milk) to find a submerged platform. Animals with frontal cortical lesions never learn to find the platform directly. They always continue to find the submerged platform using an indirect strategy, such as looping or searching. Thus their performance may improve, but they continue to show the qualitative deficit of not being able to locate the platform directly. Grafts of fetal cortex tended to improve
performance only when tested early after transplantation. At later time intervals, grafts resulted in poorer performance.

Dunnett and coworkers/31/ described studies of cognitive function using a model involving fimbria-fornix lesions, which disconnect the hippocampal formation from its subcortical inputs. Effects of intrahippocampal grafts of septal or raphe neurons were used to study the possibilities for functional restoration. Two operant behavioral tasks (that is, tasks based on lever-pressing) were described, delayed matching-to-sample and “differential reinforcement of low rates” (DRL), a form of timing behavior. Deficits in delayed matching-to-sample recovered gradually, over the course of 6 - 35 weeks. The DRL task was ultimately more useful, as the deficits were long-lasting and stable. This task involves imposition of a 20 second delay, following which a lever-press response is reinforeced with a food pellet. Responses which occur prior to the 20 second delay interval are not reinforced and reset the 20 second clock. This forces the animals to time the 20 second interval accurately. The performance of the animals can be evaluated in terms of percent efficiency. Thus, if an animal responds 12 times and receives 3 reinforcements, it has responded with a 25% efficiency. This test is highly sensitive to hippocampal damage.

THE BLOOD-BRAIN BARRIER

Study of the blood-brain barrier in transplantation involves several issues, including new vessel growth, astroglial participation and immune responses. Pappas and Sagen/106/ have described the use of opioid peptide-producing chromaffin cells for pain reduction (see /119/). When chromaffin cells are grafted, the endothelia retain their fenestrated phenotype and are leaky to protein. These investigators suggested, however, that the vessels in such grafts also have the important function of endocytosis and subsequent elimination of immunologically active compounds in the graft circulation that might cause graft rejection. Therefore, the graft vasculature is important for endogenous metabolic functions. The paper by Krum/77/ emphasized the relationship of astroglia and barrier competent vessels and has been described previously under the section on “Transplants for the study of development”. Broadwell and associates/13/ provided a review of the blood-brain barrier under normal conditions and the different approaches to examining changes at the light and electron microscopic level. The authors displayed various permeations in the blood-brain barrier and angiogenesis following a large series of central and peripheral grafts. Their studies of syngeneic, allodense or xenogeneic grafts suggested that neither the barrier nor the purported immunological privilege of the CNS is absolute. Finsen and colleagues/37/ described the expression of immunological markers on activated astroglial cells following xenografting and other non-graft models of brain injury, such as chemical lesioning. Rejection following xenogeneic grafting caused infiltration of reactive cell types. MHC antigens Class I and Class II were induced on microglia, and Class I only on some astrocytes. Injury in the hippocampus following ischemia or kainate lesioning caused widespread induction of Class I on microglial cells. Brightman and Ishihara/12/ used skeletal muscle autografts to examine the phenomenon of neurovascular specificity. The muscle grafts, when placed within the ventricle, were vascularized by vessels that resemble those normally found in muscle. Interestingly, in bilaterally ganglionectomized hosts, choroidal vessels which were fenestrated, unlike vessels in muscle, entered into the muscle grafts. The authors suggested that there may be an autonomic determinant of vessel type. The muscle autografts were also used to examine the entry of intravascularly injected dye-labeled macrophages through the muscle and into the brain parenchyma.

IMMUNOLOGY

Papers presented on immunology included studies on immune mechanisms/59,138/ as well as papers on the relative susceptibility of grafts to rejection/6,68,79,111,132/ depending on various techniques, including transplantation methods and genetic factors.

The opening paper was given by Streilein/138/, who described studies of the mechanisms of
induction of immune unresponsiveness induced by the implantation of various immunogenic materials, such as tissue grafts, into the anterior chamber of the eye. This phenomenon is termed “anterior chamber-associated immune deviation” (ACAID). Data suggesting a possible role of TGF-β in ACAID were presented. Studies of transplantation of neonatal retinas into both the subconjunctival space (a non-privileged site) and the anterior chamber were described. Grafts in the subconjunctival space were rejected readily. Grafts into the anterior eye chamber also did not survive permanently; however, they displayed a range of survival times depending on genetic disparity: Allografts with major MHC disparity survived for 17 - 35 days, while allografts with only minor MHC disparity survived for more than 70 days. Even isografts did not survive permanently. The rejection was unusual, in that inflammation was not seen, even for the allografts. It was suggested that the deterioration of the isografts was caused by transplantation-induced sensitization to retinal antigens.

Sloan and coworkers /132/ presented data on the induction of sensitization to brain tissue allografts by a second graft of donor tissue into the kidney capsule. Removing the second graft five days after it was implanted did not cause an interruption in the reaction to the CNS graft. Thus, CNS immune reactions can be self-sustaining once initiated. It was also noted that there was better survival and less reaction to brain tissue grafts in the brain parenchyma, as compared to grafts in the third or lateral ventricle.

Poltorak and associates /68,111/ found that even when the host animals are systemically sensitized, there are certain strain combinations that resist brain graft rejection. Whereas rejection of grafts from Brown Norway donors was readily induced in all host strains, grafts from Lewis or Fisher 344 donors (both RT1a) survived even after systemic sensitization in many host strains. Conversely, for some strain combinations, including one that did not reject grafts after sensitization (Lewis donors to AO-RT1u hosts), there was evidence of graft damage even without systemic sensitization. It was also noted that expression of MHC Class I and II antigens did not predict graft rejection, as grafts located in the third ventricle frequently showed strong expression of MHC antigens but were not rejected. It was also noted that cellular infiltration into brain grafts results in rejection only when the infiltrating cells include T-helper and T-cytotoxic lymphocytes, as well as perivascular infiltration. Cell infiltration may also consist of microglia expressing MHC Class II antigens, and this latter form of cell infiltration does not result in graft rejection.

Hickey /59/ (also cf. /60/), in the concluding paper, described a series of experiments on the mechanisms through which T-cells are able to enter into the CNS. The model used involved the use of T-cells derived from Lewis rats, which could be recognized by an antibody specific to the Lewis RT-1a MHC molecule. The T-cells were activated by various treatments, and then injected into either Dark Agouti (RT-1a) hosts, which do not produce the RT-1a molecule, or Lewis hosts. When non-stimulated lymphocytes depleted of lymphoblasts were injected, virtually none were found in the brain following injection. Activation of the lymphocytes using the mitogenic lectin concanavalin A, to induce the cells to enter a lymphoblast phase, allowed the cells to enter the CNS freely, regardless of antigenic specificity of the cells. The peak concentrations of cells in the brain were found between 3 and 12 hours after injection. It had previously been shown that anti-myelin basic protein T-cells produce EAE only if stimulated prior to injection into animals (e.g. /105/). These data may help to explain the fact that rejection of brain grafts can be induced by systemic sensitization, even though T-cells cannot normally gain access to the CNS. Apparently, the reaction of T-cells with the CNS requires activation of the cells in addition to the appropriate antigenic specificity.

A population of T-cells derived from a normal, unstimulated animal will contain a proportion of blast cells. A continuous low level of entry of these lymphoblast cells, with various antigenic specificities, was suggested to continuously "survey" the CNS. After CNS allografts, Hickey /59/ demonstrated the presence of rare donor-specific T-cells in the spleen and lymph nodes of the host. Hickey /59/ suggested that the fact that
these cells are rare, and that even fewer would be in blast phase at any one time, would result in an inability of T-cells with appropriate specificities to "find" the graft. In other words, even though CNS allografts induce a small degree of host sensitization, the level of sensitization is not sufficient to result in graft rejection unless the animal is systemically sensitized or receives a second graft.

The question naturally arises whether allografts can be rejected in non-immunosuppressed hosts, and what circumstances might lead to graft rejection. The ability of brain allografts to survive is ultimately an empirical question; there are several studies which suggest that brain allografts usually survive, but can be rejected in certain host-donor combinations, at least in some circumstances. Hickey's hypothesis /59/ suggests that rejection of brain allografts might potentially be provoked by two factors: either (i) a specific stimulation of the host, resulting in an expansion of the population of donor-specific T-lymphocytes, or (ii) non-specific host stimulation, resulting in a greater proportion of cells in the lymphoblast phase, or some combination of the two. In fact, the relative role of the two factors in sensitization-induced brain allograft rejection has not been systematically studied, as systemic sensitization generally induces both a specific sensitization in addition to a non-specific T-cell activation.

Studies of graft rejection and tolerance have been almost entirely focused on the role of surface antigens, and especially histocompatibility complex antigens. Lampson /79/ reported findings which suggest that, at least under some conditions, even internal antigens may play a role in graft rejection. The enzyme β-galactosidase (β-gal), derived from E. Coli, is often used as a cellular marker for studies of genetic alteration of cells. Cells can be made to express this enzyme constitutively by retroviral transfer of the β-gal cDNA. In Lampson's experiments /78/, the 9L gliosarcoma cell line, derived from F344 rats and altered to express β-gal, was transplanted into F344 hosts. F344 animals were systemically immunized with purified β-gal prior to transplantation of β-gal-positive 9L cells into the brain. It was found that prior immunization with β-gal significantly reduced the growth of the transplanted β-gal-positive 9L cells. It was pointed out that T-cells recognize antigen in combination with MHC antigens, rather than free antigen. The complex of MHC with the target antigen might be formed either within the transplanted target cell, or by an antigen-presenting cell after ingestion of the target antigen, culminating in rejection indirectly through a "bystander" type response. Thus, it is possible that non-MHC antigens may provoke graft rejection, even when there is no MHC disparity.

Finally, an observation reported by Ibarra et al. /65/ was that acute spinal cord injury markedly reduced the absorption of the immunosuppressant drug cyclosporin A. After oral administration of cyclosporin A, serum levels were markedly reduced in animals with acute spinal cord injury, whereas blood levels of cyclosporin A were increased after intraperitoneal administration. Absorption was normal for both oral and intraperitoneal administration in animals with chronic spinal cord injury. These differences were attributed to delayed gastric emptying (oral administration) and vascular dilatation (intraperitoneal administration). Altered drug absorption after spinal cord injury would have to be taken account of in any studies of spinal cord injury which require drug administration.

CELL IMPLANTS: USE OF SUPPORT MATRICES AND ENCAPSULATION

One theme expressed in several contexts throughout the meeting was the possibility of the use of support systems for the implantation of cell lines into the CNS. The topic of biomaterials and encapsulation is discussed in an accompanying summary by Sanberg and coworkers /121/. One type of support system involves the use of semi-permeable capsules in which cells are contained and are implanted into the brain. The pore size of the capsules is sufficiently small (approximately 50 kDa) to exclude cells and presumably to exclude large protein molecules and antibodies, thereby providing an immunoprotective effect. Small molecules, such as neurotransmitters and growth factors, can freely diffuse out of the capsules.
New findings regarding transplantation of encapsulated cells included a report on the possible use of pancreatic β-cell lines for GABA-releasing encapsulated grafts /144/. There have been several suggested potential applications for GABA-releasing neural implants, including Huntington’s disease, epilepsy, and Parkinson’s disease. Pancreatic β-cells are known to express glutamic acid decarboxylase, the GABA synthetic enzyme. Two immortalized cell lines derived from rat and mouse β-cells, designated NIT-1 and RIN, were found to release GABA. In vitro, both cell lines were found to survive encapsulation and continued to release GABA. Preliminary data on intracerebral implantation of these encapsulated cells were also reported. If methods for the intracerebral transplantation of cells capable of releasing large amounts of GABA could be developed, it might lead to a number of new applications for intracerebral transplantation.

New data on encapsulated PC12 cell grafts in animal models of Parkinson’s disease were also reported /1,36,157-159/. Emerich and coworkers /36/ described a series of preclinical experiments on the development of encapsulated PC12 cells as a potential treatment for Parkinson’s disease, including studies on encapsulation techniques, assessment of biocompatibility and immunoprotection, and functional efficacy. Winn and coworkers /157/ reported that encapsulated PC12 cell grafts could be retrieved from the host brain intact, with the encapsulated cells remaining viable, and producing minimal damage to the host brain. Winn and coworkers /158/ also compared tissue reactions following capsule implantation. Stereotaxic implantation either directly or using a teflon cannula resulted in minimal tissue reaction, whereas biocompatibility was poor when the capsules were inserted using a glass cannula or when sterility was compromised.

A second category of cell support systems seeks to use polymers or protein matrices to support cell attachment without encapsulation. Such systems serve to localize and stabilize cell populations, but do not provide immunoprotection or containment. Where simple diffusion of products from cell to host brain targets is not sufficient to produce a functional effect, enclosed capsules cannot be used. This would be the case where any form of physical interaction between graft and host is required, including invasion of cells, ingrowth of processes, or extension of neurites from grafted cells into the host brain.

Such cell matrices may allow for physical contact between implanted cells and host brain, possibly resulting in synapse formation and cell-cell interactions which require membrane to membrane cell contact. Several of these cell support systems were described and used in experimental transplantation systems. These included implantation of hybridoma cells in a Millipore filter matrix /125/, and studies by Silver in which differences between immature and mature glial cells in terms of their ability to become incorporated into implanted Millipore filters were described /95/. Another support system, employed by Ebendal et al. /35/, involved the use of a collagen gel as a matrix for the implantation of NGF-producing cells into lesion cavities in the fimbria-fornix. Wöral /160/ described the use of collagen and methacrylate matrix co-polymers, and described several structural formulations which might be used for transplantation, either by injection of cells into the matrices or by entrapment of cells by rehydration of the polymers in the presence of a cell suspension.

GLIAL CELLS

Silver /95/ described differences between the glial reactions which are observed when nitrocellulose Millipore filters are implanted into the brains of immature or mature rats. In general, when these filters are implanted into the brains of immature rats, cells, consisting of microglia and “flat” cells, invade the filters, followed by axons and blood vessels. When these filters are implanted into the brains of mature rats, the implant is instead walled off by a glial scar. In host animals 8-14 days of age and older, there is a greater glial fibrillary acid protein (GFAP)-positive glial reaction around the implant, and a much smaller migration of cells into the implant. Thus, the glial reaction tends to wall off the implant from the rest of the brain, rather than incorporating the implant into the brain structure. Also, in older hosts, a
patterned formation of blood vessels, as indicated by laminin staining within the implant, does not take place, the blood vessels are leaky, and laminin is irregularly deposited.

The filters can also be explanted and used as a substrate for cultured neurons. In all cases they support neuronal survival, but there is a substantial difference between the explants from the younger vs. the older host animals in terms of the degree to which they support neurite extension; the implants from the younger animals are several times more effective in supporting the extension of neurites. This difference appears to be related to the expression of proteoglycans, which inhibit neurite growth via a repulsive (non-permissive), but non-toxic, mechanism. These molecules are associated with boundaries between neuronal structures, similar to what has been found for tenascin.

The role of glial maturity in this phenomenon was investigated by incorporating young or old astrocytes into the filters, and then transplanting the glia-containing filters into the brain. When filters containing mature glia were transplanted, a GFAP-immunoreactive scar formed in the surrounding medium, and there was blood-brain barrier leakage and poor blood vessel formation. When immature glia were implanted with the filters, the GFAP-positive scar in the surrounding medium did not form, and there was normal formation of blood vessels with much less leakage of the blood-brain barrier. It was also noted that the cells did not migrate out of the implants.

Similar phenomena are observed even if conditioned media from mature vs. immature astrocyte cultures are used to impregnate the filters, rather than the cells themselves. When the filters are impregnated with conditioned medium from immature astrocytes plus mature astrocytes, the regrowth is inhibited and resembles that which is seen with mature astrocytes alone. This again confirms that these phenomena are caused by an inhibitory property of mature astrocytes, rather than a stimulatory property of immature astrocytes /95/.

Geller /92/ described experiments on the effects of bFGF on extracellular matrix expression in astrocyte cultures. bFGF was found to increase tenascin expression and decrease expression of laminin. bFGF treatment of astrocyte cultures reduced neuronal adhesion. Following neuronal injury, it may be that the action of growth factors plays a role in inhibiting recovery and synaptic restructuring. In some cases, this type of extracellular environment may inhibit graft integration into the host brain. Simply transplanting cells into the brain in such a neurite-inhibiting environment may not be sufficient to result in functionally significant restructuring. Several papers, in fact, discussed aspects of the effects of the local glial environment or microenvironment on graft-host connectivity /18,24,44/. For example, Castro et al. /18/ found that there were substantial differences in connectivity between cortical grafts and host brain, when the grafts were placed into excitotoxic cortical lesions, as compared to aspiration cavities. Dellman and Carithers /24,25/ found that neurosecretory axon regeneration required living glial cells; cryotreated neural lobe or sciatic nerve grafts did not support neurosecretory axon regeneration, as compared to intact neural lobe and intact sciatic nerve.

A related study was presented by Nieto-Sampedro and coworkers /98/, who employed the technique of coating tissue culture dishes with plasma membranes isolated from normal or gliotic brain tissue. Membranes from the gliotic tissue were found to inhibit neurite extension. The inhibitory activity was found to be present in a proteoglycan, which was sensitive to digestion with glycosaminoglycan lyase. A core protein with 48 kDa molecular weight was found to undergo a large increase in injured brain tissue and was able to inhibit neurite extension, and to induce neurite retraction when added to cultures in soluble form. This factor was distinct from neurite inhibitors contained in myelin, and was localized to microglial membranes by immunostaining.

GENETIC ENGINEERING

Several topics related to genetic engineering were presented in sessions throughout the meeting. For example, Glorioso /49/ presented a detailed summary of the obstacles to the use of viral vectors derived from Herpes Simplex viruses (HSV) for insertion of genes into CNS cells, as well as information on possible solutions. These viruses
have a tendency to establish lifelong infections in neurons, in a latent state without lytic infection. The major difficulties related to this approach include the development of mutant viruses which do not destroy brain tissue, and the development of a means to induce expression of the transferred gene during latency. Directions for the development of solutions to these problems were explored. For example, control of the ability of these viruses to damage brain tissue might involve the use of certain mutants, such as a deletion mutant which blocks viral replication in neurons but not in culture. Certain latency-associated transcripts are expressed while the virus is in a latent state. Natural promoters in the latency-associated transcript region of the gene might be employed to drive foreign gene expression. Further development of these possibilities may result in HSV-derived viruses which can be reliably employed to stably transfer genes into neurons in situ. Data on the expression of tyrosine hydroxylase through the use of an HSV-derived vector was presented by During and coworkers /32/. These authors showed data on TH immunoreactivity in cells around the site of viral injection into the brain, and data on behavioral changes in animals receiving these injections.

An important factor in the development of applications for transplantation of genetically-engineered cells is the development of a means to control gene expression. Studies reported by LaGamma and colleagues /78/ have begun to look at this possibility. The strategy used was to employ inducible promoters, in conjunction with the gene for chloramphenicol acetyl transferase, in primary cultures of rat striatal astrocytes. Expression of the chloramphenicol acetyl transferase gene product was measured by immunocytochemistry and by a biochemical assay. One promoter used was the human preproenkephalin promoter, which is known to be regulated by the cAMP-dependent second messenger system. Dopamine can regulate cAMP production in astrocytes, and was found to induce increases in chloramphenicol acetyl transferase expression, which were mimicked by dopamine agonists and blocked by dopamine antagonists. When transplanted into the brain, chloramphenicol acetyl transferase expression was increased 2-fold by amphetamine. Gifts placed into the striatum of animals with unilateral dopamine depletion showed 30% less activity as compared to grafts in the intact striatum. Further development of the possibility of graft regulation may greatly facilitate both experimental studies and ultimately application to human disease.

There were several reports on the development of immortalized cells /21,66,104,113,141/, most often using retroviral transfer of oncogenes such as the temperature-sensitive form of the SV40 large T antigen (tsA58). This general approach was reviewed by McKay /21/, who discussed the process of cell differentiation in the CNS and described the origin of neurons and glia. The HiB5 cell line, derived from fetal rat hippocampus using tsA58, was found to differentiate, forming cells with both neuronal and glial properties when implanted into the neonatal rat hippocampus or cerebellum (cf. /113/). Another cell line, described by Takashima and coworkers /141/, was derived from E14 mesencephalon also using tsA58, and showed increased expression of neurofilament immunoreactivity in response to growth in brain-conditioned medium. This cell line stopped growing when confluent, even at the permissive temperature. Another cell line, derived from rat Schwann cells using the 12S E1A gene, was found to produce insulin-like growth factor-1 (IGF-1) and NGF receptor /66/. These cells did not show growth in soft agar, and did not produce metastases when implanted subcutaneously into animals. On the other hand, these cells grew in a hyperplastic manner, essentially forming localized tumors. These cells were not able to infect co-cultured NIH-3T3 fibroblasts, but ultrastructurally showed occasional viral budding.

Another interesting technology, which will almost certainly be employed more widely in the future as an experimental model, is the use of transgenic animals as a source of cells for transplantation. Aramant and Seiler /2/ employed this technique as a method for labelling and identifying donor cells following transplantation to the retina. Transgenic NSE-lacZ mice contain the β-galactosidase gene coupled to the promoter for neuron-specific enolase /38/. Thus, when transplanted cells express neuron-specific enolase,
the β-galactosidase gene will also be expressed and can be used as a marker. In the retina of this mouse strain, β-galactosidase is expressed in the ganglion cell layer, part of the inner nuclear layer, and the inner and outer plexiform layers. When used for transplantation to the retina, labelled cells could be identified using this method. It was pointed out that, since not all donor cells express β-galactosidase, not all transplanted cells could be identified using this method, although a fraction of the transplanted cells could be identified clearly. Kershaw et al. /72/ employed tsA58 transgenic mice /71/ as a source of tissue for transplantation, and found that the grafts developed normally, without forming tumors. Other related techniques, involving derivation of cells from transgenic animals, are likely to be applied in transplantation models in the future.

NGF, CO-GRAFTS, CHOLINERGIC SYSTEMS, AND ADRENAL MEDULLA TRANSPLANTATION

Studies of adrenal medulla transplantation, methods for delivery of NGF to the brain, co-grafts of peripheral nerve segments, and other effects of trophic factors have become so interlinked that it is impossible to discuss these issues separately. Thus, studies of delivery of NGF into the brain by genetically-altered cells have potential applications for use in cholinergic systems, and related methods may also be used for delivery of other trophic factors (see section on trophic factors, above). However, several studies of NGF delivery presented at the meeting were directed at enhancing the survival of adrenal medulla grafts, and the entire topic of NGF delivery into the brain, along with related methods, will be discussed in the context of adrenal medulla transplantation.

Adrenal medulla grafts:

Unisicker et al. /146/ discussed growth factors found in adrenal chromaffin cells. Growth factors and cytokines which are found in adrenal chromaffin cells include interleukin (IL)-2, IL-6, insulin-like growth factor (IGF)-1, IGF-2, transforming growth factor (TGF)-β1, TGFβ2, TGFβ3, bFGF, aFGF, and a CNTF-like molecule. The contents of chromaffin cell granules include, in addition to catecholamines, ATP, chromogranin, and peptides. Release of catecholamines and chromogranin can be induced using carbachol. Unisicker described the use of a chromaffin cell-conditioned medium assay to measure the promotion of survival of neurons. Carbachol-induced release of catecholamines was found to be paralleled by a release of trophic activity. Verapamil blocked all three (catecholamines, chromogranin, and trophic activity release) similarly.

bFGF is found in chromaffin cells; it is localized to the granules, not the cytosol. This is surprising, because bFGF lacks the signal peptide needed for release. Although bFGF gets out of the cells, the mechanism of release is not known. Unisicker has not been able to show stimulated release of bFGF. It was found that whole chromaffin cells contain two bFGF-immureactive molecules, with molecular weights of 32 and 46 kDa; granules contain the 46 kDa form almost exclusively. bFGF is 18 kDa. This 46 kDa molecule is biologically similar to bFGF, but seems to be a different molecule. Adrenal medullectomy causes death of preganglionic spinal cord neurons which innervate the adrenal medulla. Growth factors which can prevent this cell death and are present in the adrenal medulla include bFGF, as well as TGFβ3, TGFβ2, and the CNTF-like molecule.

Chromogranin A is present in the adrenal medulla in large amounts. It was affinity purified using a monoclonal antibody. Purified chromogranin A was found to have trophic activity for dorsal root ganglia neurons. Surprisingly, this trophic activity was increased seven-fold by boiling. Additional increases in trophic activity were obtained by further purification; the trophic activity was highest in a 28 - 32 kDa molecular weight band. After purification, its efficacy was similar to that of NGF. This activity was not blocked by several monoclonal antibodies, including NGF antibodies. However, chromogranin A produced by 3T3 transfected cells did not have trophic activity. Possibly there is some additional processing which takes place in the adrenal medulla which is required for the trophic activity.
In the mouse MPTP-lesion model, bFGF was found to protect against dopamine depletion when applied at the same time or shortly after MPTP. Although it has a protective effect, it did not restore depletion when applied long after MPTP. TH is also protected; TH-immunoreactive fibers recover only immediately adjacent to the bFGF-impregnated gelfoam, but TH activity recovers bilaterally. It was noted that bFGF binds strongly to glial cells /146/.

Studies on the mechanisms of action of adrenal medulla grafts were described by Becker and Curran /8/. These experiments focused on two topics: first, the relationship between effects of adrenal medulla grafts and changes in striatal dopamine metabolism, and second, the possible role of changes in blood-brain barrier permeability in the functional effects of adrenal medulla grafts. Adrenal medulla grafts have been shown to cause blood-brain barrier permeability /116/. Curran and Becker /22/ showed that the behavioral response to adrenal medulla grafts, especially the reductions in amphetamine-induced rotation, are associated with an increase in permeability of the blood-brain barrier to dopamine. Additional studies by this group /142/, comparing adrenalectomized and normal rats with unilateral 6OHDA lesions of the SN, have shown that adrenal medulla grafts appear to function through at least two separate mechanisms to decrease apomorphine-induced rotation. The first, which is abolished by adrenalectomy, appears to be associated with increases in blood dopamine and is similar for animals that receive grafts of adrenal medulla and sciatic nerve or sham transplantation. The second is a specific effect of adrenal medulla grafts, which is similar in adrenalectomized and normal animals. Since decreases in amphetamine-induced rotation, rather than apomorphine-induced rotation, appear to be related to changes in blood-brain barrier function, it appears likely that adrenal medulla grafts can induce changes in behavior through more than one cellular mechanism.

A theme which was developed throughout the meeting was the possibility of improvements in the efficacy, as well as the survival, of chromaffin cells in transplanted adrenal medulla. Transplantation of adrenal medulla is an area which has been motivated by two ultimate goals; first, the treatment of Parkinson’s disease /41,50/, and second, the use of adrenal chromaffin cell implants for the treatment of intractable pain /117,119/. Studies related to Parkinson’s disease will be discussed in more detail below, and studies in chronic pain will be described in the concluding section on new models and applications.

A thorough review of the effects of adrenal medulla autografts, including the multi-center trial and registry data, was presented by Goetz /50/. Although positive effects of adrenal medulla grafts in patients with Parkinson’s disease have been reported without the use of any of these additional methods to enhance cell survival, the effects of these grafts are relatively modest. There were substantial improvements for up to one year, but several of the symptoms which had shown earlier improvement began to deteriorate after extended periods. Most of the beneficial effects disappeared after two years. There was, however, a significant increase in total “on” time, which remained significantly improved four years after transplantation. One unanswered question is whether the increase in “on” time represents a simple change in the temporal pattern of on-off cycling, as opposed to an improvement in function which is perceptible to the subjects only during certain periods of the on-off cycle. It is conceivable that patients interpret periods of improved function as “on” time, rather than as improved function during “off” periods.

Garcia-Flores /45/ has also conducted long-term studies of patients with adrenal medulla grafts, and reported data which were generally consistent with the results reported by Goetz /50/, notwithstanding differences in methods of evaluation. Whereas Goetz /50/ reported on overall mean changes in performance scores, Garcia-Flores /45/ concentrated on the responses to treatment in individual patients. Four-year follow-up data were available for 17 patients. Of the original 24 patients in the study, five had died and two others were lost to follow-up. Two patients showed a substantial long-term improvement; these patients were distinguished by early age at onset of the disease (24 and 41), and neither had received regular L-DOPA treatment. Parenthetically, it might be
mentioned that these characteristics are consistent with a possible diagnosis of striatonigral degeneration (see section on "substantia nigra grafts"). Four additional patients showed no improvement as compared to baseline disability, but did not show progressive deterioration, suggestive of a possible slowing in the progression of the disease. The remaining patients showed no apparent effect of the transplantation procedure, and in these patients there appeared to be a normal continuing disease progression. In addition, numerous surgical and medical complications were seen. These included some suggestions of possible deleterious effects of the adrenalectomy, such as decreased cortisol, increased incidence of diabetes mellitus, and a possible reduction in stress tolerance. In this latter study /45/, therefore, beneficial effects of adrenal medulla transplantation were detected, but substantial long-term improvement was seen only in a minority of patients, who were atypical.

In the seven published autopsies, few or no surviving chromaffin cells have been found. These clinical data taken together suggest that: (i) at least some of the effects of adrenal medulla grafts may be due to non-specific mechanisms which do not require chromaffin cell survival; (ii) technical improvements which result in increased cell survival may lead to improvements in the performance of these grafts clinically, and (iii) the high frequency of side-effects is a major problem for this procedure. Nonetheless, adrenal medulla transplantation continues to be attractive, in part because it eliminates the problem of obtaining fetal tissue.

Basic studies: Methods to improve performance

The improvement of chromaffin cell graft performance and survival was addressed in several experimental studies. One of the most consistent themes was the enhancement of chromaffin cell survival by methods which are intended to increase the delivery of NGF to chromaffin cells. These studies took several forms, involving delivery of NGF through chronic pumps /46,102,103/, co-transplantation of cells genetically modified to deliver NGF /20,107/, co-grafting of peripheral nerve or other tissues in combination with chromaffin cells /4,23,85,90,151,156/, and the development of methods to enhance penetration of NGF through the blood-brain barrier /43,51/. Some of these methods, including co-transplantation with peripheral nerve fragments, and direct delivery of NGF into the brain using mechanical pumps, are being employed in clinical trials of adrenal medulla transplantation in humans.

Cunningham et al. /20/ reported on the genetic modification of Type 1 astrocytes purified from rat cerebral cortex to constitutively express mouse $\beta$-NGF. These genetically-modified astrocytes release NGF into the medium at a rate more than 10-fold higher than comparable non-modified astrocytes. Effects of co-transplantation of these astrocytes with adrenal chromaffin cell suspensions were examined. Chromaffin cells transplanted alone resulted in survival of a mean of 221 ± 129 TH-immunoreactive cells after 10 weeks, as compared to 99 ± 5 cells in animals with adrenal medulla plus normal astrocytes, and a mean of 1168 ± 143 surviving TH-positive cells in the animals that received adrenal medulla plus NGF-modified astrocyte grafts. The chromaffin cells in the NGF-astrocyte co-graft group also showed an increased expression of a neuron-like phenotype, including a large soma and extension of TH-positive processes.

Along the same lines, Patterson and coworkers /107/ described the use of fibroblasts genetically engineered to produce NGF for co-transplantation with adrenal medulla. When adrenal chromaffin cells were transplanted into the striatum of adult rats in combination with these NGF-fibroblasts, the chromaffin cells expressed microtubule-associated protein (MAP)-2, NGF receptor, neurofilament, and TH. The cells exhibited greatly enlarged soma size and extensive process development. The number of surviving TH-positive cells was increased 2.5-fold as compared to chromaffin cells co-transplanted with normal control fibroblasts. As in other studies of NGF effects on adrenal medulla grafts, the outgrowth of processes was largely confined to within the graft.

An additional advance in the possibility of enhancing chromaffin cell survival by cotransplantation was reported by Date and coworkers /23/, based on studies which had shown that transection of peripheral nerve increases the
synthesis of NGF (e.g., /58/). Chromaffin cells were transplanted into the brain either alone, or in combination with sciatic nerve that had either not been previously transected, or that had been transected one day previously. The number of surviving chromaffin cells was increased approximately two-fold by the non-transected nerve, and increased about three-fold by the co-grafts of nerve that had been previously transected. Measurements of TH immunostaining intensity adjacent to the grafts showed that the adrenal medulla + pretransected nerve grafts increased immunostaining in the 0.3 mm x 0.3 mm segment of host striatum immediately adjacent to the grafts, but not further away. The immunostaining intensity was increased, as compared to the adrenal medulla-only animals, by both the pre-transected and non-pretransected nerve co-grafts. This represents a possible further refinement of the peripheral nerve co-grafting method, which is potentially applicable to clinical studies.

An interesting potential method for delivering NGF to the brain by conjugation to an anti-transferrin antibody was described by Granholm and coworkers /43,51/. This method could be applicable to promoting the survival of adrenal medulla grafts, in addition to other possible applications of NGF or other growth factors which require delivery to the central nervous system. The effects of NGF delivered by this method were measured by changes in the size of intraocular grafts of fetal septal tissue. The blood-brain barrier was shown, by exclusion of Evan's blue dye, to have developed in these grafts. Stain was found in blood vessels in the host iris and in the grafts, but not in the graft neuropil. Animals received either NGF alone, NGF-antibody conjugate, antibody alone, or no treatment by a single injection every two weeks. It was reported that grafts in animals treated with the NGF-antibody conjugate grew significantly larger. These grafts also expressed more NGF immunoreactivity and a larger number of choline acetyltransferase immunoreactive neurons than grafts in the controls. NGF concentrations in brain were measured, and preliminary results also suggested some elevation.

Gash /46/ discussed preliminary findings of the effects of infusion of NGF followed by autotransplantation of the adrenal medulla in an MPTP-lesioned primate. This study employed a bilateral infusion model, in which MPTP was infused separately into both carotid arteries. In contrast to the unilateral MPTP infusion, or systemic injection of MPTP in which both sides are simultaneously and similarly lesioned, the bilateral model allows for the possibility of the animal to recover from the first set of lesions before the other side is lesioned via the second carotid artery. In this first animal tested, a catheter was placed into the putamen to infuse NGF, and one week later adrenal medulla autografts were placed around the catheter. The NGF infusions caused an increase in motor activity, analogous to decreased bradykinesia, and an improvement in ratings of motor impairment. The adrenal medulla grafts did not produce a substantial additional effect. The ratings slowly deteriorated when the NGF infusions were discontinued. Thus, in this first animal, the improvement observed was related more to the NGF infusions than to the transplantation of adrenal medulla (also cf. /19/). This finding, although so far reported for only a single animal, is reminiscent of the report by Pezzoli et al. /110/ in which NGF infusions increased the efficacy of adrenal medulla grafts, but were similarly effective when combined with grafts of other types of tissue, such as adipose tissue or peripheral nerve.

An additional study on adrenal medulla grafts in primates, reported by Bakay and coworkers /4/, examined co-grafts of adrenal medulla and sural nerve, as compared to controls that received sham surgery. Following unilateral MPTP administration, Parkinson-like symptoms were evaluated by a variety of techniques. These techniques included rotational behavior and an operant assessment of movements task, in which time required for an animal to activate a touch-sensitive screen in response to a visual cue was measured. The co-grafts caused an 84% decrease in apomorphine-induced rotation, as compared to a 52% decrease in the sham-operated controls. The decreases were seen over the course of 3 months after surgery, and were stable for up to one year. Improvements in movement times were seen in the co-grafted animals and in only one of the sham-operated controls. Although this study did not compare co-
Several clinical studies addressed the issue of improving the performance of adrenal medulla grafts. One possibility, which has largely been ignored in the basic literature, is the perfusion of adrenal medulla prior to transplantation /86,87/. Perfusion of adrenal medulla prior to transplantation will remove blood cells which may promote graft rejection, and might accomplish some of the same goals as isolation of chromaffin cells prior to transplantation, as reported by Schueler and coworkers /123/. Lopez-Lozano /86,87/ described clinical studies in which patients received grafts of adrenal medulla which had been perfused with a calcium and magnesium-free buffer. Patients who had received perfused adrenal medulla grafts had been followed for up to three years, and were found to have shown improvement on the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr Scale over the course of the first seven months after transplantation. From seven months to three years after transplantation, some patients maintained the improvements, and for others the improvements were slowly lost, although the overall mean scores were fairly stable over the seven month to three year follow-up. Data reported by Lopez-Lozano /85,86/ on co-grafts of adrenal medulla with peripheral nerve suggested that the major difference from the grafts of adrenal medulla alone was that the phase of gradual improvement lasted longer, for approximately 9 - 12, instead of seven, months after surgery.

Watts and coworkers /150,151/ reported on initial results from three patients (ages 45 - 55) who had received adrenal medulla/intercostal nerve co-grafts. Patients received grafts into two sites in the caudate and one site in the putamen. Patients developed slightly increased dyskinesias, and otherwise tended to show improvements which were somewhat variable. In patient number two, a substantial improvement in relative "on" and "off" times was noted. There was no change in the third patient, who had been followed for only three months. On timed motor tests, patients number one and two were improved somewhat in pronation/supination, hand/arm movements, and a stand/walk/sit test, but only slightly improved in finger dexterity.

Data from Madrazo and coworkers /90/ on four patients, ages 41-51 years, who had received adrenal medulla and peripheral (intercostal) nerve co-grafts, were presented by Franco-Bourland. Although some improvements were seen in all patients, in three of the four the improvements in UPDRS scores were considerably less than the mean improvement seen in a series of 18 patients who had received adrenal medulla-only grafts. These improvements were said to be similar to the improvements seen in poorer responders to adrenal medulla-only grafts. Substantial improvement, similar to the mean improvement in patients with adrenal medulla-only grafts, was seen in only one patient. Taken together, the three studies /85,90, 150,151/ do not provide, at least up to the present time, strong support for the possibility that the clinical effects produced by the co-grafting method are more substantial than the changes produced after adrenal medulla grafts alone.

The final presentation, from Olson et al. /103/ presented by Hoffer, described a possible alternative method for improving the clinical effects of adrenal medulla grafts by combining the grafts with infusions of NGF. Data from three patients were presented. The first patient, who has already been described in a published paper, showed some prolonged improvements /102/. In a second patient, there were some improvements, including a dampening of "on"-"off" cycling, but these had disappeared after about six months. In a third patient, who had so far been followed for only about six weeks, there were substantial improvements in walking, standardized tests of motor function, and Brightschaft potentials on the operated side. These patients will continue to be monitored.

In addition to the possibility of increasing chromaffin cell survival and efficacy by co-grafts or NGF administration, two additional techniques to increase chromaffin cell survival were described. A very interesting study /76,123,124/ demonstrated convincingly that purified bovine chromaffin cells survived transplantation much better than a mixed...
cell preparation from bovine adrenal medulla. Bovine adrenal medulla cells were dissociated and transplanted into the striatal parenchyma of rats immunosuppressed with cyclosporin A. Purified chromaffin cells were prepared by Percoll gradient separation and differential plating. Three different cell preparations were employed: (a) The mixed preparation of bovine adrenal medullary cells; (b) the purified chromaffin cells, and (c) a reconstituted preparation, consisting of both chromaffin cells and the other medullary cells. It was found that cell survival was greatly superior in the purified chromaffin cell preparation, as compared to both the original mixed cell preparation and to the reconstituted preparation of chromaffin cells plus non-chromaffin cells.

Another technique, described by Dubach et al. /28,29/, involved the transplantation of adrenal medulla tissue in elongated "ribbons", using a method which allowed the tissue to be delivered into the brain so that the tissue was in contact with host brain along its entire length without deformation /30/. Behavioral effects of the grafts were examined in primates using a rotational behavior model /28,29/ which involved assessment of behavioral changes in individual subjects with time-series analyses. Transplantation of adrenal medulla using earlier methods, which result in poor tissue survival, did not cause significant decreases in rotational behavior. There were no improvements in behavior in the first two animals receiving adrenal medulla grafts by the ribbon method; however, in four subsequent animals which received more widely distributed ribbon grafts with longer total graft lengths, there were significant behavioral improvements. The behavioral improvements were associated with the total length of the graft ribbons. Thus, a relatively simple surgical technique which increases graft-host contact resulted in greatly improved tissue survival, reinforcing the conclusion that adrenal medulla grafts can produce behavioral changes which are related to the number and distribution of surviving chromaffin cells /29/.

Although so far preliminary results of clinical co-grafting studies are not especially promising, there are several techniques which have been described for improving the functional effects and survival of adrenal medulla grafts which could be employed clinically. These give several possible avenues for improving the performance of these grafts, perhaps even including optimizing the method by combining various methods such as improved surgical technique /28-30/, purified chromaffin cells or perfusion /86,87,123/, and co-grafting /23,156/ or possibly a method for delivery of NGF /20,46,102,107/. Such an approach might very well result in dramatically improved graft performance.

SUBSTANTIA NIGRA GRAFTS

Information presented at the meeting pertaining to substantia nigra (SN) grafts ranged from basic studies of the development of dopaminergic cells to long-term studies of clinical efficacy.

Basic studies

Several presenters reported on the effects of growth factors on in vitro characterization of the properties of human ventral mesencephalon /19,26,128/. For example, Dong et al. /26/ reported that treatment of primary cultures of human second trimester fetal mesencephalon with bFGF or with a combination of bFGF and NGF caused an increase in the number of neuron-specific enolase immunoreactive cells, with no change in the number of GFAP-positive cells, and a two-fold increase in catecholamine content. NGF alone had no effect, but potentiated the effect of bFGF.

Although the development of rodent dopaminergic cells has been studied extensively, there has been relatively little work on the development of human dopaminergic mesencephalic neurons. Silani and coworkers /128/ studied the development of human fetal ventral mesencephalon from 6 to 11 weeks. Neurite extension from TH-immunoreactive cells took place from 8 to 9 weeks; at 8 weeks in vivo the cells began to form a crescent-shaped structure; by 9 weeks the dopaminergic afferentation of the striatum began to form, and by 11 weeks most TH-positive neurons had developed processes. The ratio of neurons to GFAP-positive astroglia was approximately 0.7 to 1, and the number of TH-
positive cells comprised between 1 and 2.5% of the total neurons. Effects of various growth factors on the number of TH-positive neurons surviving and neurite extension in vitro were examined. In agreement with Dong et al. /26/ both survival and neurite extension were increased by bFGF, but not by several other growth factors. bFGF also induced glial cell proliferation, and a specific adherence between TH-positive neurons and GFAP-positive astrocytes was noted, raising the possibility that the effect of bFGF on dopaminergic neurons is mediated by an effect on glial cells.

Stromberg and coworkers /139/ reported on studies showing that the outgrowth of fibers from human mesencephalic cell grafts in rats is specific for the appropriate target brain regions. A study was described in which human fetal tissue from the SN, locus coeruleus, and arcuate nucleus was transplanted to the striatum of rats with unilateral SN lesions induced by 6OHDA. Only the SN grafts produced reductions in rotational behavior and electrophysiological inhibition of host neurons. There was a substantial ingrowth of neurites from the SN grafts into the striatum. For the locus coeruleus grafts, there was some ingrowth of fibers into the host brain, although very slight, while no ingrowth of neurites from the arcuate nucleus grafts into host striatum was seen. This study reinforces the conclusion that the effects of SN grafts are specific and are related to reaferentation of the host brain, at least in the 6-hydroxydopamine (6OHDA)-lesioned rat model.

Interesting data on the effects of SN grafts in rodent models were also presented. Hattori and coworkers /55/, for example, employed running on a variable speed treadmill to assess motor abilities following SN lesions and transplantation. Normal control rats, without lesions, were able to run at any speed up to the maximum (1800 cm/min). Lesions decreased the speed at which the animals were able to run, and SN grafts produced a partial recovery of running speed. The experience of running was found to cause increases in extracellular dopamine as measured by in vivo microdialysis. A study presented by Kondoh and Low /75/ showed that dopamine release in the striatum in animals with SN grafts is regulated by glutamate agonists in a manner similar to that seen in normal striatum, although the effects were smaller. For example, 10 μM of kainic acid induced dopamine release in normal striatum, whereas 100 μM kainic acid was required to induce dopamine release in animals with SN grafts. In normal striatum, the glutamate uptake inhibitor dihydrokainic acid induced a 21-fold increase in dopamine release, while a 3.5-fold increase was induced in animals with SN grafts.

Another study, presented by Triarhou and coworkers /145/, examined behavioral deficits in weaver mutant mice, which have deficiencies in the nigrostriatal system, as well as motor abnormalities including postural and gait instability. The weaver mutation was maintained in mice of two different strains, derived from BALB/c and C57Bl/6 mice. Behavioral abnormalities observed were different for the two mouse strains. Grafts of dissociated mesencephalic dopaminergic neurons induced behavioral improvements, which depended upon the mouse strain in which the weaver mutation was maintained. Further studies may help to elucidate the reasons for the different behavioral abnormalities and the mechanisms through which the grafts produce behavioral improvement.

Yanai and colleagues /162/ investigated rotational behavior in domestic fowl, with unilateral lesions of the avian SN equivalent induced by 6OHDA. In these animals, strong contralateral rotation was induced by apomorphine, although relatively higher dosages were used (3 mg/kg), as compared to the minimum dosages needed to induce rotation in rats (less than 0.05 mg/kg). Amphetamine did not, however, induce locomotor activation or rotation in these fowl. Following transplantation of embryonic (E7) SN equivalent into the striatum in the form of cell suspensions, rotational behavior was decreased by about 6% after one week, and by more than 90% after one month. These studies provide a model in which the effects of grafts on apomorphine-induced rotation are very large, and the authors point out that a significant advantage in the use of fowl is the availability of large numbers of embryos at the exact stage of development required.

A refinement in the traditional rotational behavior model of transplantation was described by Schallert et al. /122/, based on a method for examining the behavior of animals with unilateral
SN lesions. Animals were held, so that only the contralateral forelimb was supporting the animal's weight. It was shown that the animals were unable to initiate steps with their contralateral forelimb, but when the animal was moved forward manually, the contralateral limb was able to step to keep pace. The ipsilateral forelimb was able to step normally. Amphetamine and apomorphine both increased the stepping rate of the ipsilateral forelimb, but the contralateral forelimb was unable to initiate steps even after amphetamine or apomorphine. Thus, during rotational behavior tests when the animal is allowed to move freely, the contralateral limb makes only catch-up steps, which is the cause of the turning behavior. In amphetamine-induced rotation, the ipsilateral limb tends to make movements in the ipsilateral direction, while in apomorphine-induced rotation, the ipsilateral limb tends to make crossing movements in the contralateral direction. It was noted that, in animals with unilateral SN lesions, "The ipsilateral forelimb is capable of initiating stepping movements that have major weight shifting consequences whereas the contralateral forelimb almost exclusively makes reactive steps to regain support of a displaced center of gravity..." /122/. Thus, these behavioral findings tend to validate the rotational behavior model as an indicator of Parkinson-like akinesia or bradykinesia; nonetheless, these data suggest that measurement of the ability of the contralateral forelimb to initiate stepping movements would be an important means of assessing the potential efficacy of various therapeutic modalities in the unilaterally-lesioned rat model.

Taylor and coworkers /143/ presented data on the relationship between the initial severity of Parkinson-like symptoms induced by MPTP lesions in primates and the degree of spontaneous recovery and functional improvement induced by SN grafts. Each of 70 animals was assigned a score of "0" to "4", based on ratings and measurements of behavioral impairment following MPTP administration. The score of "0" was used for normal, untreated subjects, and "1" represented subtle deficits in object retrieval but not gross motor deficit. Scores of "2" through "4" represented increasingly severe parkinsonian motor impairment. The more severe the initial deficits, the less rapidly the animals showed spontaneous recovery. Monkeys with ratings of "1" showed spontaneous recovery within one month, while animals with scores of "4" did not recover during 5 months of assessment. Improvements after SN transplantation were observed in category "1", "2", "3", and "4" subjects. Improvements were not seen after control surgical procedures. Category "4" subjects tended to have medical complications and were difficult to maintain; SN grafts were found to increase the percentage of category "4" subjects which survived for more than three months. Thus, there was evidence of functional improvements in primates receiving SN grafts into the caudate nucleus, but control procedures including sham surgery, transplantation of cerebellum into the caudate nucleus, and transplantation of SN into the cortex did not produce improvement.

Prior studies by Brundin and coworkers /14,15/, using grafts of dissociated human mesencephalic cells, had found that tissue from human donor embryos of more than 11 weeks of fetal age were not effective when transplanted into immunosuppressed rats. In these experiments, tissue from donors of age 6.5-9 weeks was effective, while tissue from 11-19 week gestational donors was not. The cut-off age for human fetal donors must therefore be around 9 weeks for dissociated cells. It was generally assumed, however, that it would be possible to use slightly older donors, possibly 10 or 11 weeks, if solid grafts were used. This assumption was based on data suggesting that rat tissue can be approximately one day older when using solid tissue grafts /129,140/. A paper was presented by Freeman et al. /42/ in which this question was studied directly, using xenografts from human donors to immunosuppressed rat hosts. Donors ranged from 4.5 weeks (7 - 9 mm crown-rump length) to 10.5 weeks of fetal age. Data were evaluated in terms of survival of TH-immunoreactive cells and host reafferentation. It was found that dissociated cell grafts survived best when the donors were between 5 and 8 weeks of age (E34 to E56). Poor cell survival was obtained when the donors were older than 9 weeks (E65). For solid tissue grafts, however, E37 donor tissue resulted in only modest cell survival. The best cell survival was obtained with donors between 6 and 9
weeks (E43 to E65). No survival was obtained for grafts older than E72, or slightly greater than 10 weeks. These data provide the first direct comparison of solid and dissociated cell grafts in terms of age requirements for cell survival, and confirm previous data suggesting that tissue no older than 9 weeks for dissociated grafts and 10 weeks for solid grafts must be used. If anything, these data suggest that the age limit for solid tissue grafts is somewhat earlier than previously thought. It seems probable, therefore, that tissue of fetal age 11 weeks or greater is not likely to reaferent host striatum when used for clinical studies.

One issue which is a concern regarding the use of fetal SN grafts in human patients, is whether the grafts may be damaged by drugs - especially L-DOPA - which are routinely administered to patients with Parkinson's disease. Steece-Collier and coworkers /135,136/ reported that L-DOPA impairs the survival and neurite outgrowth from primary dopaminergic neurons in tissue culture. Effects of chronic L-DOPA treatment on SN grafts, made by injection of solid fragments of SN directly into the striatum, were also examined. Morphological development of the TH-immunoreactive neurons seemed to be impaired, in terms of neurite development and size of the neurons, although the number of surviving neurons was not decreased. Steece-Collier /136/ presented additional data on the effects of L-DOPA treatment on SN grafts. It was found that the effects of SN grafts on amphetamine-induced rotation, measured six weeks after transplantation, were greatly diminished in animals that had received chronic administration of 50 mg/kg of L-DOPA i.p., twice per day, as compared to saline-treated controls. The administration of L-DOPA was discontinued after six weeks. In the saline-treated controls, the decreases in rotational behavior were maintained when retested at 12 weeks. In the L-DOPA-treated animals, six weeks after withdrawal there was a non-significant reduction in rotation, although not to the level of the controls. When data from individual animals was examined, it appeared that there was a tendency for recovery in half of the animals, but the other half of the animals did not recover. Van Muiswinkel and coworkers /147/ found that, although L-DOPA treatment of dopaminergic neurons in culture induced signs of degeneration (a loss of dopamine uptake), this effect could not be duplicated by chronic administration of a D₂ receptor agonist. It thus remains a strong possibility that the efficacy of SN grafts may be impaired by L-DOPA treatment, at least under certain conditions (also cf. /10,147/). Discontinuation of the L-DOPA treatment may not invariably result in the reversal of this impairment.

There was also some consideration of methods to improve the effects of SN grafts, using animal models. Collier and coworkers /19/ described experiments showing that sciatic nerve co-grafts enhance the effects of SN grafts. Co-culturing experiments with fetal neurons, and studies of polymer-encapsulated sciatic nerve in aged animals in combination with SN grafts, suggested that sciatic nerve produces substances with trophic effects on dopaminergic neurons. Experiments by Yurek et al. /163/ examined the possibility that the efficacy of SN grafts could be enhanced by using both intranigral and intrastriatal SN grafts, and reported that animals with grafts in both regions showed a more rapid recovery than animals with grafts in only the striatum. Sladek and coworkers /131/ examined the effects of combined fetal SN and striatum in primates. Although neuronal survival was not markedly enhanced by the co-grafting procedure, there were increases in dopamine concentrations adjacent to the grafts, suggestive of a possible recovery of host dopaminergic systems. Taken together, these three experiments /19,131,163/ suggest that it may be possible to improve the efficacy of SN grafts by various procedural or surgical modifications.

Gervais and Vawter /48,148/ presented information related to the development of ethical guidelines and the unique concerns related to the transplantation of fetal tissue. One set of guidelines which may serve as a model is the cadaveric donation framework. These guidelines treat the fetus as a cadaver but do not, however, address the possible concerns or role of the mother as a donor. Additional provisions may be needed related to the special circumstances of fetal transplantation, to provide for protection of both the fetus and the mother. Details of the guidelines that have been proposed or set up for various countries were
described and compared. These guidelines, in general, tend more toward treating the mother as a living donor. A major issue is the provision for varying degrees of insulation in information transfer, regarding the separation of donors, researchers, and patients. Another issue is the possibility of modifications to the abortion procedure that would be required for tissue donation, including testing of blood and tissue. It was suggested that a complete set of guidelines for fetal tissue transplantation would include aspects of both the cadaver donor framework as well as the living organ donor framework.

Clinical trials

Data on two human patients with MPTP-induced parkinsonism who had been followed for two years after receiving fetal tissue grafts were presented by Widner /154/. Each of the two patients was transplanted with a large amount of tissue, consisting of the ventral mesencephalon from 6-7 human fetuses. Following the surgery, patient #1 was maintained on a stable dose of L-DOPA, while the dose of L-DOPA for the second patient had to be decreased by 75% due to side effects. Gait, as measured by number of steps, was substantially improved in patient #1. Patient #2 did not have a severe gait problem, and in this patient the grafts produced no changes in gait. Rigidity improved slowly over the two year observation period, and continues to show a trend toward improvement. Stop-start movement speed also showed a similar pattern of slow improvement over the course of the two year observation period. This unique study of transplantation in MPTP-induced parkinsonism may comprise an important bridge between animal and human studies. For example, interpretation is not complicated by continuing progressive degenerative changes which occur in idiopathic Parkinson's disease. Such continuing changes not only complicate the measurements of outcome, but also could conceivably result in progressive damage of the implanted tissue. Another advantage of MPTP-induced parkinsonism in humans for the study of transplantation is that the degenerative disease process present in the patients' brains may be relatively less widespread.

Three patients reported by Dymecki and coworkers /34/ received fetal tissue grafts from 11-12 week gestational donors into the head of the caudate nucleus using a method similar to the Madrazo /91/ technique but with a specially designed instrument. These patients had been followed for 30, 20, and 12 months. Improvement according to a number of parameters was observed starting 3 to 6 months after surgery and was sustained for the entire observation period. This study included assessments of motor performance using timed tasks; for example, there were improvements in foot lifting, pronation/supination, and finger dexterity of the order of 30 to 50%. Percentage of the day spent in "off" phase decreased from 55% before transplantation to 17-18% from 9 to 30 months after transplantation.

Lindvall /84/ discussed the results of fetal SN transplantation, using dissociated cell grafts, in four patients with idiopathic Parkinson’s disease. The two patients with MPTP-induced parkinsonism described by Widner (see above; /154/) were also discussed. Each patient received grafts from several fetal donors. Some of the earlier results from three of the patients have been described in prior publications /82,83/. Patients numbers 3 and 4 both received grafts into the putamen only. Patient #3 has shown significant improvement. Patient #4 especially has shown continued improvement from one to three years after transplantation; this patient now has no "off" periods. Rigidity has decreased, but this effect did not begin to appear until one year after transplantation. Gait was not improved, and tremor has not improved in any of the patients. Thus, there seem to have been some effects, such as decreased rigidity, that were not seen until more than one year after transplantation. Interestingly, the disease process seems to have continued, as measured by decreased fluorodopamine uptake in positron emission tomography (PET) scans or by the progressive deterioration in tremor and gait, even while progressive improvement was observed in other measures such as "on"-"off" periods and rigidity. These data suggest that graft function may not be adversely influenced in concert with the progression of the disease, and that SN grafts may produce therapeutically significant improvements in Parkinson’s disease.
Freed /39/ reported on clinical changes in patients that had received fetal SN grafts. The first of these patients had been followed for nearly four years. Alternate patients were immunosuppressed or not immunosuppressed. Clinical improvement was observed in 5 of 7 patients overall, most of whom received bilateral grafts into 12 to 14 sites in the putamen, with tissue obtained from a single embryo. Improvements were seen in rapid alternating movements, postural control, speech, and “on” durations, which occurred gradually over the course of several months. In the first patient, there were modest signs of clinical improvement during the first year after transplantation, although walking speed did not improve and in fact was slightly worse during the first year. Between 15 and 45 months after transplantation, however, there was a marked progressive improvement in walking speed. This may have corresponded to evidence of continued transplant development, from 18F-DOPA PET scanning, between 9 and 33 months. The slow, long-term improvement seen in the first patient is a very interesting observation.

Molina and coworkers reported on studies of transplantation of fetal mesencephalic tissue into patients with Parkinson’s disease, including long-term studies of 30 patients who had received grafts into the caudate via a transfrontal open surgical approach /94/ and four patients who had received stereotoxic implantation of dissociated cells into the caudate and putamen /93/. In the thirty patients with grafts to the caudate, there were clinical improvements, including changes in “on” and “off” periods, changes in L-DOPA dosages, and changes in UPDRS scores, which persisted for the 26 - 53 months of postsurgical follow-up. The largest changes in UPDRS scores were seen during the first three months after transplantation, following which scores were stable or showed further small improvements for up to three years /94/. The four patients with stereotoxic dissociated cell grafts had been followed for only a few months. For this procedure also, improvement was seen in several measures including, for example, rigidity scores and “on” - “off” fluctuations. Surprisingly, the improvements developed very rapidly, with considerable changes occurring over 2 - 3 months, and some improvements even after 30 days. There was no suggestion of an improvement occurring over a long period akin to that reported by Widner et al. /154/, Lindvall et al. /84/, or C. Freed et al. /39/.

Hitchcock and coworkers /61/ (also cf. /57/) reported on 12 patients that received grafts of ventral mesencephalon from human donors of 11 to 18 weeks gestational age, implanted into a single site in the right caudate nucleus. Note that the age of the donor tissue in this study appears to exceed the optimal range. The range of patient ages was from 41 to 67 years, with 10 of the 12 patients aged 53 to 60 years. All implantation procedures were performed stereotaxically, and the patients were not immunosuppressed. Using the Webster rating scale, which rates motor dysfunction, three of the 12 patients showed improvement after three months which was sustained when retested at 6 and 12 months after transplantation. The other nine patients either showed no change or showed improvement after 6 months followed by deterioration to baseline levels of performance or slightly below. Interestingly, timed tests of motor function showed clear group improvements, in some cases including improvements that were not reflected clinically. For example, in one patient, whose functioning according to the Webster scale improved but deteriorated back to baseline levels from 6 to 12 months, performance on pronation/supination remained improved. Group performance on the pronation/supination test showed a clear tendency for contralateral improvement (cf. /57/). In other experiments, patients received unilateral grafts into the putamen, the caudate and putamen, or bilaterally into the heads of both caudates. In addition, a group of patients that did not receive surgery was followed as a “control” or comparison group, for purposes of comparison with the operated patients. There was no clear indication of superiority of any of the four implantation site regimens. A particularly valuable aspect of this series of studies is that it employed videotapes which were blindly rated by independent neurologists. One patient has died; although results of the post-mortem examination were not yet complete, no TH-positive cells have been found and some TH+ fibers around the graft were seen.
The final paper of the symposium, also on transplantation of fetal SN into human subjects, was given by Redmond et al. /112/. This study had several unique aspects, which were in some respects advantages and in other respects were drawbacks. First, this study is the closest approximation to a controlled study to date. Half of the patients served as non-operated controls for one year, following which they received the same transplantation surgery as the experimental group. This does permit a direct comparison between the groups; however, as discussed above, recent data suggest possible effects which take place over the very long-term (more than one year), and the one-year crossover design would not allow these changes to be detected. Second, the tissue used was cryopreserved, which allows for donor-host separation, screening, and scheduling. Tissue was implanted into the caudate nucleus only, and all patients received immunosuppression with cyclosporin for 6 months after transplantation. Patients were videotaped performing timed motor tasks, while wearing a cap and gown to conceal whether they had received surgery or were controls. There were two series of patients; the first received unilateral implantations into the caudate nucleus from one 7-11 week gestational donor each. The second series received tissue bilaterally into the caudate nucleus from 1-3 donors of 9-11 weeks gestation. Preliminary results from patients in the first series were reported. There were improvements in the operated patients which were consistent for several tasks, including walking, pronation/supination, foot-tapping, and fist clenching. These improvements exceeded those of the controls. An interesting observation with implications for other clinical studies was that ratings using the UPDRS showed improvements in the operated patients and also in the controls; nonetheless, the improvements in the operated group were somewhat smaller. Also, the controls self-rated themselves as being considerably improved, but the objective raters did not observe as much improvement in the control group.

There was one death, which appeared to be unrelated to the experimental procedure. Histology of this brain revealed that the graft did not have surviving TH-immunoreactive cells and, moreover, that this patient had striatonigral degeneration, not Parkinson’s disease. This latter observation can be interpreted as raising an interesting caution. This diagnosis represents a minority of the population of patients with Parkinson’s disease. Nevertheless, there are several factors which may substantially increase the probability of including patients with striatonigral degeneration in clinical transplantation trials. It may be that patients with striatonigral degeneration, who are unresponsive to L-DOPA, show a relatively high probability of non-responsiveness to therapy, and a correspondingly high probability of meeting the selection criteria (severe illness, unsatisfactory response to conventional therapy) for inclusion in experimental trials. Although patients with striatonigral degeneration can be distinguished by a poor response to L-DOPA, it is often not possible to make this diagnosis reliably except by post-mortem examination (cf. /33/). Patients with striatonigral degeneration are also relatively younger than patients with Parkinson’s disease (cf. /73/). In several clinical trials, it has been considered desirable to employ relatively young patients. Several very young patients, in fact, have been employed in clinical transplantation trials. The tendency to include young patients may further increase the probability of including patients with striatonigral degeneration. Although it is not impossible that dopaminergic tissue transplants would improve function in patients with striatonigral degeneration, such an improvement, of course, would not have been predicted from the literature on transplantation in animal models.

This latter study /112/ is certainly a potential model of experimental design for future studies, in terms of the controlled design, blind ratings of quantifiable motor tasks, and consistency which was permitted due to the use of cryopreserved tissue. On the other hand, there were drawbacks to this particular study, including the relatively short (one-year) controlled evaluation period, implantation of tissue into the caudate only, and the use of donors which were in some cases of a gestational age slightly exceeding what appears to be optimal. The development of an effective transplantation methodology is bound to be a complex process which will involve improvements
in procedures and experimental design over the course of a number of clinical trials.

Several reports on transplantation in human patients have employed tissue of a gestational age which exceeds the optimum that would be expected from studies of transplantation of human fetal tissue into rat hosts. Freeman /42/ (see above) presented data which suggest that it is not likely that human tissue older than approximately 10 weeks gestational age would be effective. It is therefore conceivable that the improvement in some of the clinical studies is due to some factor other than graft survival and host brain reafferentation.

On the other hand, it is also possible that synapses are irrelevant for the clinical improvement. This could be the case even for younger tissue which does reafferent host brain and even produces synapses, since improvement has been observed in some studies using tissue more mature than what would seem to be optimal. Since the improvement to some degree (especially for the near-term effects, i.e., up to one year) seems to be generally similar for all of the transplantation techniques, perhaps reafferentation is not important. Another caveat should be pointed out as well: The conclusion that clinical transplantation will require tissue younger than 10 weeks is based largely on studies of human tissue transplantation into rats, in vitro studies and examination of human fetal tissue. Although this extrapolation is quite reasonable, the possibility that survival and development of more mature donor tissue can be observed for transplantation of human tissue into human brains, and especially into the brains of patients with Parkinson's disease where unusual trophic interactions may be present, cannot be ruled out entirely.

The one facet of clinical improvement that, so far, has been reported only for transplants using young donor tissue is the very long-term gradual improvement observed between one and three years after transplantation, by Widner et al. /154/, Lindvall et al. /84/ and C. Freed et al. /39/. Each of these studies used donor tissue younger than 10 weeks, which would be expected to be at least capable of reafferenting host brain. Nonetheless, there were other unique aspects of these studies including transplantation into the putamen. It is also notable that, in animals, the functional effects which are observed following SN grafts generally require tissue which is capable of growing new dopaminergic fibers into the host brain. A reasonable hypothesis then, regarding the clinical results, is that the relatively short-term effects, seen up to approximately one year, involve a mechanism other than reafferentation of the host brain, as generally similar effects can also be produced by several types of fetal tissue transplantation and even, at least to some extent, transplantation of other tissues, including adrenal medulla and superior cervical ganglia. A gradually-developing improvement that continues over the very long term, that is, more than one year after transplantation, may be suggestive of effects which involve the development of new connections between graft and host brain. This hypothesis, however, is far from confirmed and will require considerable additional evidence.

NEW MODELS AND NEW POTENTIAL APPLICATIONS

Nakao and associates /97/ presented data on transplantation of superior cervical ganglia (SCG) to the brain in animal models of Parkinson's disease. In rats, SCG from 2-3 day postnatal rats was grown in culture for four to six weeks. For transplantation, these cells were scraped off of the culture dishes and resuspended, but not entirely dissociated. Either SCG cells or sciatic nerve, as a control tissue, was transplanted into the brains of rats with unilateral lesions of the SN. Rotational behavior was gradually decreased over the course of 12 weeks, finally reaching a decrease of approximately 70% as compared to the control group. Excellent examples of grafts with numerous surviving catcholaminergic cells were shown using histochemical fluorescence. Wu et al. /161/ also showed that the development of kindled seizures in norepinephrine-depleted rats could be delayed by SCG grafts into the amygdala and piriform cortex. Five animals with substantially surviving grafts showed a greater than four-fold increase in the number of stimulations required to induce seizures,
while no effect was seen in another five animals with poorly-surviving grafts.

In primates, using the MPTP model, long-term improvement in three monkeys was reported. This was accompanied by a slight increase in plasma homovanillic acid /70/. Convincing evidence of long-term survival of the grafts after two years was shown using catecholamine histochemical fluorescence. In the primate studies, although improvement was reported in the animals that had received grafts, there was not a very clear difference between the experimental and control groups.

Itakura also presented data suggesting the possible use of transplantation of stellate ganglia in human patients with Parkinson's disease /69/. Eight patients, 45 to 59 years of age, received unilateral stereotaxic autografts of stellate ganglion, cut into small fragments. No L-DOPA was administered starting one week before transplantation and during the entire follow-up period of 2 to 12 months. Gradual improvements in bradykinesia and gait disturbance were reported in seven of the eight patients. From timed tests of motor function, the improvement appeared to be bilateral. There was a transient worsening of tremor in seven of the eight patients from two to four weeks after transplantation. Other complications of the surgery included probable manifestations of stellate ganglion removal. In one severely affected patient, who did not improve, it was noted that the catecholaminergic cells in the stellate ganglion were damaged.

A model presented by Sharp and coworkers /127/ approaches the traditional “replacement” model of neural tissue transplantation from a quite different perspective. Lesions of the frontal cortex induced atrophy of ventroposteromedial thalamic neurons. Within 5 hours of cortical lesions, whisker simulation was no longer able to activate thalamic neurons. Nonetheless, at this time the thalamic neurons were still intact, and cortical tetrodotoxin did not eliminate the ability of whisker stimulation to activate these thalamic neurons. These data suggest that cortical inputs to the thalamus are not directly required for whisker activation of thalamic neurons; but, following cortical removal, the synaptic connections from brainstem to thalamus lose their efficacy. The neuronal death that occurs following cortical removal is associated with thalamic hypometabolism as measured by local cerebral glucose utilization. Transplantation of fetal cerebral cortex into the sites of cortical lesions maintained thalamic glucose utilization and, in some animals, restored the ability of whisker stimulation to activate ventroposteromedial thalamus. It is suggested that the cerebral cortex provides a factor which is required for the survival of neurons in the ventroposteromedial thalamus and for the maintenance of synaptic connections from the brainstem to the thalamus. Cortical transplants may provide this trophic factor, thereby facilitating the maintenance of these brainstem-ventroposteromedial thalamus synaptic connections.

Another model of transplantation in cortical injury, described by Bermudez-Rattoni et al. /9/ examined the effects of insular cortical lesions on conditioned taste aversion. Lesions of the insular cortex impaired conditioned taste aversion learning, and transplantation of insular cortex into the lesion site produced significant recovery of taste aversion learning in insular cortex-lesioned rats. Transplantation of occipital cortex did not produce recovery. It was found that the insular cortex grafts, but not the control occipital cortex grafts, released acetylcholine in response to depolarization, suggesting a role of acetylcholine release in the behavioral recovery. Afferents from the cortical grafts were found to extend into the host thalamus and amygdala, with these connections developing over a 60-day time course, roughly paralleling the time course of behavioral recovery. Based on the hypothesized role of acetylcholine in the behavioral recovery, effects of NGF were examined. NGF was found to accelerate the time-course of behavioral recovery after insular cortex grafts, but had no effect on the long-term outcome. NGF had no effect alone or when combined with occipital cortex grafts. One interesting aspect of these experiments /9/ is that they suggest a specific neurochemical mediation (i.e., acetylcholine) of a behavioral response to cortical tissue transplantation.

A novel transplantation model described by Sortwell and Sagen /133/ involved the use of
"learned helplessness" and "forced swimming" models of depression. "Learned helplessness" describes a paradigm in which animals subjected to inescapable stress show deficits in their ability to avoid subsequent escapable stress. This deficit can be alleviated by antidepressant drugs. Sortwell and Sagen /133/ found that grafts of either adrenal medulla (to produce catecholamines) or pineal gland (to release serotonin), or a combination of adrenal medulla and pineal gland grafts in the frontal cortex prevented the learned helplessness response, but control grafts of muscle tissue had no effect. The "forced swimming" test involves the measurement of immobility induced by forced swimming in a confined enclosure, and is also used as an animal model of depression. Transplantation of adrenal medulla or pineal gland grafts, but not control grafts of sciatic nerve, were found to reduce immobility scores six to eight weeks after transplantation. Biochemical and immunohistochemical studies suggested that both the adrenal medulla and pineal gland grafts survived well. This is a new model which may suggest applications of neural tissue grafting in cortically-mediated phenomena.

Senatorov and associates /126/ suggest that functional graft-host connections may be present which cannot be activated under normal conditions. Among other receptor systems, excitatory synapses in the cerebral cortex may be mediated by the N-methyl-D-aspartate (NMDA) receptor complex, and activation of this receptor is regulated by magnesium ions. When magnesium ion concentrations are increased, greater levels of glutamate occupancy are required for ionic conductance by the channel. To determine whether graft-host connections are present which are "silent" under conditions of normal magnesium ion concentration, cerebral cortex grafts were studied using a slice preparation. Slices containing fetal cortical grafts were examined in lesion cavities and surrounding host cortex. In a medium of standard artificial cerebrospinal fluid (CSF), electrical stimulation of the host brain elicited field potentials in four of seventeen preparations. When the medium was changed to magnesium-free artificial CSF, field potentials were recorded in 10 of the 17 rats. The amplitude and duration of the evoked field potentials were increased in magnesium-free medium, for both graft responses to host stimulation and vice versa. NMDA antagonists decreased the amplitude of the evoked field potentials. These data suggest that connections between transplanted cerebral cortex and adjacent host brain may be mediated by NMDA-type glutamatergic synapses. There is also a possibility that some of these synapses are inactive under normal conditions of endogenous synaptic activation. These data would be consistent with several findings that functional effects of grafts can be enhanced by pharmacological stimulation; for example, when SN grafts are activated by amphetamine administration in animals with SN lesions (cf. /40/), or when nicotinergic drugs are employed to activate adrenal medulla grafts in pain models (cf. /118/).

Transplantation was employed in a model of Down's syndrome, using the trisomy 16 mouse. These trisomy 16 animals do not survive past late gestation, but fetal brain tissue from these animals can survive as transplants in the brains of normal mice. Hohmann and co-workers /63/ reported that these grafts did not show obvious abnormalities, other than a transient increase in the expression of amyloid precursor protein in trisomy 16 grafts as compared to controls, which was seen two weeks after transplantation but had disappeared by one month. Hohmann /63/ also observed abnormal amyloid precursor protein immunoreactivity in the CA3 region of the host hippocampus in many of the animals bearing trisomy 16 grafts. Stoll et al. /137/ also failed to find obvious signs of Alzheimer's-like degeneration in trisomy 16 grafts. On the other hand, Holtzman and colleagues /64/ observed time-dependent atrophy of cholinergic neurons within trisomy 16 grafts, using cell suspension grafts into the hippocampus. This atrophy was not a gross or obvious neuronal loss or degeneration, but was manifest as a modest but statistically significant decrease in the size of cell somata (126 µ² versus 156 µ² for the controls). The small size of the change suggests that it would not be seen unless quantitative measurements of cell soma size were used. NGF administration was found to reverse this cholinergic neuronal atrophy, in that it increased the size of all cholinergic cell
somata. The effects of NGF were not specific for trisomy 16 grafts, however, in that the size of all cholinergic neurons was increased. These papers are further described in the accompanying report by Geller /47/.

An example of the use of transplantation to study physiological regulatory mechanisms was presented by Murphy et al. /96/. These experiments involved the use of normal Wistar rats and a spontaneously hypertensive rat strain to study blood-pressure regulation. Transplantation of the antero-ventral third ventricle region of the hypothalamus, an area which has been implicated in control of blood pressure, from hypertensive to normal rats resulted in chronic blood pressure elevations in normal Wistar rats. The ability of tissue grafts to elevate blood pressure appeared to depend strongly upon donor tissue age. Hypothalamic tissue from E19-20 donors elevated blood pressure for 30 - 60 days, while tissue from younger E15-16 donors elevated blood pressure for at least five months. When the younger E15-16 donors were used, even grafts of cerebral cortex were able to induce blood pressure increases for at least four months after transplantation. These data suggest that the genetic control of blood pressure is anatomically localized, does not involve complex circuits, and that the abnormality may be "transmissable" by transplantation of relatively isolated tissue fragments. As the effect can be transferred by even cortical tissue, the defect may even be a general property of CNS from the abnormal rat strain, rather than a hypothalamic abnormality. This is an example of the use of transplantation to localize primary versus secondary control of neuronal circuit functions /96/.

Studies by several groups have shown that transplantation of the suprachiasmatic nucleus into the hypothalamus of rats with suprachiasmatic nucleus lesions, and consequently disrupted circadian rhythms, can restore circadian rhythmicity. Lehmann et al. /80/ described their experiments on this topic. It was noted that the restoration of circadian rhythmicity displays the pacemaker properties of the donor cells, demonstrating that the donor cells themselves continue to express their intrinsic pacemaker activity in the host brain. Another experiment related to functions of the suprachiasmatic nucleus was described by Aravich et al. /3/. Animals that are given voluntary access to running wheels, but are placed on a time-restricted feeding schedule, develop a disorder termed "activity-based anorexia", which involves a progressive increase in running and a substantial loss of weight as compared to rats that are placed on a restricted feeding schedule only. This syndrome is aggravated by lesions of the suprachiasmatic nucleus or continuous illumination. Immediately after receiving lesions of the suprachiasmatic nucleus, animals received transplants of rostral hypothalamus (containing the suprachiasmatic nucleus) or control grafts of fetal cerebralcortex. After 30 days, the animals were subjected to conditions designed to induce activity-based anorexia; namely, 1.5 h per day access to food and 22.5 h per day access to a running wheel. Susceptibility to activity-based anorexia, defined as a 25% weight loss, was seen in 69% of the controls but in only 31% of the animals with hypothalamic transplants. The animals with the hypothalamic transplants showed a significantly greater food intake than the animals with control grafts of cerebral cortex, and no significant difference in amount of running activity (wheel turns). The animals with hypothalamic transplants, however, tended to run more (not less, as might be expected) than the controls. This is an interesting example of the use of transplantation to examine the neural circuits involved in a model of a neuropsychiatric disorder.

An interesting technique for graft implantation into spinal cord, which might be used in a modified form in other anatomical regions such as cerebral cortex, was described by Grijalva and colleagues /53/. These investigators sought to improve graft-host adhesion by enzymatic manipulation of the site of a spinal cord lesion. Rats received spinal cord lesions by the weight-drop method, and after nine days received fetal spinal cord grafts following aspiration of necrotic tissue at the lesion site. In the experimental group, the lesion site was bathed in a solution of 0.25% collagenase and 0.1% hyaluronidase for 20 min, followed by application of 0.1 M EDTA for 30 seconds. The enzymatic
treatment did not change the total number of animals with surviving grafts, or the total amount of surviving graft tissue. There was, however, approximately a 2.7-fold greater surface of graft-host contact in the animals treated with enzyme solution. In addition to spinal cord, similar methods might be useful in other circumstances where graft-host contact is impaired by scar formation.

Weiss /114,115,152/ presented data on a method to generate cells from the CNS which express neuronal and glial properties by relatively simple manipulations in tissue culture. Cells obtained from the brain under certain conditions can be induced to proliferate in tissue culture under the stimulating influence of epidermal growth factor (EGF), generating groups of cells which they have termed “neurospheres”. These neurospheres can be propagated and will continue to divide; however, when they are subsequently grown on coated surfaces without EGF, cells from these neurospheres differentiate to form cells with both neuronal and glial properties. It might eventually be possible to use methods such as these to generate, from the human CNS, glia, neurons, or partially differentiated cells with sufficient neuron-like properties to be useful for transplantation. Although most readily generated from fetal tissue, some such cells can even be obtained from the periventricular striatum of mature animals. These studies suggest that stem cells representing neural/glial progenitor cells continue to be present even in the mature CNS to some extent. Under appropriate conditions, it may be that these cells can be induced to differentiate to form mature neurons or glia.

Possibly the most exciting new development was presented by Sagen and associates /117,119/, who discussed the possible use of adrenal medulla transplants for chronic pain. Sagen first reviewed their findings on the effects of adrenal medulla transplants in animal models of pain, including data showing that these grafts are effective in alleviating manifestations of pain, such as vocalizations and weight loss, in chronic pain models /54,120,149/. Adrenal medulla grafts were tried in terminal cancer patients with severe pain who had a prognosis of six months of less. Each patient received an allograft of 1.5 precultured adrenal medulla into the spinal cord by lumbar cisternal puncture, and immunosuppression by cyclosporin for approximately two weeks. Three patients aged 52-69 years, with carcinoma of the colon and suffering from severe pain, showed substantial improvement, consisting of a lowering of self-assessed pain scores. All three became nearly pain-free between 3 and 16 weeks after the surgery. Each of the three carcinoma patients remained pain-free thereafter, and in two cases lived for 11 to 12 months free of pain. Of the other two patients in the trial, aged 41 and 49 years, with breast carcinoma and Gardiner’s syndrome, one reported no pain relief, and only transient improvement was observed for the fifth patient. These data suggest that adrenal medulla transplantation may eventually be found to be a significant alternative or adjunct to narcotics for severe chronic pain /117,119/.

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