The Collection, Storage and Preparation of Viable Cadaver Marrow for Intravenous Use

By JOSEPH W. FERREBEE, LEONARD ATKINS, HARRY L. LOCHTE, JR., ROBERT B. MCFARLAND, A. RICHARDSON JONES, GUSTAVE J. DAMMIN AND E. DONNALL THOMAS

INTRAVENOUS ADMINISTRATION of viable marrow cells has been shown in animals to bring about a repopulation of marrow spaces rendered aplastic by radiation,\(^1\) chemotherapy\(^2,^3\) or natural processes.\(^4\) Cells from acceptable donors, isologous,\(^5\) homologous\(^6\) or heterologous,\(^7\) may be used either fresh or after storage for considerable periods in glycerol at \(-80\) C.\(^8,^9,^{10}\) Efforts to adapt the implications of these observations to problems current in clinical medicine have led to an investigation of methods for collecting and storing human marrow.\(^10,^{11}\) Preliminary observations have shown that one or two billion nucleated cells may be obtained from a fetus, the usual rib removed at surgery or an aspiration biopsy of the ilium.\(^11,^{12}\) Table 1 shows amounts obtained from partial collections of ribs and vertebrae of adult cadavers. The adult cadaver appears to be the largest potential source of the necessary cells. Particular attention has been given, therefore, to the special problem of collecting and storing cadaver marrow in a form suitable for intravenous administration. The procedure outlined here has shown sufficient promise of ease, quantity of yield and quality of preservation\(^13\) to warrant description. Yield is a function of the amount of bone removed and the cellularity of the marrow. Marrow of elderly individuals tends to be fatty.

MATERIAL AND METHODS

Adult cadavers with the necessary legal permission for postmortem examination are selected on the basis of presumptive cause of death, recent clinical history and time of death. Subjects dying of blood loss, vascular accident, coronary-artery disease or other nonseptic and non-neoplastic conditions are suitable, provided no more than approximately four hours have elapsed since death occurred, although an interval of one or two hours is preferable.

The cadaver is placed in the usual supine position on the autopsy table with a block under the shoulders so that blood from the head and neck tends to drain away from the trunk. The stretcher used in transporting the body to the autopsy room is draped and...
VIABLE CADAVER MARROW FOR INTRAVENOUS USE

Table 1.—Preliminary Study of Quantities of Bone Marrow Obtainable from Partial Collections of Ribs and Vertebrae of Adult Cadavers

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Time from Death to Freezing of Cells (hours)</th>
<th>Ribs Yield (Billions of Nucleated Cells)</th>
<th>Vertebrae Total</th>
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serves as an instrument table. Table 2 shows a list of equipment and instruments used. A Lakeside cart used in transporting the wrapped supplies and instruments to the autopsy room may be used as a "prep table."

The skin of the torso is cleansed with soap, alcohol and tincture of Zephiran solution. The body is draped with sterile sheets, and the usual Y-shaped autopsy incision is made. The skin knife is discarded, and the drapes are clipped with towels to the incised edges of skin. The skin folds are reflected back. The abdominal wall and peritoneum are incised along the midline and costal margins and reflected laterally, care being taken not to puncture the intestine. The breast plate is removed in the routine manner, and the edges of the ribs are covered by towels. The pericardial sac is opened, and blood for typing and a serologic test for syphilis is aspirated from the inferior vena cava at its point of entrance into the right atrium. The great vessels of the neck are dissected, clamped and divided. As long a length as possible of trachea, together with adherent esophagus, is dissected free and clamped with two Rochester-Ochsner straight hemostatic forceps placed as far apart as possible. The midportion of this segment of trachea and esophagus is divided and the knife discarded. The severed ends are carefully covered with rubber dam and clamped with Rochester-Ochsner straight hemostatic forceps. The leaves of the diaphragm are severed from their attachment to the thoracic wall, and the thoracic and abdominal organs are eviscerated en bloc starting above with the clamped trachea and esophagus in the upper thorax. The organs are reflected inferiorly over the pelvis and covered by a drape. Thus the vertebral column from the upper thoracic portion to the level of the fifth lumbar vertebra is exposed. Half sheets are placed in both paravertebral areas to absorb excess blood.

The anterior portion of the vertebral column is excised with a bone saw and chisel.
## Table 2.—Equipment for Sterile Autopsy

### Pack #1

**"Prep." tray:**
- 3 sponge bowls
- 2 packs of sponges
- 6 sponge-holding forceps

### Pack #2

- #1320 bone saw handpiece*
- 1 large sectioned blade and arbor*
- 1 large bone scoop*
- 1 Keyes bone-splitting chisel with thin, straight blade, %4 in.
- 1 pattern gouge, 10 mm., length 8 in.
- 6 Rochester-Ochsner hemostatic forceps, straight, 8 in.
- 6 Rochester-Carmalt hemostatic forceps, curved, 6%4 in.
- 18 towel clamps, 3 in.
- 2 towel clamps, 5%4 in. for clamping gown
- 2 bone-cutting forceps, straight, 10 in.
- 2 tissue forceps with teeth, 10 in.
- 2 tissue forceps with teeth, 6 in.
- 2 operating scissors, straight with blunt tips, 6%4 in.
- 4 Bard Parker handles #4
- 4 Bard Parker Blades #22
- 4 towels
- rubber dam

### Pack #3

- 2 syringes (50 ml., Luer-Lok)
- 2 test tubes with stoppers for collecting blood for typing and STS
- 2 needles (15 gauge)
- 1 large dish with cover (100 mm. in diameter, 80 mm. in height)
- towels

### Pack #4

- stainless steel tower with chopper, gaskets and plastic nuts†
- plastic tubing

### Pack #5

- 1 stainless steel cylinder with screens, gaskets and cover†
- plastic tubing
- 2 three-way stopcocks

**Other Sterile Equipment**

- 2 gowns (1 towel packed with each)
- 2 packs of half sheets (2 each)
- 3 ortho sheets
- 6 pairs rubber gloves
- 1 can scrub brushes with 1 sponge bowl for holding soap solution
- 1 cable for motor

**Other Equipment and Solutions**

- 1 can with caps and masks
- scrub shirts and pants
- 4 600 ml. Fenwal transfer pack
- #1300 motor with on-off footswitch*
- tincture of Zephiran
- stand for suspending motor
- soap solution
- 70% alcohol
- 500 ml. Hanks' solution containing 10% serum (type AB)
- 1 Payr clamp

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*Orthopedic Frame Company, Kalamazoo, Mich.
†Edward A. Olson Company, Ashland, Mass.
A bone scoop, a heavy bone chisel and a pattern gouge are then used in sequence to remove marrow in pieces 1 or 2 cm. in diameter from the exposed vertebral bodies, including the excised anterior slab. Intervertebral discs are avoided. Usually 100 to 200 cu. cm. of cancellous marrow bone are obtained.

The collected pieces of marrow are loaded into a stainless steel chopper (fig. 1). Approximately 2 volumes of suspending fluid (Hanks' solution containing 10 per cent human serum, type AB) are run into the chopper from a reservoir. Ten minutes of mild chopping and mixing with the cutting plunger is sufficient to put most of the marrow cells into suspension. The marrow suspension is then sucked through a 30-mesh, stainless steel screen at the lower end of the chopper into a syringe and discharged into a 600 ml. Fenwal transfer pack.* The remaining marrow cells are rinsed through by repeating the above process with two fresh washes of 50 ml. of suspending fluid.

The inlet tube of the transfer pack containing the marrow is tied off, and the pack is placed with the inlet and outlet ports down in an International Centrifuge cup #353.

*Ethicon, Inc., Somerville, New Jersey.
containing sufficient rice to support the pack in an upright position free from kinks. Centrifugation for 10 minutes at 1000 rpm (International Centrifuge head #976) is sufficient to sediment the nucleated marrow cells and bring the fat to the surface. After centrifugation the pack is continued in its inverted position. A Payr clamp is applied to separate from the main volume of fluid and supernatant fat a small volume of fluid phase and packed cells lying just above the outlet ports (fig. 2). With gentle manual massage the cells are resuspended in the liquid phase below the clamp. Another 600 ml. Fenwal transfer pack (pack 2) is attached to one of the outlet ports, and the resuspended cells are allowed to flow by gravity and massage into this second pack. The fluid reservoir is detached from the chopper and attached to the second port of pack 1. Fluid from the reservoir is used (100 to 200 ml.) to flush all cells from pack 1 into pack 2 and to provide a suspension suitably dilute for screening.

Pack 2 is tied off from pack 1 and attached by a Fenwal connector through one of its ports to the upper connection of a screen tower containing in series 3 screens of 60, 120 and 200-mesh stainless steel. A 100 ml. syringe and 3-way stopcock is attached to the lower connection of the screen tower and the marrow suspension from pack 2 is drawn through and ejected into a third 600 ml. Fenwal transfer pack (fig. 2). Fat, DNA slime or strands of connective tissue may occasionally occlude the screens. This material is easily dislodged by back-washing with the liquid in the 100 ml. syringe. Pack 2 is tied off and centrifuged upside down at 1000 rpm for 10 minutes. The sedimented cells are clamped off and removed to a fourth pack by a procedure similar to that used in the case of pack 1, except that no wash is used from the reservoir and an effort is made to keep the liquid volume as low as convenient. Pack 4 is detached and weighed. The volume of contained cells and fluid is estimated from the difference between known weight of the empty pack and observed weight using "1 Gm. equal 1 ml." as approximate conversion formula. An equal weight (volume) of 30 per cent glycerol, 70 per cent serum type AB is injected through the inlet tube of pack 4 with a needle and syringe, the pack being gently shaken to promote thorough mixing during the injection. The inlet tube is tied off containing a small sample (1 ml.) for sterility test and cell count. The pack is immediately laid flat in a cooling system designed to drop the temperature at approximately 1 C. per minute to −15 C. and then at approximately 2 C. per minute to −80 C.∗

Marrow processed and stored as described above is prepared for intravenous use by thawing briskly with gentle but continuous agitation of the frozen bag in a large volume of water at 37 C. The thawing operation should be completed in 1 minute or less, and the contents of the bag during this time should remain cool. A half volume of 35 per cent glucose in water is immediately added with continuous mixing through one of the inlet ports. At the end of 2 minutes, 1 volume of isotonic saline is added, followed in 2 minutes by a further volume of saline (total dilution 1 to 6). The additions are conveniently made through appropriate Fenwal connector tubing with gentle shaking to accomplish continuous mixing. The final suspension contains about 20 billion nucleated marrow cells in 2.5 per cent glycerol and slightly hypertonic glucose-saline.

**DISCUSSION**

Glycerolized cells of cadaver marrow similarly prepared on a smaller scale and stored at −80 C. for several weeks have been administered intravenously to patients of similar but not identical blood group. These cells, after removal of glycerol, have shown evidence of continued viability in vitro (DNA synthesis) and have appeared to make final osmolar adjustments in vivo from levels of 2.5 per cent glycerol concentration without loss of viability and without production of signs or symptoms of pulmonary embolism.

We have not infused glycerolized cells in man without first reducing the

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*Canal Industrial Company, Bethesda, Maryland.
concentration of glycerol to 2.5 per cent or less, as above described. In mice, several observers have found that glycerolized marrow may be infused at glycerol concentrations of up to 15 per cent without untoward effect. The cells infused, however, have been administered rapidly in a relatively large volume of glycerol, 0.5 ml., into a relatively small, 25 Gm., animal. Under such circumstances there is a rapid build-up of glycerol concentration in the blood stream followed by a relatively slow and evidently satisfactory adjustment of osmolarity between cellular and extracellular fluid spaces. Experiments are in progress in dogs to determine whether a similar satisfactory adjustment occurs when the volumes infused are administered more slowly and are smaller in relation to blood volume, thereby approximating more closely the rates of change expected in man with infusions of the order of 100 to 200 ml.

Marrow withstands postmortem autolysis for several hours, but it does not well withstand the superimposed traumas of postmortem autolysis and gly-
ferred low-temperature preservation. Cadaver marrow obtained as above but not glycerolized or frozen has also been used in the treatment of patients. In vitro measurement of its viability has indicated that a useful degree of preservation may be obtained at 4 C. for one or two days. Human marrow frozen to -80 C. without glycerol loses its ability to synthesize DNA. However, mouse marrow that is similarly injured by freezing without glycerol still retains its ability to repopulate marrow spaces when infused in vivo. Whether human marrow retains a similar ability has not been determined.

After marrow has been removed from the body and broken up by the chopper (fig. 1) it consists of particles of marrow, fragments of vascular and connective tissue and pieces of bone. It is necessary to remove the undesirable material and to disperse the marrow particles so that intravenous administration of the suspension does not produce pulmonary emboli. This is accomplished by passing the marrow through a series of stainless steel screens. By starting with a coarse screen and passing the marrow through successively finer screens, the foreign material can be removed and the marrow particles broken into a suspension of single cells without the use of force and without trauma to the cells. Observations of morphology, heme synthesis and oxygen consumption have shown no evidence of damage to marrow cells after repeated passage through the screens. Histologic sections of the material remaining on the screens show only an occasional marrow cell. DNA slime can be avoided by making collections as soon after death as possible and by the use of deoxyribonuclease. Fat in high concentrations may clog the screens, and for this reason it is important to screen only marrow suspensions that are well diluted and well mixed.

Maintenance of sterility during collection of cadaver marrow has not proved a great problem. Nine out of 10 recent collections have been sterile. Contaminated collections on culture, for the most part, have grown out Staph. albus, suggesting simple breaks in manipulative technics rather than in situ contamination with intestinal organisms.

The described method of collecting cadaver marrow has the merit of providing marrow in quantity adequate for most in vivo and in vitro studies. It disturbs the classical autopsy procedure minimally. In vitro evaluation of preservation and collection procedures is continuing. Clinical studies are in progress to determine the usefulness of cadaver marrow in restoring marrow functions after their destruction by radiation, drugs and disease.

**Summary**

A procedure for collecting and storing viable cadaver marrow in a form suitable for intravenous use is described. Vertebral bone marrow is removed under sterile conditions within a few hours after death. The marrow cells are screened and suspended after centrifugation in an equal volume of a liquid medium consisting of 30 per cent glycerol and 70 per cent human serum and frozen to -80 C. in plastic bags. Suggestions for thawing, deglycerolizing and administering the cells intravenously are given.
Summario in Interlingua

Es descipte un technica pro le collection e immagazinage de viabile medulla de cadavere in un forma appropriate al uso in administrationes intravenose. Medulla de osso vertebral es obtenite sub conditiones sterile intra pauc horas post morte. Le cellulas medullar es cribirate, centrifugate, suspendite in un equal volumine de un medio liquide que consiste de 30 pro cento de glycerol e 70 pro cento de sero human, e congelate a -80 C in saccos de plastico. Detalios technic pro le disgelation, disglycerolation e administration intravenose del cellulas es includite.

REFERENCES
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