Displacement Bone Marrow Transplantation Can Correct Some Inborn Errors of Metabolism

John R. Hobbs

Charig Cross & Westminster Medical School, Department of Immunology, Westminster Hospital, 17 Page Street, London, SW1P 2AR, United Kingdom

The objective of displacement bone marrow transplantation (DBMT) is to obtain 100% healthy donor-type marrow which can correct an inborn error that was expressed in the bone marrow of the recipient, and at the same time endow the recipient with the immunology of the donor so that the healthy gene product will enjoy immune tolerance and not be subjected to humoral or cellular reactions by the host. These could be the major problem when gene transfer is undertaken in vivo after birth. DBMT is only applicable to about 7% of understood genetic diseases, where correction has been achieved for over 40 previously fatal genetic diseases, with partial correction for another six, but there has now been failure in at least five diseases. While Good and colleagues (1) were able first to treat a genetic disease, severe combined immune deficiency, by a simple bone marrow transplant with no induction, the need for a displacement induction was demonstrated by work done in 1970–1973 at Westminster, where bone marrow transplant was also extended to include other family (2,3) and volunteer unrelated donors (4) needed for the four out of five patients who do not have a healthy matched sibling. Bone marrow transplantation can correct genetic diseases in two main ways: (a) by replacing genetically deranged or absent blood cells [as when Steinmuller and Motulsky (5) corrected spherocytosis in an animal; or Good’s team installed helper T-cells in a boy (1)] and (b) by implanting bone marrow cells that will deliver a normal protein to the tissues of the host, as was discovered by the team of Hobbs in 1970 when they showed that the lymphocytes of a healthy brother could transfer the capacity to produce migration inhibitory factor (MIF) to the cells of an elder brother, achieved both in vitro and then in vivo by the transplant (6). It was the evolution of the latter work that led to the displacement concept and the proposal by Hobbs in 1978 to a Working Party of the European Bone Marrow Transplant organization that over 30 previously fatal genetic diseases might respond to DBMT. The introduction of busulfan for bone marrow transplant induction in mice by Santos encouraged its successful use in a boy with Hurler’s
TABLE 1. Principles to correct inborn errors by displacement bone marrow transplantation

1. The inborn error should be expressed in bone marrow stem cells.
2. The patient’s abnormal marrow should be displaced.
3. Donor marrow factory produces the normal gene product.
4. The host must be immunologically tolerant to that product.

*For enzymes, proteins, etc.*
5. Leukocyte production can be 50–300 g/day
6. Leukocytes circulate, release the component, or deliver cell to cell.
7. Defective tissues should be able to accept the component.
8. The component finds its functional site to a degree adequate to correct the defect.

disease, and the proposal was then published (7). Since then, DBMT with immunoprophylaxis has been extensively used and reviewed (8–11). The principles are summarized in Table 1, lists of the treatable diseases in Tables 2 and 3, and optimal conditions for elective DBMT in Table 4. In this chapter I outline the procedures, including immunoprophylaxis, and consider the results, always remembering that the most serious complications of infection and graft-versus-host diseases (GVHD) can cause serious morbidity (8,10) so that DBMT is confined to otherwise fatal inborn errors for which no better treatment has yet been established.

**PROCEDURE**

If optimal results are to be obtained (see Table 4), it is imperative to assess the patient for referral before irreversible damage has occurred. Such assessment is undertaken by the referring team, our own team, and when the disease is one with which our team is not familiar, by a further expert opinion. Such assessments will indicate any urgency, or permit wider donor search, and even the setting up of in vitro assays to test potential transfers and to see if any binding antibodies have been generated in the patient by previous exposures to normal components. Such antibodies can persist postgraft (12) and have been found in nine patients to date.

The final decision with regard to a donor depends on the exact status relevant to the inborn error (normal homozygotes are preferred), and a two-way mixed lymphocyte culture (MLC) result with a transformation index (TI) under 1.64 (13), which has been the most reliable parameter in over 220 of our transplants. Use of such donors has caused <7% fatal GVHD without the use of T-cell depletion [which can cause rejection rates up to 15%, or unstable partial chimerism: without 100% donor-type bone marrow, the graft can be lost any time up to 7 years later (7)]. Appropriate measures (14) are taken for any blood group differences. Full viral studies are undertaken for patient and donor to plan appropriate measures during the procedure (e.g., use of CMV-negative blood products) and experienced psychological and social workers are involved in the consultation and advice to the parents, including a home
TABLE 2. Inborn errors where displacement bone marrow transplantation can provide normal cells

<table>
<thead>
<tr>
<th>Already successfully corrected</th>
<th>Possibly correctable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular dysgenesis (leukocytes)</td>
<td>Thalassemia major (RBC)</td>
</tr>
<tr>
<td>Sex-linked (helper T + B)</td>
<td>Sickle cell (RBC)</td>
</tr>
<tr>
<td>Swiss-type autosomal recessive (T + B)</td>
<td>Spherocytosis (RBC)</td>
</tr>
<tr>
<td>SCID with cartilage-hair dysplasia (T + B)</td>
<td>Osteopetrosis (osteoclasts)</td>
</tr>
<tr>
<td>Late adenine-deaminase (new T + B)</td>
<td>Possibly correctable</td>
</tr>
<tr>
<td>Late purine nucleoside phosphorylase (new T + B)</td>
<td>Severe elliptocytosis (RBC)</td>
</tr>
<tr>
<td>Late Di George (new T)</td>
<td>Erythropoietic porphyria (RBC)</td>
</tr>
<tr>
<td>Connatal GVHD (new T)</td>
<td>Severe pyruvate kinase (RBC)</td>
</tr>
<tr>
<td>Wiskott-Aldrich (new T + B + phagocytes)</td>
<td>Other severe hemoglobinopathy (RBC)</td>
</tr>
<tr>
<td>Autosomal recessive (helper-T)</td>
<td>Homozygous G6PD deficiency (phagocytes)</td>
</tr>
<tr>
<td>Bare lymphocyte (new class II)</td>
<td>Severe myeloperoxidase (phagocytes)</td>
</tr>
<tr>
<td>Bare lymphocyte (new class I)</td>
<td>Lysozyme (phagocytes)</td>
</tr>
<tr>
<td>Bare lymphocyte (new class I + II)</td>
<td>Lipochrome histiocytosis (phagocytes)</td>
</tr>
<tr>
<td>Nezelof/Matsaniotis (T)</td>
<td>Lactoferrin (phagocytes)</td>
</tr>
<tr>
<td>Interleukin II receptor (T)</td>
<td>Strauss defect (phagocytes)</td>
</tr>
<tr>
<td>Late onset childhood SCID (T)—not AIDS</td>
<td>Tubulin (phagocytes)</td>
</tr>
<tr>
<td>Nonfunctional B (B ± T)</td>
<td>Severe myosin (phagocytes)</td>
</tr>
<tr>
<td>Adult onset (T + B)—not AIDS</td>
<td>Severe histiocytosis X (phagocytes)</td>
</tr>
<tr>
<td>Chronic granulomatous disease (phagocytes)</td>
<td>D. Miller’s reticuloendotheliosis (phagocytes)</td>
</tr>
<tr>
<td>Chediak-Higashi (phagocytes)</td>
<td>Bruton’s (B)</td>
</tr>
<tr>
<td>Kostmann, recessive (neutrophils)</td>
<td>Sporadic hypogammaglobulinemia (B)</td>
</tr>
<tr>
<td>Autosomal dominant agranulocytosis (neutrophils)</td>
<td>Isolated IgM (B)</td>
</tr>
<tr>
<td>Lazy phagocyte (phagocytes)</td>
<td>Isolated IgG (B)</td>
</tr>
<tr>
<td>Cyclic neutropenia (phagocytes)</td>
<td>Severe IgG2 (B)</td>
</tr>
<tr>
<td>Adhesive proteins (phagocytes)</td>
<td>Severe isolated IgA (B)</td>
</tr>
<tr>
<td>Diamond-Blackfan (RBC)</td>
<td>Duncan’s X-linked proliferative (T + B)</td>
</tr>
<tr>
<td></td>
<td>Severe azotemia (T + B)</td>
</tr>
</tbody>
</table>

*A review (26 papers), Correction of Certain Genetic Diseases by Transplantation; is available by sending £22 Sterling payable to Westminster Medical School Research Trust, 17 Horseferry Road, London SW1P 2AR, G.B.

visit. Only after this, at the second interview, are the parents or the child asked to give their final decision about DBMT. The present schedule of induction is outlined in Table 5 and the reasons for it are given elsewhere (10), as are those for avoiding irradiation of children (7).

Immunophylaxis

After busulfan, infusion of donor buffy coat allows presentation of any normal antigens to the recipient’s immunocompetent lymphocytes, where T-helper function must be available. If exactly 24 hours later cyclophosphamide is given, it results in the deletion of any primary immune responses that might have been made by the recipient. To reduce the risks of GVHD, cyclosporinA (CsA) begins intravenously
TABLE 3. Inborn errors where displacement bone marrow transplantation can provide a transferable component

<table>
<thead>
<tr>
<th>Already successfully corrected</th>
<th>Known inadequate correction by 100% engraftment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic mucocutaneous candidiasis (MIF)</td>
<td>GM1 gangliosidosis (acid-β-galactosidase)</td>
</tr>
<tr>
<td>Hurlers (α-iduronidase)</td>
<td>Pompe’s (acid-α-glucosidase)</td>
</tr>
<tr>
<td>Sanfilippo B (acetyl-α-glucosaminidase)</td>
<td>Niemann-Pick A (sphingomyelinase)</td>
</tr>
<tr>
<td>Gaucher’s Type III Norrbottian (cerebroside-β-glucosidase)</td>
<td>Krabbe’s (galactosylceramidase)</td>
</tr>
<tr>
<td>Gaucher’s, onset &lt; 3 years (cerebroside-β-glucosidase)</td>
<td>Faber’s lipogranulomatosis (acid ceramidase)</td>
</tr>
<tr>
<td>Gaucher’s, onset 3–16 years (cerebroside-β-glucosidase)</td>
<td></td>
</tr>
<tr>
<td>Fabry’s (α-galactosidase)</td>
<td>Possibly correctable</td>
</tr>
<tr>
<td>Refsum’s (?)</td>
<td>Scheie’s (α-iduronidase)</td>
</tr>
<tr>
<td>Immunodeficiency (γ-interferon)</td>
<td>Hurler-Scheie compound (α-iduronidase)</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy (arylsulfatase A)</td>
<td>Sanfilippo C (α-galactosaminidase-acyltransferase)</td>
</tr>
<tr>
<td>Wolman’s (acid esterase)</td>
<td>Sanfilippo D (acetyl-α-galactosaminidase-6-</td>
</tr>
<tr>
<td>Fucosidosis (fucosidase)</td>
<td>sulfatase)</td>
</tr>
<tr>
<td>(Biotinidase)</td>
<td>Mannosidosis (mannosidase)</td>
</tr>
<tr>
<td>Niemann-Pick B (sphingomyelinase)</td>
<td>Sialidosis (sialidase)</td>
</tr>
<tr>
<td>Fanconi’s (DNA-repair enzyme)</td>
<td>Mucolipidosis III (? mannose-phosphorylase)</td>
</tr>
<tr>
<td></td>
<td>Niemann-Pick D (?)</td>
</tr>
<tr>
<td>Already partially corrected</td>
<td>Maple syrup urine (leukocyte correctable)</td>
</tr>
<tr>
<td>Hunter’s (iduronidase-sulfatase)</td>
<td>Galactosemia (galactose-1-phosphate-</td>
</tr>
<tr>
<td>Sanfilippo A (heparan-sulfatase)</td>
<td>uridylyltransferase)</td>
</tr>
<tr>
<td>Morquio B (β-galactosidase)</td>
<td>Ataxia telangiectasia (DNA-repair enzyme)</td>
</tr>
<tr>
<td>Maroteaux-Lamy (arylsulfatase-B)</td>
<td>Xeroderma pigmentosa (DNA-repair enzyme)</td>
</tr>
<tr>
<td>Adrenoleukodystrophy (peroxisomal enzyme)</td>
<td>Morquio A (galactosamine-6-sulfatase)</td>
</tr>
<tr>
<td>Lesch-Nyhan (hypoxanthine-guanine-phosphoribosyltransferase)</td>
<td>Batten’s (? peroxisomal enzyme)</td>
</tr>
<tr>
<td>1-cell, mucolipidosis II (mannose processing enzyme)</td>
<td></td>
</tr>
</tbody>
</table>

* A review (26 papers). *Correction of Certain Genetic Diseases by Transplantation*; is available by sending £2.50 Sterling payable to Westminster Medical School Research Trust, 17 Horseferry Road, London SW1P 2AR, G.B.

TABLE 4. Optimal conditions when referring patients for elective displacement bone marrow transplant

1. Before complications have occurred (such as septic foci, transmitted viral diseases, transfusional sensitization, severe bony deformities, irreparable CNS damage).
2. If splenectomy is needed, with immunization planned in advance.
3. If prior enzyme therapy is tried, immunoprophylaxis should have been used (10).
4. In general, the younger the better.
5. Ideally, with a matched sibling who is a normal homozygote.
6. To a center with gnotobiotic facilities, especially for transplants from alternative donors.
### TABLE 5. Induction for displacement bone marrow transplantation

<table>
<thead>
<tr>
<th>Day</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>−11</td>
<td>To theater for Hickman line and autologous BM harvest; return to sterile laminar flow room.</td>
</tr>
<tr>
<td>−10</td>
<td>Busulfan&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>−9</td>
<td>Busulfan</td>
</tr>
<tr>
<td>−8</td>
<td>Busulfan</td>
</tr>
<tr>
<td>−7</td>
<td>Busulfan</td>
</tr>
<tr>
<td>−6</td>
<td>Donor buffy coat</td>
</tr>
<tr>
<td></td>
<td>Exactly 24 h later&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>−5</td>
<td>Cyclophosphamide&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>−4</td>
<td>Cyclophosphamide&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>−3</td>
<td>Cyclophosphamide&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>−2</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>−1</td>
<td>Day of rest</td>
</tr>
<tr>
<td>0</td>
<td>Transplant</td>
</tr>
</tbody>
</table>

<sup>a</sup> 80 mg/m<sup>2</sup> corrected to not < 4 and not > 6.

<sup>b</sup> 2 g/m<sup>2</sup> mercaptopoethane sulphonate and diuresis are used daily with the cyclophosphamide.

<sup>c</sup> T-helper function is needed here, so cyclosporin A or ATG must not be given before day −3.

on day 3, and methotrexate is usually used only where the donor is an unrelated volunteer or where problems can be anticipated. Where there is failure of engraftment, confirmed between days +14 to +21, our usual procedure is to rescue the patient with autologous marrow immediately and not to attempt regraft for at least 3 months, whereafter a second full induction has caused no problems. Should chronic GVHD occur, continuous penicillin is used postgraft until it resolves, as it can be associated with autosplenectomy.

Where possible, we routinely use intravenous CsA, carefully monitoring the free plasma level (plasma being separated at 37° within half an hour of the blood collection) and have found that a window of 70–180 ng/ml is helpful and does not cause too much hypertension or renal failure. Such intravenous CsA continues until day +20 or later if the gut has not recovered to a normal xylose absorption allowing oral doses. If a matched sibling DBMT is followed by no GVH, CsA is abandoned at 3 months but where GVHD above grade 2 has occurred, or in all transplants from mismatched or unrelated donors, CsA is continued for 1 year at least, if possible (15). Prophylactic IgG can be used but live vaccines are not permitted until full immunological recovery has been verified. The splenectomy situation is considered more fully under Gaucher's disease. In general, we expect the majority of our patients to be off all treatment within 1 year of the transplant and this is the case in >90% of our patients.
RESULTS FOR SPECIFIC DISEASES

These are listed in Tables 3 and 4, and the type of donor is indicated as MS (matched sibling), VUD (volunteer matched unrelated donor), or HS (a family donor sharing at least one full genetic haplotype).

Reticular Dysgenesis

In a known family the birth was conducted under aseptic conditions and an MS bone marrow transplant undertaken without any conditioning, to achieve full correction (16). If diagnosed after birth, it would be important to eradicate any infection (e.g., using irradiated leukocytes from one parent so that the other could be an HS donor).

Severe Combined Immune Deficiency (SCID)

The first MS bone marrow transplant, without conditioning (1), has resulted in a healthy chimera, now stable for 21 years. The donor female helper T-cells fortunately cooperate with the recipient’s remaining B-cells. In some patients this has not occurred, so where there is any doubt, it would be advisable to use DBMT to achieve all-donor cooperating cells (17). T-helper deficiency is the commonest form of SCID, and simple bone marrow transplant from HS donors, while possible, has often failed to achieve complete correction (18).

Non-Sex-Linked SCID (Swiss Type)

Bone marrow transplant without conditioning has been successful (19), but it is important to deal with existing infection, with antibiotics, intravenous IgG, and even irradiated immune T-cells (e.g., from one parent), to improve the chances of success. Emergency bone marrow transplant has produced good results in less than 60%. Of 12 patients with disseminated BCG, the only two survivors had immune T-cells immediately. Presentation with acute GVHD, transplacental or from unirradiated blood products, is serious: the graft might be possible after rescue by antilymphocyte globulin (ALG), and so on.

SCID with Cartilage Hair Dysplasia

The SCID and hair have been corrected by bone marrow transplant without conditioning (18,20). Today it is felt the cartilage disorder might have responded better to DBMT.
T-cell Deficiencies Diagnosed after Age 3 Months

Today it is important to exclude HIV infection, where reliance cannot be placed on serology: detection of the HIV genome can be achieved within 2 hours (21). At present there is no justification for the use of bone marrow transplantation to treat HIV as growing T-cells propagate the virus and over 40 failures are known. Deficiencies of adenosine deaminase, purine nucleoside phosphorylase, or of the thymus can be corrected by substitution therapy begun within 6–8 weeks of birth, but after the age of 3 months transient responses are more the rule. Since the common form of adenosinedeaminase deficiency arises by deletion of a large part of the genome (22), the normal enzyme generates antibody production to its own inactivation. DBMT is the treatment of choice, preferably correcting infection, prolonged malnutrition, treatable viral infections (HSV, etc.) and even using thymic therapy where indicated, although this can generate GVHD. If enzymes or cells are infused before DBMT, it would be important to practice immunoprophylaxis (10) to abrogate a primary immune response.

Wiskott-Aldrich Syndrome

The underlying membrane protein defect (23) indicates early DBMT (7). Immunomodulation failed to prevent bleeds and malignant transformation (24).

Autosomal Recessive Helper T-Cell Defect

The OKT4 hapten can be absent with completely normal helper function (25). Where malfunction is proven, DBMT is essential to achieve cooperating T- and B-cells, unless the donor is a perfect MS.

Bare Lymphocyte Syndrome

Defects of class I and class II expression (26) require DBMT for full correction. Donor selection can be complicated by failure to stimulate in the MLC. It is hoped molecular biology methods may be developed to assist matching.

Nonfunctional Lymphocytes

Since secondary T-cell deficiency is so common (27), total absence of the suspected cytokine deficiency and failure to induce it with immunomodulators (27) must be shown before undertaking DBMT. Our first patient (6) with candidiasis since birth did not produce migration inhibitory factor (MIF) to all immunogens tested and it was not inducible in vitro: five similar patients not given bone marrow transplants all died, confirming the seriousness. Deficiency of gamma interferon can also be so
serious (28) that the only treatment seems to be DBMT. More patients with normal numbers of T- and B-subsets are being found with serious malfunction (18). Improving knowledge and assays for cytokines and their receptors may better identify them and the best treatments. The provision of lymphocytes delivering cytokines at short range seems preferable to the complications of lifelong systemic therapies, where malaise, growth failure, and even amyloidogenesis may result. In older patients it is possible that autoimmune mechanisms are active, and where life becomes intolerable, intervention with total body irradiation (TBI) could eradicate the autoimmune process with an MS bone marrow transplant establishing normal immune function and good health.

Errors Intrinsic to Phagocytes

Bone marrow transplantation intended to replace abnormal by normal phagocytes was first undertaken in April 1973 (4) for chronic granulomatous disease and from it arose the concept of DBMT. The traditional use of TBI in animals by serendipity deleted the abnormal phagocyte stem cells of mice to cure Chediak-Higashi syndrome (29) and of dogs to correct cyclic neutropenia (30), and in the latter condition, recombinant human granulocyte colony stimulating factor (GM CSF) therapy has had successful initial responses and long-term results are awaited. In contrast, ordinary bone marrow transplant failed for lazy leukocyte syndrome (31) and though achieved after TBI, the subsequent pneumonitis killed the child (31). Kostman’s syndrome was corrected by bone marrow transplant after TBI (32) but today may be corrected by treatment with GM CSF (33), avoiding the risks of bone marrow transplant. Cyclophosphamide induction alone achieved only 30% engraftment in another form of congenital neutropenia (34), but today a trial of GM CSF should perhaps first be made before proceeding to proper DBMT. Adhesive protein deficiency also failed to respond to ordinary bone marrow transplant (35) but was successful after total body irradiation. The same sequence occurred for human Chediak-Higashi syndrome (36).

Severe Genetic Anemia

While explaining why previous failures had occurred when only cyclophosphamide was used to try to correct such conditions, it was predicted that DBMT should be successful (7), so it was a pleasure to read that thalassemia major was first successfully corrected using dimethyl busulfan added to the induction (37). The use of Bu/Cy now in over 300 children for MS DBMT has been successful in some 90% of children under 5 years, and in around 80% from 6 to 16 years (38). The alternative of continuous transfusion, despite the use of chelates to reduce iron overload, has had a mortality of 37% within 10 years (39) together with the risks of transmission of CMV, viral hepaties, and worst of all, HIV (36 children who have contracted HIV by blood transfusion for thalassemia are known to the author). Experience has
shown that tentative dosages of Bu/Cy result in unstable chimeras with regression to the disease state (40), so that full doses are indicated (41); the risk of veno-occlusive disease has been overestimated, for it rarely occurs (42). The DBMT option seems preferable to conservative management (43) and where parents have to bear the cost is much more effective. In older children who have been less well maintained the risks are higher for either DBMT or continuance, but pregraft high-dose intravenous chelate can remove much iron, and complex isoimmunization can be treated by giving Thymostimulin Serono 1 mg/kg with the buffy coat in our protocol; this recruits many of the secondary memory B-cells and encourages them to commit suicide. For such patients the choice between DBMT and continuing transfusions will be an individual decision.

Congenital spherocytosis in animals was corrected by DBMT serendipitously because total body irradiation was used (5), and now that we know the ongoing risk of splenectomy under 5 years of age (some 30% will die), a MS DBMT seems attractive. Similarly, irradiation intended for leukemia also achieved a DBMT to correct sickle cell anemia (44), which has since been treated successfully with DBMT (45). Blackfan-Diamond syndrome has also been completely corrected by DBMT (46) but may respond to cytokine treatment, where, again, long-term results are awaited. With improving characterization to identify the severe forms of elliptocytosis, pyruvate kinase deficiency, and so on, these might also be best treated by DBMT.

**Osteopetrosis**

There appear to be four different errors in animals giving rise to this syndrome, but as yet in humans only two major varieties have been identified; the infantile form (which, however, varies in rate of progress between families), which is due, at the time of writing, to an unidentified error in osteoclasts; and the late onset or benign form, due to an absence of carbonic anhydrase type II (47). It is the infantile form which justifies DBMT because bone overgrowth crushes the cranial nerves to cause the blindness and deafness and other paralyses and eventually obliterates the bone marrow to cause death. Successful parabiosis led Walker to the more practical use of bone marrow, but with spleen, to restore bone resorption in microphthalmic mice (48). In 1977 it was shown that bone marrow alone would do, and in mice displacement was not needed (49). Alas, this experiment could not be transferred to the human situation for simple bone marrow transplant (50) failed, as did a heavy induction (51). In 1979, Lamendin (52) recorded success after adding total body irradiation, a practice confirmed in Minnesota (53), but today Bu/Cy is preferred (18, 41). Mobilization of the bone postgraft demands control of the hypercalcemia. In some late patients, the bone is so dense that even with DBMT the marrow cannot get established so, ideally, it DBMT should be undertaken as early as possible, even inducing at 36 weeks of gestation; in this way the cranial nerves can usually be saved. Vitamin D (calcitriol) can delay petrosis (54) during a donor search and brave sur-
geons can enlarge the optic foramina to buy time. At diagnosis it is important to establish whether the child is blind and/or deaf, so that the parents can be fully informed and may decline a graft option. Current evidence does suggest that the osteoclast may have its own precursor in human bone marrow (55), which is fortunately transferred during the transplant. Alas, at present there is no way of transferring the osteoblast, which, of course, might correct quite a few other diseases.

Mucopolysaccharidoses

Infants presenting with swollen abdomens or hernia (56) should be screened for storage diseases, including mucopolysaccharidoses (MPS), so that diagnosis can be made before damage becomes irreversible. Where the CNS is involved with deposits, clearance beginning under the age of 2 years would permit 1 year of normally continued dendrite growth, and new connections are possible until the age of 3 years. Thereafter, rehabilitation would depend on residual pathways. Nine years' experience has shown that under elective conditions, MS DBMT can achieve over 90% good survival, except that some bone abnormalities cannot be reversed. Normal enzyme has to be delivered across the blood-brain and cartilage barriers in adequate amounts to correct the defect, and this may not always occur. While much transfer of free protein enzyme seems unlikely, in a child iduronidase rose from 0 to 4% of normal (57), and this has been confirmed in dogs, with up to 10% normal (58). Donor cells do cross the blood-brain barrier and become part of the microglia both in mice (59) and in humans (Krivit et al.; to be published). A review (57) of available evidence showed that other enzymes, such as α-N-acetylglucosaminidase (Sanfilippo B), and arylsulfatase A (metachromatic leukodystrophy) are transferred in amounts adequate to reverse neurologic lesions in the young. Some enzymes such as β-glucuronidase, catalase, and arylsulfatase B are not transferred, and the enzymes for Niemann-Pick A and GM1-gangliosidosis are not transferred in amounts adequate to reverse human disease. Sulfamidase (Sanfilippo A) can reverse early lesions, but so far, iduronate sulfatase (Hunter's syndrome) has not had good results, although the survivors were all older children. For each disease, separate studies and maneuvers will have to be undertaken, and sometimes a comparable animal model does not always transfer to the human situation [e.g., young Twitcher mice get better (59), whereas Krabbe's disease does not improve] (Krivit, personal communication). New cases would best have MS DBMT from a donor with the full normal level of circulating missing enzyme. Certainly, the IQ of many survivors is very pleasing and may be adequate for them to lead a normal life, although none have yet reached the age of 12 years.

Hurler's Disease

Hurler's disease was first treated by the Westminster team (60) and today 5 of 6 MS transplants, 3 of 3 VUD grafts, and 4 of 15 HS attempts have produced survivors who have done well. Eight of the survivors are beyond 3 years postgraft and are
attending normal schools. Those whose donors had full normal levels of enzyme showed rapid normalization of liver and spleen, clearing of corneal clouding by 3 months, and marked improvement in heart failure, so that the soft tissue results were excellent. With regard to the bones, the children stand up straight and walk normally, but the beaked vertebra at the main point of the original lumbar gibbus does not change and, so far, three of our patients have had spinal fusion across this lumbar vertebra; the growth of the spine lags somewhat behind the growth of the limbs. All retain enlarged metacarpal bones, but lose their claw hands and are able to undertake fine skilled movements. Some can flatten their fingers together in the prayer position but others (mostly whose donors were heterozygotes) cannot. Postgraft, the accelerated skull growth ceased. In a boy who died at 15 months from a pneumococcal septicemia, α-iduronidase was found at 4% of the level in normal brain after correction had been made from the hemoglobin level for the contained blood (control MPS brains from ungrafted children show no enzyme activity at all); in this patient the rib cartilage showed normalization in its pattern. However, now 8–9 years after their grafts, two of the children are suffering pains around their knee joints which is inhibiting the freedom of movement they had enjoyed; one has stopped playing football and the other no longer jumps on trampolines. As the cartilage thickens, further enzyme may not be delivered to the chondrocytes, which then use up their limited supply and revert to Hurler type. It appears that the hips have become unstable in one patient with a heterozygous donor, and another child at 9 years of age has developed a limp in one leg, despite having a homozygous normal donor. These developments some 8 years after the DBMT are a cause for concern and are being closely followed. In contrast, the membrane bones do well and the Hurler facies and nasal sinuses have normalized, apart from some thickening at the top of the nose (a cartilage bone area). Hearing must be continually assessed, using grommets, if needed, to ensure that the children can benefit from their education; most of our patients are maintaining IQs from 85 to 115, the higher values being in those grafted around 1 year of age. By computed axial tomography and magnetic resonance imaging the brains of the treated children seem vastly improved compared to untreated controls (10). Contrasting the results of all 12 survivors with the natural progress of disease in their families (who cannot all have the Scheie variant with normal IQ), DBMT is at present considered well worthwhile, but we are following the progress of the large joints in our older children, and hoping that when the epiphyses fuse the cartilage will be less thick and perhaps those bones may enjoy the benefits seen in the membrane bones.

San Filippo-B Disease

Nonidentical twin sisters who had HS DBMT (61) when just over 2 years of age have not, in the subsequent 7 years, followed the disastrous progress of their two elder brothers who were each severely affected by the age of 4 years. Their donor was heterozygous, and progress was complicated by severe GVHD and leukopenia.
One of the girls appears to have recovered almost completely from chronic GVHD, but the other is still showing effects. Both have led a reasonably normal life and go to school, but require extra assistance in their education. DBMT under the age of 3 years still seems justifiable but, of course, follow-up must continue.

**Gaucher's Diseases**

The classifications of this group are continually being revised in the light of new molecular biology. As yet, there is no evidence that the infantile acute neuronopathic form can respond to DBMT. For the mainly nonneurological other forms we prefer to adopt a classification as "fast" (symptoms before age 3 years), "medium" (symptoms 3–16 years of age), and "slow" (symptoms only after 16 years of age). This would encompass the Norrbottian (62) variety which probably has different genetic origins. Enzyme replacement evaluated in old types I and III (63) had very limited success and it has never been clear whether antibody formation occurred against the enzyme as prepared. While gene transfer has been accomplished in the test tube (64) there was no test against the mature immune system of a patient, and as yet we are unaware of any treatments with recombinant enzyme. The classical Gaucher cell is a mononuclear phagocyte, but early attempts (Hammersmith and Philadelphia) did not use displacement and failed to establish successful grafts. While total body irradiation (65) achieved successful engraftment, the response seemed slower, with longer persistence of the Gaucher cells than in our patient (66), where CAT scans (67) show rapid improvement before all the host monocytes could possibly have been replaced. Thus the idea that all that is necessary is to change the Gaucher cell for the normal phagocyte seems wrong and it is much more likely that enzyme is actually transferred from the engrafted phagocytes to their neighbors. For Gaucher cells locked in fibrous cords in the liver clearance is much slower, possibly because of delivery problems, so some Gaucher cells persist up to 2 years, whereas in the bone marrow they clear within 6 months. It is also possible that the nonuse of immunophrophylaxis (62,65) could have generated antibodies, to explain their high enzyme levels (not found in our nine cases) in the phagocytes for some months after the graft; IgG-tagged enzyme would go back into the white cells to be measured by the assay: delivery elsewhere would be impaired. Splenectomy under the age of 5 years is followed by many septicemic deaths (65,68), so we initially tried transplants, leaving spleens intact in two children with neutrophil counts above 1,000 and platelet counts above 75,000 µl⁻¹. These patients required up to 356 units of platelets, and one never achieved a neutrophil count above 50 µl⁻¹, dying subsequently of aspergillosis. The other recovered completely to normalize spleen, liver, and lung function. Three other splenectomized patients had much easier grafts (67), so four subsequent patients had elective splenectomies pregraft. Pre-splenectomy, they can be immunized with pneumococcal, meningococcal, and hemophilus vaccines, to set up memory status in B-lymphocytes and antigen-processing cells. Immunizing their donors pre-bone marrow transplant ensures that immune recipient and donor cells can
cooperate postgraft when a booster dose is given and good antibody levels are obtained. The spleen has an important role in initiating responses against capsular antigens (69), then transferred as memory cells to the bone marrow. The spleen may also have a vital role in filtering the blood (70), so that surgeons should be encouraged to try and leave some spleen. If, however, they leave too much, severe neutropenia can cause postgraft deaths, as in two patients after day +56, although donor red cells were well engrafted. Survivors should take penicillin for life, and while some strains of the offending microorganisms do become resistant, this does not seem yet to be the case for the DF2 type organisms common in cats and dogs, which are known to be able to kill post-splenectomy patients. A major problem in Gaucher’s diseases, found in all our patients in pregraft liver biopsies, was quite extensive fibrosis. The impaired liver function tests and raised IgA have normalized in all our survivors postgraft, but biopsies up to 2 years later have not shown very much improvement in the degree of fibrosis, although there has been a large amount of clearing of Gaucher cells. Nevertheless, the children have developed a marked increase in well-being (“new children” say their parents) and achieved active lifestyles. A 16-year-old girl abandoned her 2-year-old leg irons at 3 months, gave up her crutches at 4 months, and now regularly rides a bicycle. Two other girls who had pregraft hip damage now behave as if it did not exist and our orthopedic surgeon is not going to intervene until there is a better indication than a bad X-ray. This is a most rewarding disease to treat by DBMT, although long-term liver results are awaited.

**Fabry’s Disease**

Small increases in α-galactosidase A level achieved by fetal liver transplantation benefited three patients (71). DBMT could do better and confer tolerance to the enzyme for the lifetime of the patient.

**Refsum’s Disease**

Similar benefit followed fetal liver transplantation (71), so DBMT could work even from a heterozygous donor. Current treatments with aphereses and difficult dietary regimes are not satisfactory for all patients.

**Metachromatic Leukodystrophy**

The first patient to receive DBMT died before the heterozygous enzyme level could affect her progress (72), and another patient continued to progress after ordinary bone marrow transplantation (73) but did improve when DBMT was done. A further patient (74), and two others have also shown measurable improvements in their CNS functions.
Wolman’s Disease

The severe form of acid esterase deficiency shows storage visible within leukocytes, and the biochemical abnormalities were fully corrected by DBMT, but alas, the infant died on day +80 from aspergillosis (75).

Fucosidosis

Because affected fibroblasts could be cleared of deposits by adding normal leukocytes to a culture, DBMT was proposed for this disease (76). Hopefully, the human blood-brain barrier can be crossed as easily as in the dog. In dogs, DBMT achieved fucosidase brain levels as high as 48% of normal, with reversal of CNS lesions in dogs done before 4 months of age (77).

Biotinidase Deficiency

Biotin replacement therapy does not fully correct this syndrome (78), which must be correctly identified (79) from other defects which do respond to replacement therapy (80). Older descriptions (e.g., Omenn’s syndrome) are inadequate, but patients have been corrected by DBMT (18).

Niemann-Pick Disease

A patient diagnosed as ‘type B,’” who showed initial improvement after a DBMT (81), is now showing some evidence of neurological lesions. Her defect is being fully evaluated and while it still shows many features of Type B, the neurological developments suggest that the initial diagnosis will have to be changed; there may be even more variants in this group.

Fanconi’s Syndrome

The underlying defect is a failure to repair DNA, so excess somatic mutations occur throughout life which can end with neoplastic transformation; the tissues are also much more susceptible to test doses of irradiation or cyclophosphamide, whereby challenge tests enable early diagnosis to be made, before over-transfusion, and so on. Late patients with aplasia have responded to ordinary bone marrow transplants, although procarbazine is the preferred induction drug (82,83). Fatal GVHD occurred in some 60% of patients over the age of 6 years (84), but the success rate is now approaching 50% (85), and while HS donors have had poor results, two VUD bone marrow transplants have been successful (86). In our first patient (82) neoplastic epithelia around the eye and in the bladder normalized post-transplant, so the repair enzyme appears transferable.
Hunter’s Disease

There now appear to be “fast,” “medium,” and “slow” forms of this disease (87). Our two survivors had fast disease and only had their DBMT at 5 years of age, both developing severe chronic GVHD with low levels of leukocyte and enzyme. Neither has yet shown any real evidence of mental improvement, although hepatosplenomegaly has gone. It is not known whether a graft from a normal homozygote under the age of one year can achieve better results. A boy with a slow variety had a successful DBMT at 7 years of age and appears to be progressing satisfactorily (88). Assuming that random neutralization of the X-chromosome occurs for neurons, the mother’s cells unable to synthesize enzyme must obtain an adequate supply from their neighbors. It remains to be seen whether an adequate supply can cross the blood-brain barrier after early DBMT.

San Filippo A Disease

Our patient grafted at 5.4 years showed marked improvement of the systemic features, but at 5 years postgraft we are disappointed by mental deterioration and would not at present graft any patient over the age of 4 years. A French child who had DBMT at 2 years is apparently doing well.

Morquio’s Disease

Here again, severe and mild forms exist (87) with at least three distinct enzyme deficiencies. One of our patients with advanced disease did not receive buffy coat immunoprophylaxis and produced postgraft IgG that bound to the normal enzyme and greatly reduced its activity; that child had improvement only in liver and spleen (89) and finally died after dislocation of her odontoid process. Our second patient had HS DBMT from his father but developed severe chronic GVHD with leukopenia and low enzyme delivery, and again while the liver and spleen has improved, his orthopedic progress has been disappointing.

Maroteaux Lamy Syndrome

A 13-year-old patient who received DBMT for this disease (90) showed improvement in hepatosplenomegaly, corneal clouding, and lung function, but little progress in the joint deformities, her age precluding much new cartilage formation. In a 7-year-old boy, DBMT ensuring immunoprophylaxis not only produced the improvements noted above but also a clearly improved range of movement within 6 weeks of the graft. We are still assessing his further progress, but at least can detect no antibodies to arylsulfatase B.
Adrenoleucodystrophy

DBMT achieved normal circulating leukocytes in the first patient (91), who, nevertheless, died at +145 days with no reduction of the lesions. Immunoprophylaxis had not been used, so it is not known if the “peroxidation corrector” reached the required sites. Since most X-linked carriers have no lesions, those neurons unable to produce enzyme must receive it from adjacent cells. Two further patients are showing improvement after somewhat better transplants.

Lesch-Nyhan Syndrome

The original patient received an MS DBMT at 21 years of age and his metabolic disease appears completely corrected, but there has been no improvement in his psychosis in the subsequent 2 years (92). Two further attempts have been made in children under 1 year of age, but alas, early post-transplant deaths have prevented evaluation.

I-Cell Disease

Biochemical improvement has followed an MS DBMT from a heterozygote (93) and the usual downhill course has been prevented. Long-term follow-up is awaited.

GM1 Gangliosidosis

By the time the infant is born, there now appears to be extensive deposition, and DBMT at 9 months and even at 3 months from a normal homozygote have been unable to reverse the inexorable progression of the disease (94).

Pompé’s Disease

Three attempts at 5–6 months of age were followed by two deaths from heart failure and one from pneumonia (95). Before transplanting our patient, we kept samples of her voluntary and smooth muscle alive in tissue culture for 24 h, by which time the added donor leukocytes had completely cleared them of glycogen; that this happened in vivo was confirmed at postmortem, even though the graft had only just taken. We could not test heart tissue in vitro and it is not known if there are sufficient coated pits to allow adequate access of donated enzyme.

Niemann-Pick A Disease

In a mouse mode (96) and in a young infant (Kravit, personal communication) bone marrow transplantation has not been able to reverse the neurological damage present
at diagnosis, although it appears that immunophylaxis was not practiced in either case. Clearly, any antibodies that bound to donor enzyme would almost certainly prevent its entry into the central nervous system, but their postgraft presence has not been sought.

Krabbe's Disease

In the Twitcher mouse model, bone marrow transplantation, which must be before the age of 11 days, greatly improves the progress (59). As this simulates the human disease, two patients have been treated, but with very disappointing results despite full engraftment (Krivit, personal communication).

Farber's lipogranulomatosis

A boy with a severe deficiency of acid ceramidase and brain lesions had a DBMT at 18 months ago from his compatible sister, who had a normal homozygous level of enzyme. This achieved 100% donor-type engraftment with only GVH grade II. The patient nevertheless showed no improvement in the progress of his disease (Dr. G. Souillet, Lyons; to be published).

FUTURE DEVELOPMENTS

While a majority of the original diseases proposed have been corrected by a properly undertaken DBMT, Tables 2 and 3 list other diseases that might be correctable through the same principles. While a bad family history guides judgment to the seriousness, it is in those very situations that future births would best be prevented, if at all possible, by studies of chorionic villus biopsies or, indeed, amniotic biopsies. In Britain, some 80% of these diseases tend to be sporadic, with no known family history, and are not preventable. For others, prenatal diagnosis is not yet possible. Some diseases seem to be intrinsic to the B-lymphocyte and, indeed, absence of IgA has been both conferred upon a BMT recipient (97) and corrected by one of our DBMTs, just as is recorded for the atopic state (98). The severe diseases indicated can render a patient’s life quite miserable, and intravenous IgG has not prevented acquisition of serious viral infection and other complications. It is possible that cytokine deficiencies may underlie some of them and that recombinant peptide therapy may become the future choice as for Kostmann's. On the other hand, in some centers, the over 90% success rate for elective MS DBMT before complications occur may provide a better choice to the parents than more expensive alternatives. It is to be hoped that DNA probes and monoclonal antibodies will better identify those severe forms justifying DBMT, and for many of the currently suggested diseases it is possible to set up fibroblast cultures from affected patients in media without corrective factors (e.g., avoid fetal calf serum) and to compare these with what happens after
the addition of prospective donor leukocytes or plasma (99), and extending this to more relevant tissues such as brain or heart when this becomes feasible. However, just as for gene replacement therapy, DBMT must be tested in an intact animal with all its physiologic barriers and a normal immune system. It should also be remembered that some animal successes (simple bone marrow transplant in micro-ophthalmic mice, correction in Twitcher mice) have not been transferable to the human situation.

The cost-efficiency of current DBMT is excellent and, updating old estimates (7), some 100 children born each year in England and Wales could have DBMT for £1.8 M, to achieve lifelong correction for over 60 years, as against £8 M being expended before most of them die from a miserable existence that afflicts their families. Currently, most attempts at gene therapy have only been successful in the test tube (e.g., ref. 64), but have failed in whole animals, (e.g., ref. 100). It seems initially that many will be based on harvesting autologous bone marrow from an affected animal, transfecing it with the gene, and restoring it to the host; this is doomed to failure unless DBMT is undertaken, for it is very important to remove the competition of the remaining host stem cells. There is also the problem that the basic primordial stem cells seem to be turning over in such a way that only 1:8 is ever being used at any given time; this makes them harder to eradicate, as in young infants transplanted where, rarely, a 100% donor-type bone marrow has disappeared in a year due to replacement by host cells that had persisted. Nevertheless, methods are being developed for the positive selection of the earliest bone marrow stem cells which might be totally transfecable without impairing their power to displace those of the host and avoid the risks of allogeneic bone marrow transplant. There will still remain the real danger that the host’s remaining B-cells will mount an immune rejection of the new gene product. If total displacement requires an unopposed new start, there may be a return to a total body irradiation induction with its risks (7) and, of course, the always present risk of infection. It seems at present that such gene transfers would have to be undertaken with exactly the same facilities and experience that exists in those teams undertaking DBMT and initially for many of the same diseases.

Clearly, there are many factors that will influence the final decision for any given patient, that will have to be made by the parents whom we must advise as best we can. At the time of writing, the initial bone marrow transplants for genetic diseases have been extended from matched siblings (1) to other family (2) and unrelated volunteer donors (4), both to replace abnormal cells and to evolve the concept of DBMT with immunoprophylaxis (7) (first put to a European Working Party in 1978), to confer a transferable component and widen the range of previously untreatable serious genetic diseases that might be corrected.

ACKNOWLEDGMENTS

Much of this review has arisen from the work of the Westminster Children’s Bone Marrow Transplant Team (many of whom are named elsewhere [76]) who are grateful
REFERENCES

DISCUSSION

Dr. Saudubray: From a theoretical point of view, you divided inborn errors in two major categories: one in which DBMT can provide a transferable component and the other when DBMT can provide normal cells. I guess this separation is really too schematic and in the list you propose at least four disorders are not really well adapted to this classification (i.e., Refsum disease, X-linked adrenoleukodystrophy, MSUD, and galactosemia). In these four disorders, if DBMT is effective, and maybe it is effective, it is because the circulating leukocytes can clear circulating toxic components. So this is another category, which is different from the one that provides a transferable component. In these four diseases it is not the transferable component that is circulating, it is the toxic compound itself. So the problem is not the problem of quantitative calculation and if we can calculate that the production of branched-chain amino acids in MSUD can be cleared, or at least a significant amount of these amino acids can be
DISPLACEMENT BONE MARROW TRANSPLANTATION

cleared through the provision of a sufficient amount of leukocytes, this procedure will work.
I suggest in your classification that you add a third group, that is a list of the diseases where toxic compounds are circulating and can be cleared.

Dr. Hobbs: I quite accept that. I did start with that but it got too complicated, so I tried to simplify into two main groups. In adrenoleukodystrophy I do believe the evidence is that enzyme is delivered to the brain and to the peripheral nerves, because there are three American children and one French child whose lesions have regressed, as shown by MRI and also by peripheral nerve biopsy. As I can't see a white cell getting into a peripheral nerve axon and clearing it, I suggest that enzyme must have been transferred.

Dr. Van Hoof: If I did not misunderstand you, you do not give too much credit to experiments in animals. I was personally impressed by the work of Hoogebrugge et al. (1) on the mouse model of Krabbe's disease (deficiency of the lysosomal galactosylceramidase). This generalized enzyme deficiency profoundly affects the central and peripheral nervous system. Bone marrow transplantation had beneficial effects (presence of donor macrophages in the brain, which increased enzyme activity and caused some degree of remyelination) when performed between 7 and 12 days after birth, but not later. The development of mouse brain at birth is much less advanced than that of humans, and I am afraid that the period during which donor cells could reach the brain in humans would already have passed at birth.

Dr. Hobbs: I said that very often experimental results on animals have not been confirmed in the human situation. There are at least 18 conditions in which this is so. You have chosen Krabbe's disease in mice. This was beautiful work by Dr. P. Hoogerbrugge et al. and I have studied it well. If an affected twitcher mouse is transplanted before it is 11 days old, the disease progresses is largely prevented. On the basis of that work, Professor W. Krivit in America has undertaken two transplants in children with Krabbe's disease under the age of 7 months and 9 months. In a human life of 70 years, 8 months is about 1% and in a mouse life of 730 days, 11 days is about 1.5%, so the timing corresponded reasonably well. In fact, there has been a total failure to deliver any enzyme into the human central nervous system, verified at the postmortem. Although it is delivered in the mouse it is not delivered in the human. There are other situations, such as osteopetrosis, where the work in mice held up progress for 10 years. Everybody said that in osteopetrosis all you have to do is provide the cells and they will do the work themselves. There were 18 attempts to cure osteopetrosis by infusing bone marrow cells. Every one of them failed. In the human situation you have to displace a normal marrow. In a mouse you don't have to. In a mouse all you have to do is put in the cells. So mouse osteoclasts behave differently from the human ones. They just find they own way and clear the marrow. In humans you have to do a proper transplant. All I can say is that in the end, whether we like it or not, the final experiment probably has to be done in a family and the families have to understand that. We have done 18 of the first transplants for different genetic diseases and we have two discussions with the families. We have the first one where we explain what we are trying to do and what might go right or wrong. We always let them go away and think about it before we have the final discussion, where they make up their own mind. Of course, an experimental animal does not get this advantage; he does not have any chance to think about it or any chance to go away and make up his mind.

Dr. Van Hoof: Another question about the reported correction of a Wolman patient. Did you use natural or artificial substrates to measure acid lipase? Several esterases can act on artificial substrates to give a false positive result. If the presence of enzyme activity was demonstrated biochemically on a liver sample, could you exclude the possibility that this activity belonged to the white blood cells from the donor, present in this piece of tissue?

Dr. Hobbs: I only used the methods that were available when we did that transplant in 1981,
and those were the two activities that we measured in those days to make a diagnosis. I quit accept your point, but as a histopathologist who had some training I do not think you can clear cholesterol crystals with white cell enzymes, so something went into those liver cells that enabled the crystals to be cleared.

**Dr. Van Hoof:** How do you know that it was not just the antirejection drugs you gave the children that did the job?

**Dr. Hobbs:** That has been proven already. There have been liver transplants, with the same drugs being used, where the child did not get any correction of the Wolman's diarrhea and finally died, with typically diseased gut still present.

**Dr. Mowat:** Do the long-term survivors develop problems?

**Dr. Hobbs:** Some do. We have to admit that in Hurler's disease, children who have had normal physical activity for 8 years are now suffering from bone problems. Two of our oldest children have trouble with walking. This is due to problems in the hip joint, and one of them has just had an osteotomy which helped. We don't think that the problem is totally solved. We think that the correction in Hurler's disease has been well worthwhile for the first 9 years, but we still don't know what their brains will be doing when they are 12 years old. At 6–9 years of age the five survivors are all at normal school, but we have this bone problem, which may perhaps be due to the cartilage getting thicker with age and becoming more difficult to penetrate. I hope one day we may see that when the diaphyseal cartilage has disappeared from the bones the problem corrects itself. We don't have all the answers. All we can say is that the present management of Hurler's disease with a transplant is much more satisfactory than the alternative of letting them slowly die for 10–20 years.

**Dr. Mowat:** Have you been able to do any biochemical studies on the bones at 9 years old?

**Dr. Hobbs:** We have taken biopsies. The superficial cartilage is beautiful, looking just like normal cartilage. The cells line up for about the first six or seven layers. Then I guess they run out of enzyme. They probably only had 4–10% of the normal amount and when that runs out they revert to the natural diseased state. There are three areas that have been difficult to penetrate: (a) cartilage, (b) the heart in Pompe's disease (all three patients who have died after transplant still had excess glycogen in the heart whereas it had gone from the other tissues), and (c) brain (in half the conditions where this treatment has been tried, brain lesions have not been adequately corrected).

**Dr. Brodehl:** After the first displacement therapy with the cytotoxic drugs, do you need any continuous treatment with drugs?

**Dr. Hobbs:** We use cyclosporin A after a matched sibling transplant for only 6 weeks–3 months. Nobody has yet developed a proven test of immune tolerance. We are trying to and we would like this to guide us. At present most of our patients are transplanted from matched siblings, and are off all drugs by 3 months of age, except that they all have ampicillin throughout the first year. They are reassessed in subsequent years to see if they are able to make antibodies. If necessary they stay on ampicillin for much longer periods (e.g., 3–5 years)—it can take a long time to recover. If the transplant is from an unrelated volunteer or from a half-match donor, we have to continue cyclosporin A for a year, after which we measure neopterin daily. Neopterin is a very sensitive indicator of immunologic activity. If neopterin values rise, the patient goes back on cyclosporin A; if they remain low, the patient stays off the drug. But when we initially stopped cyclosporin A at 1 year without such monitoring, we lost two transplants. Thus in a nonperfect match situation you have to use cyclosporin A for about a year. All our recent children have only had it for 1 year, but some will be found who need it for longer.

**Dr. Brodehl:** What is the dose of cyclosporin?

**Dr. Hobbs:** About 20 mg/day. And it does not seem to cause any renal problems in the older
children. In the young infants this can be a very serious problem and we have sometimes have
to abandon the drug because of renal complications. Then we get into trouble.

Dr. Wang: We have done marrow transplantation to correct thalassemia. I quite appreciate
that you don’t have particular trouble with infections, especially CMV. But we have a lot of
CMV infections in Taiwan. Do you have any solutions for the improvement of early detection
of infections?

Dr. Hobbs: CMV is our biggest problem as well. Our 20 thalassemic transplants have been
largely done for the Asian population in Great Britain, and they have been very badly main-
tained: according to the Pesaro grading they would be class 3 patients. When you do class 3
patients the survival is only 60%. If you can do them at 1–2 years of age when they are class
1 patients, you can expect a 93% survival, exactly the same as in our other patients. So the
answer is that you must do them before these complications occur. The only other advice with
regard to cytomegalovirus is that the current opinion is that most of the reactivation occurs
from the infusion of CMV-positive white cells in support therapy. Some centers give CMV-
negative products to all patients, even if the mother, donor, or child is CMV positive. We
haven’t got that sophisticated and it is expensive, and in the British population CMV is quite
frequent, so there are not enough CMV-negative donors. By a new process that reduces the
remaining white cells, we give “leukocyte-poor” platelets. The dose of CMV in transfusion
support, which is the major problem in thalassemia, is thus going to be much lower and this
should lead to less trouble. We don’t have statistics but I think this will turn out to be one of
the new advances. As you know it is the thalassemics that are now the largest genetic group
being transplanted. Over 400 children have now had transplants for thalassemia and around
the world, with all the problems, the disease-free survival rate has been over 64%. In good centers
with good selection, choosing class 1 patients under elective conditions, disease-free survival
for some genetic diseases is reaching 93%. The center at Tokai university in Japan has 100%
survival from their first 24 transplants. It is really quite encouraging.

REFERENCE