A Technic of Bone Marrow Transplantation in Man

By ROBERT SCHWARTZ,* DWIJENDRA K. MISRA AND WILLIAM DAMESHEK

ALTHOUGH ATTEMPTS have been made in the past to treat certain hematologic disorders, notably aplastic anemia, by means of bone marrow infusions, their uniform lack of success led to a neglect of this procedure until recently. The current revival of interest in this form of therapy has grown out of a series of animal experiments indicating that homologous, even heterologous, bone marrow transplantation is possible, provided the graft rejection phenomenon has been circumvented. With these experiments as stimulation, intensive investigations of the use of bone marrow transplantation in the treatment of radiation injury, aplastic anemia, acute leukemia and certain malignancies are being carried out in many laboratories. An important consideration is the procurement of adequate amounts of marrow from suitable donors, together with its administration to the patient. In the course of the last two years we have utilized bone marrow transplantation in a number of clinical conditions, in the course of which our technic has become gradually modified. The purpose of this paper is to describe our present method of procurement and infusion of bone marrow in the human subject.

MATERIALS

A. A "bone marrow transplantation pack" (fig. 1) is prepared in advance and wrapped for sterilization in a steam autoclave. All glassware and needles are siliconized with SC-87 Dri-Film (G.E.). This pack contains:

1. Twenty 30 ml. syringes
2. One 10 ml. syringe for local anesthesia
3. Six 13 gage Vim-Silverman biopsy needles
4. Two 24 gage, 1/4 inch needles
5. Fifteen 50 ml. centrifuge tubes
6. Fifteen rubber stoppers for centrifuge tubes
7. Two 20 gage, 1 1/2 inch needles
8. One test tube rack to hold centrifuge tubes
9. One tissue homogenizer, ground glass, 50 ml. capacity
10. One sponge forceps
11. Two Kelly clamps
12. Two 100 ml. beakers
13. Two basins to hold skin antisepsics
14. Two 10 ml. empty test tubes

B. Heparinized Earle's solution. The suspending fluid for the marrow cells contains 15 mg. heparin (Liquaema) per 100 ml. Earle's solution (Cappel List No. 82-200)

C. One 500 ml. empty blood collecting bottle (Baxter #H-90)

D. One plasma transfer set (Baxter #R-42)

E. Solution novocaine, 2 per cent

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METHOD

The entire procedure is carried out in an operating room under aseptic precautions. The operators scrub as for any surgical procedure; gowns, caps, masks and rubber gloves are worn. The donor is premedicated with alphaprodine hydrochloride (Nisentil), 30 mg. I.M. Approximately 15 to 30 minutes later the procedure is begun by placing the donor in the lateral decubitus position with the hips flexed and the knees drawn up to the abdomen. The posterior iliac spines can be easily palpated in this position, and are found medial to the posterior iliac crests in line with the third to fourth lumbar vertebrae.

The skin of the back is washed with iodine and absolute alcohol and appropriately draped with sterile towels. After palpating the posterior iliac spines, the skin and subcutaneous tissues overlying them are infiltrated with 2 per cent novocaine. The infiltrating needle is gradually advanced, and the periosteum of the entire posterior iliac spines as well as the surrounding tissues are injected with the local anesthetic.

A Vim-Silverman needle is then introduced into the posterior iliac spines until the marrow cavity has been entered (fig. 2). The stylet is withdrawn from the needle and a 30 ml. syringe containing 5 ml. of heparinized Earle's solution is inserted into the hub of the Vim-Silverman needle. Negative pressure is created within the barrel of the syringe by rapidly pulling back its piston. As the marrow flows into the syringe, the needle is rotated about 20 degrees and is simultaneously advanced about 1/2 cm. into the marrow cavity. Approximately 5 to 10 ml. of marrow material are obtained in each position of the needle (fig. 3). The needle is steadily advanced and turned while marrow is being withdrawn until the ventral surface of the posterior iliac spine is felt. The needle is then withdrawn until its tip is just inside the

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Fig. 1.—Equipment for removal of bone marrow from donor. Each syringe contains 5 ml. heparinized Earle's solution.
periosteum of the bony cortex; its angle of direction is next turned 30 degrees from the initial puncture site and the aspiration procedure is repeated as before. The entire procedure is then repeated so that 12 sectors of each posterior iliac spine are aspirated. From 150 to 200 ml. of marrow material are obtained from each posterior iliac spine, and although there is undoubtedly a considerable admixture with blood, large numbers of gray-white marrow particles can be seen within the syringe throughout the entire procedure (fig. 4).

As each syringe is filled, an assistant transfers its contents to the tissue homogenizer and gently passes the pestle of this instrument up and down about 6 times in order to disrupt the marrow clumps (fig. 5). The marrow so processed is then transferred to the 50 ml. centrifuge tubes. After all the marrow has been aspirated and processed in the homogenizer, the contents of the centrifuge tube are transferred to the vacuum bottle by inserting the longer needle of the plasma transfer set into the test tube and its shorter end into the inlet space of the vacuum bottle (fig. 6). Care is taken to avoid dissipating the vacuum in the blood collecting bottle by applying a Kelly clamp to the plastic
tubing of the plasma transfer set during successive transfers from the centrifuge tubes. After filling the vacuum bottle, its contents are shaken and two 5 ml. aliquots are removed for routine bacteriologic culture and cell counts.

The marrow in the vacuum bottle is now ready to be transfused into the patient. This is ordinarily done by the intravenous route, although in several instances instillation of donor marrow directly into the medullary cavity of the recipient has been carried out. Preliminary experiments done with Cr³¹-labeled red blood cells have shown that after administration of these
cells via the intramedullary route they appear in the peripheral venous blood within minutes. It is not known, however, whether leukocyte precursors and megakaryocytes behave in this manner. It is possible that these cells are trapped in the reticular framework of the bone marrow and thus never appear in the peripheral blood. The marrow is given intravenously at a rate of 15 to 30 drops per minute and the entire transfusion period is usually no greater than 3 hours.

Results

The average volume of material aspirated from both marrow cavities of the posterior iliac spines varied from 300 to 400 ml., while the total nucleated cell counts ranged from 5 to 18 billion (table 1). The morphology of the marrow obtained by this technic appeared normal, as judged by examination of Wright-stained films of the aspirated material. The adequacy of the homogenizing procedure was demonstrated in all cases by the absence of large cell clumps as observed in the hemocytometer chamber during the cell counts. The aliquots removed for bacteriologic studies were sterile in each instance.
POSSIBLE COMPLICATIONS

We have seen no complications following the procedure of bone marrow donation in the two year period. Vasovagal reactions, no more severe than those following blood donation, and mild low back pain lasting a few days have occurred occasionally. No donors were incapacitated and almost all resumed their usual activities directly after the marrow aspiration procedure.

Except for an occasional mild febrile reaction, the majority of marrow recipients experienced no important sequelae. However, of approximately 100 transplantation procedures, severe hemorrhagic manifestations or serious infection occurred in four recipients. These complications, which were at least temporally related to the administration of bone marrow, subsequently proved fatal. Thus, a 13 year old boy with aplastic anemia developed a marked drop in his already low platelet count after five infusions of marrow, and shortly thereafter died of a cerebral hemorrhage. This may have been caused by a “sweeping out” of his platelets from the peripheral blood by the foreign bone marrow. Such an effect has been seen in experimental animals following the infusion of colloidal materials.7

Three patients with aplastic anemia developed severe infections shortly after marrow administration. All were markedly neutropenic and had been taking high doses of corticosteroids, usually prednisone, 100 to 150 mg. daily. Cultures of the administered marrow were sterile in each instance. Although
TECHNIC OF BONE MARROW TRANSPLANTATION IN MAN

### Table 1.—Results of Bone Marrow Aspiration from 24 Normal Donors

<table>
<thead>
<tr>
<th>Donor number</th>
<th>Volume of marrow aspirated (cc.)</th>
<th>Total number nucleated* bone marrow cells ((\times 10^6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>180</td>
<td>5.6</td>
</tr>
<tr>
<td>2.</td>
<td>200</td>
<td>5.24</td>
</tr>
<tr>
<td>3.</td>
<td>200</td>
<td>16.6</td>
</tr>
<tr>
<td>4.</td>
<td>300</td>
<td>10.8</td>
</tr>
<tr>
<td>5.</td>
<td>350</td>
<td>13.9</td>
</tr>
<tr>
<td>6.</td>
<td>300</td>
<td>14.0</td>
</tr>
<tr>
<td>7.</td>
<td>200</td>
<td>8.1</td>
</tr>
<tr>
<td>8.</td>
<td>250</td>
<td>7.6</td>
</tr>
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<td>9.</td>
<td>300</td>
<td>8.2</td>
</tr>
<tr>
<td>10.</td>
<td>200</td>
<td>6.9</td>
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<td>11.</td>
<td>250</td>
<td>6.3</td>
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<td>12.</td>
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<td>21.</td>
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<tr>
<td>22.</td>
<td>300</td>
<td>9.9</td>
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<tr>
<td>23.</td>
<td>300</td>
<td>10.0</td>
</tr>
<tr>
<td>24.</td>
<td>250</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Average: 260 Average: 10.9

* Nucleated cells counted in WBC pipette with 2% acetic acid as diluent. Total number of nucleated marrow cells = \([\text{WBC count of marrow} \text{ (in mm}^3\text{)} \times 1000 \times \text{volume of aspirated marrow}] - [\text{peripheral WBC count (in mm}^3\text{)} \times 1000 \times \text{volume aspirated marrow}]\)

It is difficult to incriminate the bone marrow infusions as contributing to the demise of these patients, the temporal sequence was striking in each case. Thus bone marrow transplantation, particularly in the patient with severe neutropenia who is taking large, even massive doses of corticosteroids, may not be without danger. Whether this is due partly to microscopic emboli in various parts of the circulation is not clear. In one patient with congenital aplastic anemia, small foci of marrow tissue were found in the lung at postmortem examination. No foci of myeloid metaplasia were found elsewhere.

**DISCUSSION**

The procedure described above has proved to be a simple and practical method of transplanting bone marrow in man. With this technic it is not necessary to expose the donor to the risks of general anesthesia; multiple sternal punctures and their attendant risks have been avoided since our yield of marrow cells from the posterior iliac spines alone has been satisfactory; the surgical removal of ribs in order to obtain marrow should no longer be necessary for these reasons. While it is true that only the first 1 to 5 ml of
aspirate are rich in marrow particles in a diagnostic marrow puncture, the constantly changing position of the Vim-Silverman needle in the present technic, with exploration of the entire marrow cavity, results in obtaining large quantities of marrow-rich particles throughout the entire procedure.

The problem of the best route of administration of bone marrow has not yet been settled. Direct intramedullary instillation of donor marrow requires a preliminary centrifugation at low speed in order to concentrate the marrow cells so that the final volume of administered material is in the range of 50 to 100 ml. Of necessity, this means of administration is more uncomfortable to the patient than the intravenous route. Despite these limitations, it is possible that this method might avoid entrapment of marrow cells in the pulmonary circulation, as almost certainly occurs with intravenous administration. It should be pointed out, however, that intravenously administered marrow appears to have the ability to settle eventually in the bone marrow of experimental animals, and there is no reason to believe that this does not occur in man.

Although actual transplantation of bone marrow in the human subject is still beset by many difficulties, it is likely that these will be overcome. The results of treatment of acute leukemia with chemotherapy or radiotherapy plus autologous, isologous or homologous bone marrow transplantation, and that of homologous marrow transplants for the treatment of aplastic anemia are sufficiently encouraging to warrant further examination of this procedure in these two usually hopeless disorders. That at least temporary chimeras have resulted from the treatment of severe radiation injury to man has now been established. Although not without risk, the treatment of leukosarcomas and other malignancies by chemotherapy or radiation plus autologous marrow reinfusion shows some promise, although the further development of more effective antitumor agents than are presently available is certainly required. In any event, with the development of adequate bone marrow transplantation procedures, potential marrow damage by antitumor drugs should no longer be considered a therapeutic roadblock. Other situations in which bone marrow transplantation may prove valuable include agammaglobulinemia and the transplantation of homologous organs, such as the kidney.

The emerging possibilities of this new therapeutic modality thus show interesting, even exciting possibilities. The mechanical aspects of the problem are amenable to simple solution. The more fundamental biologic problem of the homograft reaction, although difficult to understand and even more difficult to circumvent, should not be impossible to resolve in due time.

Summary

A method for the transplantation of bone marrow in man is described. With this technic it was possible to obtain up to 18 billion marrow cells from the posterior iliac crests. The effects of this procedure on the donor and the recipient are discussed in the light of our experience with over 100 such transplants.

Summario in Interlingua

Es desribite un metodo pro le transplantation de medulla ossee in humanos. Per medio de illo, il esseva possibile obtener usque a 18 miliardas
CELLULAS DE MEDULLA AB LE CRESTAS POSTERO-ILIAC. LE EFECTOS DEL METHODO SUPER DE DONATORE SUPER LE RECIPiente ES DISCUTITE IN LE LUMINE DE NOSTRE EXPERIENCIA CON PLUS QUE 100 TAL TRANSPLANTATIONES.

ACKNOWLEDGMENT

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REFERENCES


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