A New Method for the Preparation of Human Cadaver Bone Marrow for Transfusion


CONSIDERABLE effort has been expended on studies of bone marrow transfusion and the results have been well summarized in a number of recent reviews. It is not yet established whether the transfusion of homologous human bone marrow has any therapeutic value in the clinical management of bone marrow depression syndromes. Extensive investigation of this problem requires the availability of simple and practical methods for procurement of substantial amounts of sterile human bone marrow in a fit state for administration to patients with marrow hypoplasia. The procurement of large amounts of human bone marrow has been hampered by several factors: (1) the extensive procedures required to obtain substantial yields by multiple aspirations from living normal volunteer donors, (2) the elaborate procedures which have been used to obtain marrow from human cadavers at the time of "sterile" autopsy, and (3) the need for nonstandard equipment especially designed to accomplish, under sterile conditions, extraction of the cellular marrow from the bone, homogenization of the marrow clumps and separation of the marrow fat.

The present report describes a simple, rapid and economical method for obtaining large sterile yields of fat-free, well-dispersed hematopoietic cells from bones obtained at the time of a routine autopsy procedure.

MATERIALS AND METHODS

Cadaver source and autopsy procedure. Bones were obtained within 14 hours of death from patients aged 17 to 75 years, without history of acute or chronic infection, malignant disease or hepatitis. Autopsies were performed by whatever procedure was routine for each of the four cooperating hospitals (Barnes Hospital, Jewish Hospital, St. Luke's Hospital and St. Louis County Hospital). The pathologists were given no special instructions by the authors. As soon as the viscera were removed from the body cavities, one of the investigators proceeded to remove 3 to 10 vertebral bodies (T7-L5). The simple equipment employed for this procedure (sponge, saw blade, knife, scalpel and forceps) was sterilized between autopsies but no effort was made to carry out the bone removal with sterile precautions. Blood and fluid were removed from the paravertebral areas with a sponge. With forceps and scalpel, the muscle attachments were separated from the anterolateral surfaces of the vertebral bodies. Individual vertebral bodies were then removed by cutting through the intervertebral discs with a knife and through the lateral pedicles with a Stryker autopsy saw fitted with a spinal column saw blade. The bony defect was rectified...
Fig. 1.—Comparison of vertebral bodies before and after dissection of adherent soft tissues. Front and rear views of upper bone demonstrate presence of fat, muscle and ligamentous tabs. Lower views illustrate the relatively clean surface of a bone which is ready for immersion in 70 per cent ethyl alcohol. Note also the relatively small access to the central marrow cavity presented by the cut surfaces of the lateral pedicles.

with a simple prosthesis consisting of a wooden peg, 1 3/4 inches in diameter affixed to the sacrum and thoracic vertebra by means of two 3 1/2 inch nails. After the bones were dissected free of soft tissue so as to provide a clean boney surface (fig. 1), they were suspended for one hour in 70 per cent ethyl alcohol, rinsed twice with sterile pyrogen-free saline and transferred to the processing container.

Preparation of the marrow. The processing container consisted of a specially designed 53-inch long sterile pyrogen-free plastic bag* (fig. 2). For convenience a clamp was placed across the middle of the bag until completion of the bone compression procedure. The outer surface of the end of the bag opposite the closures was swabbed with 70 per cent alcohol and the end of the bag then cut open with sterile scissors. Two sterile 16-gauge stainless steel plates (6 x 3 inches), their inner surfaces covered with stainless steel screen mesh, were then introduced into the bag, followed by the marrow suspending solution. The purpose of the wire screening over the inner aspect of the steel plates was to prevent the bone from slipping out from between the plates when pressure was applied. The suspending solution consisted of 200 ml. of sterile pyrogen-free saline containing 0.1 per cent ethylene diamine tetracetic acid (EDTA) plus 50 ml. of 25 per cent salt-poor human albumin solution.† Freshly prepared penicillin and streptomycin were added to provide final concentrations of 80 units per ml. and 0.08 Gm. per ml. respectively. The saline-rinsed bones were then introduced and the end of the bag was tightly closed by means of a Pyar clamp. Individual bones were manipulated between the steel plates, and

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*Provided through the courtesy of Fenwal Laboratories, Somerville, N. J.
†Kindly made available by the St. Louis Chapter of the American National Red Cross.
Fig. 2.—Fifty-three inch long sterile pyrogen-free plastic container within which the surface-sterilized bones are compressed and the extruded marrow is homogenized and filtered.

the marrow was squeezed out into the fluid suspending medium by compressing the plates in a vise (see inset on left, fig. 2). A single layer of soft flannel over the outside of the bag protected against puncture of the plastic during the application of pressure. After all the bones had been compressed, the clamp across the middle of the bag was temporarily released and the marrow suspension was allowed to flow into the lower segment of the bag, the plates and compressed bones remaining in the upper segment. The air was then forced into the upper segment and the clamp again closed across the bag, approximately 20 inches from the outlets. Homogenization of the marrow particles was accomplished by massaging the bag with a grooved foam rubber roller (fashioned from a wall paint applicator; see inset on right, fig. 2). The marrow suspension was then allowed to flow through a conventional transfusion filter* into two dry 150 ml. bags† which were centrifuged in the inverted position for 10 minutes at 1200 RPM in an International Refrigerated

*Recipient Set, Cat. No. HB-92D, Fenwal Laboratories, Somerville, N. J.
†Transfer pack unit, Cat. No. TA-6, Fenwal Laboratories, Somerville, N. J.
Fig. 3.—Separation of the marrow fat accomplished by clamping across the 150 ml. plastic bag after light centrifugation. The substantial amount of fat extruded by the bone compression procedure is clearly shown above the clamp. The cellular marrow appears as a gray layer constituting the lower third of the bag’s contents below the clamp.

Centrifuge, model PR-1. The fat was separated by placing a Pyar clamp across the bag (fig. 3), and the marrow suspensions from the two bags were combined in a dry 300 ml. bag.* After thorough mixing to insure even resuspension of the cellular marrow, 45 ml. was removed for culture and cell counts. The remainder was stored at +5 C. until transfused or discarded.

Estimation of marrow yield. The volume of fat-free marrow was determined from the weight of the marrow suspension prior to removal of aliquots for culture and cell count, without correction for the specific gravity of the marrow suspension. The nucleated marrow cells were counted by the standard white blood cell counting method except that the marrow sample was diluted 1:200 in a conventional red cell diluting pipette. Counts were generally based on enumeration of more than 500 cells.

Bacteriologic studies. Approximately 10 ml. of marrow suspension was inoculated into

*Transfer pack unit, Cat. No. TA-5, Fenwal Laboratories, Somerville, N. J.
NEW METHOD FOR PREPARING HUMAN CADAVER BONE MARROW

each of three flasks containing 85 ml. of culture media as follows: (a) nutrient broth to which penicillin and streptomycin were added to equal the concentrations in the marrow suspension, (b) thioglycollate broth with added penicillin and streptomycin as above, (c) thioglycollate broth to which was added 100 units of penicillinase; in addition, (d) 1 ml. of marrow suspension was diluted 1/10, 1/100, 1/1000 and 1/10,000 in nutrient broth containing penicillin and streptomycin as above. Also, 0.5 ml. of marrow suspension was inoculated directly on a blood agar plate, on a MacConkey agar plate and on a Sabouraud's dextrose agar slant. Five-tenths ml. of the 1/10 dilution of marrow suspension was also inoculated on blood agar and MacConkey's media. On the following day samples from a, b, c, and d 1/10 were subcultured on blood agar and MacConkey plates. Flasks and plates were incubated at 37 C. and observed after 24 and 48 hours. The Sabouraud's slant was incubated at 25 C. and observed for at least 6 weeks.

Pyrogen test. On the day following marrow procurement, approximately 15 ml. of the marrow suspension which had been stored at 5 C. was expressed from the marrow container and placed in a sterile pyrogen-free centrifuge tube. The tube was centrifuged for 25 minutes at 2000 RPM, to assure sedimentation of the particulate cellular material. Six ml. of the supernatant fluid was injected rapidly into the marginal ear vein of a white New Zealand rabbit weighing 2.5-3.5 Kg. The rabbit's temperature was recorded immediately prior to injection and at hourly intervals thereafter for three hours, using a Telemometer, with the probe inserted at least six inches inside the rectum. The rabbit was kept unrestrained in his cage between temperature readings. Tests were considered positive only if the temperature rose more than 1 F. above the baseline pre-injection level.

Recipients. The recipients of bone marrow transfusions were patients at Barnes Hospital or on the Washington University service at St. Louis City Hospital. The marrow to be transfused was of the same ABO and Rh groups as the recipient, but no effort was made to cross-match the recipient's serum against the marrow suspension.

Transfusion procedure. Baseline values were obtained for the recipient's oral temperature, pulse and blood pressure. The marrow was transfused through a conventional blood transfusion administration set at a rate of 2.5 ml. per minute. The recipient was observed continuously for symptoms throughout the administration of the transfusion and for the succeeding 30-60 minutes. Oral temperature, pulse and blood pressure were recorded at 15 minute intervals during administration of the transfusion, and at 30 and 60 minutes following completion of the transfusion. Thereafter, the oral temperature was recorded at hourly intervals for five hours. Because the recipients' control white blood cell counts were generally abnormal (e.g., leukemia, aplastic anemia) no attempt was made to follow alterations in the blood count immediately following the marrow transfusion.

Leukoagglutinins were tested as described by Brittingham and Chaplin.

RESULTS

Procurement of marrow by bone compression. The efficiency of compression as a means of extracting cellular marrow from the honeycomb of cancellous bone is illustrated in figure 4, where the "dry" marrow cavity of the compressed fourth lumbar vertebral body is compared with the "wet" cut surfaces of the bisected third lumbar vertebra from the same cadaver. The procedure does not appear to cause serious gross morphologic damage to the hematopoietic cells. Figure 5 illustrates a Wright's stain of marrow sampled at the completion of processing, 10 hours post-mortem; despite some degree of autolysis, a megakaryocyte, normoblasts, lymphocytes and a spectrum polymorphonuclear leukocyte precursors are readily identifiable.

Yield of marrow cells. Considerable variation was encountered in the yields of nucleated

*SchenLabs Pharmaceuticals, Inc., New York, N. Y.
†Yellow Springs Instruments Co., Inc.
‡Recipient Set, Cat. No. HB-92D, Fenwal Laboratories, Somerville, N. J.
Fig. 4.—The "dry" interior of a 4th lumbar vertebra after compression (on right) compared to the "wet" cut surfaces of 3rd lumbar vertebra (on left). Both bones are from the same cadaver. After compression, the flattened 4th lumbar vertebra was torn open to expose the marrow activity.

Fig. 5.—Wright's stain of marrow at completion of processing, approximately 10 hours postmortem. Some autolysis is evident but most of the cells can be readily identified: e.g., megakaryocyte, $mk$; normoblast, $n$; lymphocyte, $ly$; eosinophile, $e$; myelocyte, $my$; metamyelocyte, $mm$. 
marrow cells that could be obtained from different autopsy subjects. The subject's physical stature and age were of importance, higher yields correlating in general with larger vertebral bodies, and lower yields being obtained from subjects over 70 years of age, when bone compression secondary to osteoporosis was often prominent and when marrow fat was frequently greatly increased. Observations on the yields from 14 individual vertebral bones were made during the developmental phase of the investigation. The results are summarized in the upper part of table 1. Approximate equivalent yields may be expected from each of the eight lower vertebral bodies (T10-L5). The results of processing multiple vertebral bodies are summarized in the lower part of the table. As the authors gained experience with the procedure, and acquired a more efficient vise, increasingly higher yields were obtained (e.g., 80 x 10⁶ cells/9 bones, 59 x 10⁶ cells/9 bones and 73 x 10⁶ cells/7 bones).

Sterility of marrow suspensions. The results of sterility tests are summarized in table 2. Sixteen cadavers were considered “suitable” in terms of freedom from gross autopsy evidence of bacterial infectious disease; the marrow from 11 of these was sterile, and from three additional cases was almost certainly sterile (see footnote to table). Two marrow suspensions were contaminated by gram-negative rods, as manifested by growth confined to the thioglycollate flasks containing penicillinase. By contrast, five additional cadavers were considered “probably unsuitable” because of gross autopsy evidence of localized infection; e.g., extensive bronchopneumonia, unsuspected subacute bacterial endocarditis, pyelonephritis, unhealed amputation stump. Marrow suspensions from all five of these cadavers showed evidence of bacterial contamination, generally a mixed flora of gram-negative rods and gram-positive micrococci.

Table 1.—Yields of Nucleated Marrow Cells from Single and Multiple Vertebral Bodies

<table>
<thead>
<tr>
<th>Single Vertebral Bodies</th>
<th>Number of Observations</th>
<th>Nucleated Cells x 10⁶</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5th Thoracic</td>
<td>1</td>
<td>—</td>
<td>4.0</td>
</tr>
<tr>
<td>6th Thoracic</td>
<td>1</td>
<td>—</td>
<td>6.0</td>
</tr>
<tr>
<td>8th Thoracic</td>
<td>1</td>
<td>—</td>
<td>6.4</td>
</tr>
<tr>
<td>10th Thoracic</td>
<td>2</td>
<td>9.0–12.9</td>
<td>11.0</td>
</tr>
<tr>
<td>1st Lumbar</td>
<td>2</td>
<td>10.5–11.0</td>
<td>10.8</td>
</tr>
<tr>
<td>2nd Lumbar</td>
<td>1</td>
<td>—</td>
<td>13.7</td>
</tr>
<tr>
<td>3rd Lumbar</td>
<td>3</td>
<td>7.5–11.9</td>
<td>9.3</td>
</tr>
<tr>
<td>4th Lumbar</td>
<td>3</td>
<td>9.6–12.5</td>
<td>11.1</td>
</tr>
<tr>
<td>Multiple Thoracolumbar Bodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 to 6 Bones</td>
<td>10</td>
<td>15.0–49.0</td>
<td>31.2</td>
</tr>
<tr>
<td>7 to 10 Bones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>15.0–80.0</td>
<td>47.0</td>
</tr>
<tr>
<td>Employing new press</td>
<td>5</td>
<td>23.5–50.0</td>
<td>54.7</td>
</tr>
</tbody>
</table>

Table 2.—Results of Sterility Tests on Marrow Preparations

<table>
<thead>
<tr>
<th>Number of Observations</th>
<th>Nucleated Cells x 10⁶</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadavers considered suitable</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Sterile</td>
<td>11</td>
<td>70</td>
</tr>
<tr>
<td>Probably sterile*</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Unsterile</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Cadavers considered probably unsuitable</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Unsterile</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

*19 of 20 cultures sterile; 1–5 colonies on one plate, considered probable contaminant.
**Transfusion studies.** Thirteen cadaver bone marrow transfusions have been administered and have been well tolerated by the recipients (table 3). Mild symptoms related to the transfusion were noted on only three occasions. One patient (E. S.) with a history of multiple food allergies noted mild itching and the appearance of five small urticarial wheals, all of which disappeared within 20 minutes of onset. A second patient (C. B.), who had received multiple blood transfusions and gave a history of febrile transfusion reactions, experienced a moderate chill approximately 50 minutes after the transfusion was begun, followed by a 2.7 F. rise in temperature over the ensuing three hours. The patient's serum gave a strongly positive test for leukoagglutinins, a finding frequently associated with febrile transfusion reactions. A third patient (R. H.) experienced a slight chill followed by a 2.7 F. temperature elevation; her serum lacked leukoagglutinins and although the original pyrogen test had been considered negative, a repeat pyrogen test performed on the marrow residue was positive. On five occasions asymptomatic temperature elevations (0.5–2.2 F.) were observed, the peak occurring 3–4 hours after commencing the transfusion. It should be emphasized that 10 of the 13 transfusions were accompanied by absolutely no symptoms and that on six occasions the recipient's temperature remained normal during six hours of careful observation following the transfusions.

Transfusions number 1–7 were administered largely for the purpose of gaining clinical experience with the procedure and without any special therapeutic intent. Recipient A. H., with aplastic anemia, received a single transfusion (No. 8) containing a small number of cells. There has been no evidence of hematologic remission during the six months that have elapsed since he received the transfusion. Recipient R. H. has received five transfusions over a five month period and is presently in a partial clinical and hematologic remission of her aplastic anemia. It is of interest that she received a total of 124 x 10⁶ nucleated marrow cells over a period of six months without exhibiting evidence of increasing intolerance to the marrow transfusions and without developing a leukoagglutinin in her serum tested repeatedly throughout the course of the study.

Two of the recipients (C. B. and J. W.) died of complications of their underlying disease two weeks and two months respectively after receiving marrow transfusions. Careful review of the autopsy sections revealed no evidence of a deleterious effect from the marrow transfusions; in particular, sections of the lungs in each case revealed no evidence of marrow or fat emboli, nor was there any granulomatous reaction.

**DISCUSSION**

In order to establish conclusively whether or not the transfusion of homologous human bone marrow has any therapeutic value, there must be available

<table>
<thead>
<tr>
<th>No.</th>
<th>Recip.</th>
<th>Recipient's Diagnosis</th>
<th>Date of Transfusion</th>
<th>Nuc. Cells Transfused x 10⁶</th>
<th>Symptoms</th>
<th>Temp. Elev. °F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. S.</td>
<td>Lymphosarcoma</td>
<td>10-8-59</td>
<td>15</td>
<td>Mild urticaria</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>W. R.</td>
<td>Chr. Lymph. Leuk.</td>
<td>10-14-59</td>
<td>24</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>L. G.</td>
<td>Anemia, Heart Fail.</td>
<td>11-19-59</td>
<td>26</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>A. C.</td>
<td>Ca Bladder</td>
<td>12-9-59</td>
<td>8</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>C. H.</td>
<td>Thrombocytopenia</td>
<td>12-17-59</td>
<td>16</td>
<td>Mod. Chill</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>J. W.</td>
<td>Chron. Myel. Leuk.</td>
<td>1-6-60</td>
<td>24</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Chron. Myel. Leuk.</td>
<td>1-8-60</td>
<td>15</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>A. H.</td>
<td>Aplastic Anemia</td>
<td>1-22-60</td>
<td>12</td>
<td>None</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>R. H.</td>
<td>Aplastic Anemia</td>
<td>1-28-60</td>
<td>15</td>
<td>None</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Aplastic Anemia</td>
<td>2-27-60</td>
<td>25</td>
<td>Slight chill</td>
<td>2.7</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>Aplastic Anemia</td>
<td>4-3-60</td>
<td>25</td>
<td>None</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>Aplastic Anemia</td>
<td>5-4-60</td>
<td>46</td>
<td>None</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>Aplastic Anemia</td>
<td>6-17-60</td>
<td>13</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*Strong leukoagglutinin in patient's plasma.
†Repeat rabbit pyrogen test was positive.
a sufficient supply of transfusible marrow to carry out extensive clinical trials. The present authors believe that the following practical considerations are germane to the problem: (a) high yields of nucleated marrow cells can best be obtained from adult cadaver sources, (b) the opportunity to procure cadaver bone marrow will be enormously expanded if the source of marrow can be obtained at the time of a routine postmortem examination, without requiring the prosector to carry out any new or special procedures, and (c) the procedures for bone marrow procurement and processing should be simplified as much as possible and should employ easily available and economical equipment. The present investigation was designed with the above considerations in mind.

Since the exposed surfaces of bones procured in the course of a routine autopsy are grossly contaminated, a method for surface sterilization of the bones must be employed if the bones are to serve as a source of hematopoietic cells for transfusion. The vertebral body is especially well suited as a source of cadaver marrow: it is known to be rich in marrow cells, it is readily accessible at autopsy, and it is well designed for surface sterilization, since the only access to the marrow cavity is through the cut surfaces of the lateral pedicles (fig. 2, on the right). It was gratifying therefore to find that it was possible to immerse vertebral bodies in 70 per cent ethyl alcohol for at least one hour without penetration of the alcohol inside the marrow cavity with consequent damage to the contained hematopoietic cells. The results of sterility tests were generally encouraging (table 2). It was the authors' impression that contamination of the final product was more closely related to the cadaver's "suitability" in terms of freedom from gross autopsy evidence of infection than to the time elapsed between death and the performance of the autopsy (mean post-mortem time for 7 contaminated marrow suspensions was 5.2 hours, for 14 sterile suspensions 7.7 hours). However, it may well be that the frequency of sterile yields could be further increased (a) if the prosectors modified their autopsy procedure slightly to minimize gross surface contamination of the spinal column, (b) if the sterilizing solution were modified to enhance its bactericidal properties, and (c) if the time between death and post-mortem examination were considerably shortened.

With the exception of the elongated plastic bag (fig. 1), all of the equipment employed in the present method for marrow procurement and processing was of simple design, economical and readily available from commercial sources. A new mold was not required for fabrication of the long plastic bag since the bag is of standard width, with standard outlets. Originally, an ordinary wood vise was used for compression of the bones, but the authors ultimately found it more convenient and effective to employ a small arbor press located in the hospital's sheet metal shop. The time required for the entire procedure, including bone procurement, surface sterilization, bone compression, marrow homogenization and filtration and separation of marrow fat, was approximately three hours and could have been appreciably shortened if desired.

Since the bone marrow cavity resembles a sponge, it seemed logical to hope that extrusion of marrow by compression would be both simpler and more
efficient than attempting to float the marrow free from finely ground bone chips. The compression procedure proved very simple and the yields of nucleated marrow cells were gratifyingly high. The numbers of cells obtained by compression of individual vertebrae (upper part of table 1) appear to be higher than the yields reported by Haurani et al. who suspended the hematopoietic cells from chips of marrow obtained from whole vertebral bodies. The total yields from compression of multiple bones averaged more than \(50 \times 10^9\) cells during the latter phases of the investigation (lower part of table 1), which may be compared with reported average yields of \(1-11 \times 10^9\) cells obtained by multiple aspirations from living subjects\(^{1,6,7}\) and average yields of \(12.5 \times 10^9\) cells obtained from adult cadaver sources\(^{10}\).

The patients tolerated the transfusions of cadaver marrow well (table 3), it is not certain whether all of the low-grade febrile responses observed following 7 of the 13 transfusions represent an effect of exogenous pyrogens not detectable by injection of the suspending medium into rabbits, or possibly reflect a response to the rapid intravenous administration of large numbers of homologous nucleated hematopoietic cells (an infusion of \(25 \times 10^9\) cells is equivalent to the number of nucleated cells contained in seven pints of donor blood). Similar febrile responses have been reported by Schwartz et al.\(^9\) following infusions of marrow obtained from living volunteers, suggesting that the phenomenon is not related to the cadaver source per se.

The major goal of the present investigation was to modify current methods for bone marrow procurement so as to make available high yields of hematopoietic cells which could be transfused without serious hazard to the recipient. The results of the study suggest that real progress was made toward the achievement of this goal. Since we were not concerned at this time with the question of whether or not the marrow cells must be viable in order to benefit the recipient, no attempt was made to preserve cell viability during the 48 hours storage at 5 C. that preceded the transfusion of the marrow. The two day waiting period was necessitated by the detailed bacteriologic studies which were considered mandatory for cadaver material obtained at routine autopsy. If subsequent studies substantiate the importance of cell viability to achieve a favorable therapeutic effect, the marrow suspensions obtained by the present procedure should be entirely suitable for frozen storage according to technics already well worked out\(^{10}\) thereby allowing ample time for whatever bacteriologic studies seem indicated. It may be hoped that the greater availability of transfusable homologous marrow will make possible the extensive studies necessary to define its potential value in the treatment of marrow depression disorders.

**Summary**

1. A simple, economical and effective method has been described for obtaining cadaver bone marrow following a routine autopsy.
2. Individual vertebral bodies are dissected free of soft tissue and their surfaces are sterilized by immersion of the entire bone in 70 per cent ethyl alcohol.
3. Following surface sterilization, the bones are placed in a modified sterile plastic container and the nucleated marrow cells are expressed from the bone by compression of the bag in a vise.

4. After the marrow particles have been dispersed by massaging the bag, the marrow suspension is filtered and the fat is separated following gentle centrifugation.

5. Sterile yields were obtained from 70-90 per cent of cadavers which were judged suitable in terms of freedom from gross evidence of bacterial infection at the time of autopsy.

6. High yields of nucleated hematopoietic cells were obtained, averaging more than $50 \times 10^9$ cells as the investigators became more experienced with the procedure.

7. Thirteen transfusions of cadaver marrow were administered to eight patients. Minor symptoms and low-grade febrile responses were experienced on three occasions; in four additional patients entirely asymptomatic temperature elevations of 0.5-2.2 F. were observed. The remaining six transfusions were unaccompanied by fever or symptoms of any kind.

8. The possible value of the technic for definitive study of the therapeutic usefulness of bone marrow transfusion has been discussed.

**Summary in Interlingua**

1. Es describite un simple, economic, e efficace metodo pro obtener medulla ossee ab cadaveres post le necropsia routinari.

2. Corpores vertebral individual es dissecate pro liberar los de tissu molle, e lor superficies es sterilisate per immerger le integre osso in 70 pro cento de alcohol ethylic.

3. Post le sterilisation del superficie, le ossos es placiate in sterile receptaculos de plastico, e le nucleate cellulas de medulla es exprimite ab le osso per compression mechanic del receptaculo.

4. Post que le particular de medulla es dispergite per massagear le receptaculo de plastico, le suspension de medulla es filtrate, e le grassia es separate post dulce centrifugation.

5. Sterile productos final esseva obtenite ab inter 70 e 90 pro cento del cadaveres considerate como usabile pro iste objectivo a causa del absentia de grossier evidentia de infection bacterial al tempore del necropsia.

6. Alte rendimentos de nucleate cellulas hematopoietic esseva obtenite, con—al media—$50 \times 10^9$ cellulas, quando le investigatores habeva devenite plus experte in le manipulation del metodo.

7. Dece-tres transfusiones de medulla ab cadaveres esseva administrate a octo patientes. Minor symptomas e basse grados de un responsa de febrilitate occurreva in tres ocasiones. In quatro altere patientes, completamente asymptomatic elevationes del temperatura (per inter 0,5 e 2,2 F) esseva constatate. Le remanente sex transfusiones non esseva accompaniante de febre o de non importa qual altere symptomatologia adverse.

8. Es discutite le valor possibile del technica pro le studio definitive del utilitate therapeutic de transfusiones de medulla.
ACKNOWLEDGMENTS

The authors are grateful to Dr. Carl G. Harford, Acting Chief of the Department of Microbiology, for his suggestions concerning the bacteriologic studies undertaken in the present investigation. Doctor Thomas E. Brittingham kindly performed the leukaogglutinin tests.

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REFERENCES


THE SITE OF ABSORPTION OF ORALLY ADMINISTERED VITAMIN B_12 IN DOGS.


Absorption study in dogs with cobalt-58 labelled vitamin B_12 indicates that the maximum absorption of the vitamin occurs in the second half of the small intestine; little or no absorption takes place in the stomach, duodenum, jejunum or colon. Passage of vitamin B_12 across the intestinal mucous membrane is perhaps an active transfer of the material rather than passive diffusion.
A New Method for the Preparation of Human Cadaver Bone Marrow for Transfusion

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