

**Instructions  
for the  
Induction of  
Solid State  
Hypothermia  
in Humans**

**ALCOR**

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MANRISE CORPORATION

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IN  
HUMANS

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## DEDICATION

Throughout this manual we refer to those persons who will make direct use of it as the cryonics "Society Representative". For the sake of variety, we sometimes speak of the "reader" or the "user", but the meaning is the same. We are referring to those persons who have decided to shoulder the burden of responsibility for rescue, for resuscitation, and the induction of solid state hypothermia, when and as required.

These Society Representatives are the foundation of all organizations accepting anatomical gifts for the induction of solid state hypothermia. They are the persons who have chosen to grasp the knowledge, acquire the equipment, develop the skills, and devise the administrative-legal tools for dealing with almost every conceivable circumstance of legal death. Their dedication is measured by their readiness and competence. Every Donor must place his trust in a Society Representative's preparedness and ability.

Interested physicians and scientists have provided the basis for this manual, and this manual provides the basis for organized preparation, but only the presence of a dedicated Society Representative provides the basis for action in the event of the legal death of a Donor. In the eyes of the Donor, the Society Representative is the difference between the possible chance of continued life and the certainty of final obliteration.

We dedicate this manual to Society Representatives, in cryonics societies wherever they exist or may be formed. Its only purpose is to serve their ends.

Manrise Corporation

# MAIRISE SSH INSTRUCTIONS

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# 1.0 INTRODUCTION

A condition of hypothermia (abnormally low body temperature) extreme to the point of virtually arresting almost all biological and chemical processes involves the crystallization of tissues. This constitutes "solid state hypothermia" (SSH), a term which will be used to denote that condition throughout this manual. The induction of solid state hypothermia following legal death is intended to maximize chances of survival.

Methods have been developed whereby single cells and certain kinds of tissue survive freezing and thawing.<sup>1</sup> Whole organisms, including mammals, survive body core temperatures approaching the freezing point of water under proper circumstances.<sup>2</sup> In principle, it is possible that human beings can survive freezing and thawing provided proper techniques, yet to be developed, are used. No means of restoring a normal physiological state to a person placed in a condition of solid state hypothermia are known at this time. Some speculate that such means will be developed<sup>3</sup>, while others claim this is highly improbable.<sup>4</sup>

This instruction manual covers all legal and preparatory aspects as well as actual procedures involved in the induction of SSH. It is not the intent here to persuade or promote the use of SSH or the merits of life extension. The sole purpose of this manual is to coordinate and reduce isolated, individual efforts and recommendations into a single useable form. Every effort has been made to keep it simple and practical.

Since Dr. Bedford was frozen in 1967, a number of steps toward improved methods have been taken.<sup>5</sup> Many individuals in various locations are continuing to work out improvements to existing methods. This manual will stay abreast of these advances, so that theory can become practice with minimal delay.

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1. "Cryobiology", Edited by H. T. Meryman, Academic Press, 1965.
  2. "Methods of Induction and Effects of 'Cardiac Arrest' in Relationship to the Hypothermic State", by J. Hunter, Vol. 3, Part 3, (pp 485-510) of "Temperature; Its Measurement and Control in Science and Industry", Reinhold, 1963.
  3. "The Prospect of Immortality", by R.C.W. Ettinger, MacFadden, 1966.
  4. "Suspended Animation", by P. W. Prehoda, Chilton, 1969.
  5. "Brief History and Overview of Methods Used for Induction of Solid State Hypothermia (SSH)", by L. L. Chamberlain and F. R. Chamberlain, Manrise Technical Review, Vol. I, No. 1, Aug-Sept, 1971.

## 2.0 WORDS OF CAUTION

This instruction manual provides a practical basis for implementation of the latest recommendations from interested physicians and scientists in the actual practice of inducing SSH. The authors have made every effort to reduce this manual to its most useable form, and to minimize the burdens of familiarization. Still, availability of a manual is just a first step. A good deal of additional, dedicated effort on the part of the user is required.

2.1 USEFULNESS of the Instruction Manual. In most instances, danger to the life of a Donor will arise without warning. The Society Representatives on whom the Donor depends will have to act quickly. Users of this manual should prepare for such emergencies by becoming intimately familiar with this manual, acquiring equipment consistent with available resources, and maintaining skills through regular training. The correct administration of a properly organized society is of great importance and should not be overlooked. Further information on these subjects is provided in sections 5.0 through 8.0.

2.2 USEABILITY of the Instruction Manual.

2.2.1 The authors of this manual are not physicians. Although medical references are provided where available, other portions of this manual are not supported by specific documentation. The user should validate the ideas presented for himself, questioning any inconsistencies that seem to exist.

2.2.2 The authors of this manual do not assume any responsibility for actions taken by other persons in connection with the content presented herein. Individual users of this manual must bear full responsibility for their choices of action.

## 3.0 MANUAL ORGANIZATION

Sections four through nine of this manual provide information applicable to long range preparations, while sections fifteen and higher deal with operational procedures. Sections ten through fourteen serve to guide the user in selecting the procedures called for in specific sets of circumstances.

Each of the operational procedure sections contains detailed instructions, but the instruction manual is arranged so that the user who is familiar with the contents can use it as an outline, referring to details only as necessary to supplement his memory. On the other hand, a person lacking this prior familiarity can study the instruction manual and obtain a useful level of understanding in each area.

Extensive cross referencing and substantial redundancy is incorporated to facilitate the learning process. As familiarity increases, there will be less and less need for reference to the more easily retained details. Any time that a numerical reference appears in parenthesis, it indicates a part of the manual providing additional information.

Many sections of this manual will be incomplete at the time of original issue, and will later be supplied to MTR subscribers for insertion. Essential information will be presented to begin with, in most sections. All sections will be revised and expanded as comments and suggestions from readers are received. This approach puts information in the hands of the reader as quickly as possible, and will permit the most efficient correction of errors and deficiencies. Also, it establishes the principle of continuous revision for the purpose of incorporating improvements as new knowledge and techniques are developed.

The reader can easily assure the completeness of his manual at any time by cross checking it against the "list of effective pages" in the front of the manual. An updated list will be mailed with each set of revision sheets.

## 4.0

READER COMMENTS  
AND RECOMMENDATIONS

4.1 Level of Treatment. This manual is intended to be useful and comprehensible to persons with a high school education. Notwithstanding this, the reader may find many unfamiliar terms and concepts which should be simplified. Only one way exists for this to be accomplished. Readers who find the treatment "too complicated" or "not easy to understand" must let the publisher know that this is the case. Write directly to Manrise Corporation (4.3). Changes will be made to remedy such problems in whatever ways are practicable.

4.2 Completeness and Correctness of Treatment. The authors have attempted to foresee the needs of the user for information in all applicable areas, but it is almost certain that omissions will have occurred. Readers can help by pointing out additional details that are necessary and appropriate. Also, some readers will find that errors have been made or that really excellent alternative ideas have been overlooked. These deficiencies can be corrected most rapidly by direct communication.

4.3 Comments and Recommendations. Suggestions should be mailed to Manrise Corporation, Box 731, La Canada, California, 91011. A prompt reply cannot be promised, but all recommendations will receive serious consideration.

## 5.0 LEARNING SSH PROCEDURES

Some readers will study this manual directly, developing a through familiarity with the material. They will look up terms in dictionaries, check references at medical libraries, and contribute to the manual's improvement through submission of recommendations and corrections. Others will find the process more difficult. Further, even if an initial familiarity is acquired, this may be lost with time. Periodic review of the material is necessary. Some readers will find periodic rereading enjoyable and rewarding. Others will neglect it.

It is possible that a series of questions testing the knowledge of the user would be valuable both in the learning process and in reviews for "refresher" purposes. These could be in the form of examinations. Many approaches are possible, however, and choices must be made. Readers are requested to consider the following questions:

- 5.1 Is the reader of this manual interested in examinations to test familiarity with and understanding of this instruction manual?
- 5.2 Should exams be conceived to test understanding ("open-book exams") or familiarity ("closed-book" exams)? or both?
- 5.3 Would readers be more interested in "self-test" exams (answers included on separate sheets) or in "graded" exams (which are returned to Manrise Corporation for correction and comment)?
- 5.4 In the case of "graded" exams, would readers be interested in programs of examination leading to the establishment of "qualifications" as to levels of understanding and familiarity, with periodic "requalification" to assure that the level of knowledge is sustained and up-to-date?

Reader response to the above questions is invited. Recommendations as to questions that should be incorporated in these examinations will be welcome. Address your comments to Manrise Corporation, Box 731, La Canada, California, 91011.



## 6.0 ACQUIRING EQUIPMENT

This section will be transmitted at a later time. It will include charts listing equipment that should be acquired for various purposes. Categories will include "Rescue and Resuscitation", "Phase I", "Phase II". Priorities will be shown for acquisition of equipment in each category, so the user can acquire an initial capability at minimum cost, building on this as additional resources permit. Methods of packaging equipment for efficient transportation in emergencies will be discussed, as appropriate.

Pending completion of this section, the reader will find later sections on equipment to be useful. Section 59, in particular, treats Phase I perfusion equipment comprehensively and cites commercial sources of apparatus.

## 7.0 TRAINING: ACQUIRING SKILLS

Rescue and resuscitation are of overwhelming importance, and skills in this area should be acquired first. Luckily, formal courses in this area are offered in almost every city, most of them by the American National Red Cross. Manrise Corporation recommends that all persons interested in the practical aspects of SSH enroll in the Red Cross first aid program, reaching and maintaining qualifications at the "Advanced" level (27.0).

Training in other procedures involved with SSH (implementation of external cooling, cannulation, perfusion, etc.) will necessarily be more difficult to obtain. The use of experimental animals and simulators may be feasible, but no organized means of providing appropriate training oriented toward SSH objectives exists at this time.

Training in cannulation (53.0) might be most easily obtained by serving as a mortician's apprentice. Efforts should be made to use the femoral location whenever possible. "T-shaped" cannulas (56.0), if available, should be used.

Training in the use of perfusion equipment (59.0) might be effected with physiological simulators constructed of thermally conductive tubing coiled in a tank of water approximately the mass of a human being. The flow-pressure relationships of total volume contained by the tubing should approximate that of the human circulatory system. Also, the surface area of contact between the tubing and the surrounding water should be sufficient to develop the heat exchange characteristics anticipated in an actual perfusion. Finally, the surface area of the outer container, corrected for differences of thermal conductivity, should approximate that of the human body.

It should be apparent that the design of a simulator is quite complex (although hopefully the construction can be made relatively inexpensive). This section will be revised at a later time to provide details concerning the construction of an appropriate simulator.

## 8.0

### NON-PROFIT ORGANIZATIONS

Only a non-profit society with certain chartered goals can legally be the recipient of anatomical gifts, and the requirements for incorporation of such societies vary from state to state. Formation of an appropriate society is just the beginning. Administrative arrangements and procedures must be provided for dealing with practically all foreseeable circumstances.

"Donors" are those society members who have executed documents naming the society as the recipient of their persons in the event of legal death. Typically, all Donors will have made financial arrangements to cover the expenses of inducing SSH and maintaining it for an indefinite period. No. 14 (8 May 1970), The Bulletin de la Societe Cryonics de France (English Edition) contains an elaborate analysis of costs and financing arrangements for SSH.

"Society Representatives" are those trained individuals formally designated to act on behalf of the society in accepting anatomical gifts in the event of a Donor's legal death. More broadly, they are the persons who are depended upon for rapid, decisive, and competent action in the event a Donor's life is endangered.

If a number of Society Representatives function as a team, the interaction of the "team members" (Society Representatives) should be clearly organized. This consideration is accounted for throughout the manual, in sections dealing with subjects such as Emergency Communication Systems (9.0), General Coordination Procedures (18.0), Division of Activities within the Phase I Team (52.0), etc.

The society may be fortunate enough to have a research facility, but otherwise must have arrangements for the use of suitable laboratory space in the event induction of SSH is necessary. Use of an operating room in a hospital would be ideal, but the prolonged duration of perfusion and the reluctance of hospitals to assist usually renders this alternative impractical. More frequently, the society will find it advantageous to make arrangements with a mortician for the use of a "preparation room". The society may also be able to arrange for assistance in cannulation from the mortician.

A society may be prepared for Phase I activities and still lack a capability for Phase II perfusion. Arrangements in such cases may be concluded with another society which is prepared to conduct Phase II operations for assistance. After Phase I activities are completed, a Donor could be temporarily transferred between societies and Phase II procedures could be performed by the more well equipped organization.

~~Cryonics societies must provide for long term storage of Donors in the event of the induction of SSH, and this generally requires the use of liquid nitrogen and well insulated containers. The containers are preferably secured in earthquake-proof, fire-proof structures. The use of surplus missile sites has been suggested and this means is currently under investigation by cryonics storage corporations. Societies will usually find it advantageous to contract with commercial organizations for storage services, since the purchase of suitable sites and the erection of the required structures is quite expensive.~~

Societies may wish to establish reciprocal arrangements with each other so that a Donor far from home may be protected by the nearest cooperating society. Details concerning this are included in the section on administration arrangements (8.3).

### 8.1 Administrative Arrangements; Donor.

8.1.1 Anatomical Gift Certification. Various forms, signed by the Donor and witnessed, are used by cryonics societies for certifying that an anatomical gift has been made. Additional information may be obtained on the subject from the National Society for Medical Research, 1330 Massachusetts Avenue, N. W., Washington, D. C. 20005.

~~This organization publishes (free of charge) a pamphlet titled "How to Donate Organs, Tissues, or Your Entire Body for Medical Science". Forms are attached, the most interesting being a "Uniform Donor Card" proclaiming that it is a "Legal Document under the Uniform Anatomical Gift Act or Similar Laws". It is recommended that the "Uniform Donor Card" be filled out, signed, witnessed, and carried on the Donor's person at all times.~~

8.1.2 Medical Records. A detailed medical record should be completed by the Donor and filed by the Society. This should be up-dated yearly, following a physical examination, or after any major illness.

8.1.3 Release. The Donor should execute a document releasing the society and its representatives from liability in connection with efforts to resuscitate the Donor.

8.1.4 Will. The Donor should revise his will or add a codicile thereto noting his wish that SSH be induced in the event of his legal death.

8.2 Administrative Arrangements; Society Representative. Society Representatives should be so designated, in writing, by an appropriate officer of the society. The designation should specifically authorize the Society Representative to accept anatomical gifts on behalf of the society. This document, coupled with the Donor's "Uniform Donor Card" (8.1.1) should provide the necessary legal basis for the Society Representative's actions in inducing SSH.

8.3 Administrative Arrangements; Reciprocal Arrangements Between Societies. Societies which desire reciprocal arrangements should exchange documents which authorize other societies to accept anatomical gifts on their behalf. The documents should bear the seal of issuing society, and an expiration date should be indicated. In the event a Donor far from home dies, the nearest cooperating society would be notified and would assume responsibilities for induction of SSH as necessary. The Donor's "Uniform Donor Card" would establish the anatomical gift to the original society, and the reciprocal arrangement document would enable the closest-by society to act for the more distant society.

## 9.0 EMERGENCY COMMUNICATIONS SYSTEMS

Many emergency communications systems are possible, but all of them require a 24-hour alert network of some kind. One system, described in this section, may offer the most advantages to an SSH organization of moderate size which depends on volunteer services of its members (Society Representatives) in an emergency.

### 9.1 System Components.

- (1) Emergency Team Members (Society Representatives)
- (2) Radio Paging Devices (Beepers)
- (3) Radio Paging Service
- (4) Telephone Answering Service.

9.2 System Organization. The Society contracts for radio paging services from an appropriate organization. The radio paging devices are leased from the radio paging service and carried by all emergency team members.

### 9.3 System Function.

9.3.1 Mode I: Answering Service Intercepts the Emergency Call. An emergency call comes in, but is *not* answered by a Society Representative at the principal telephone. The answering service takes the call, and finds that it is an emergency. The answering service contacts the radio paging service, which pages ("beeps") all team members (each of whom is a Society Representative). The first Society Representative to answer the page "takes" the call and leaves word with the answering service as to where he can be reached. He then calls the scene and gets all the details he can over the phone (15.0, 17.0).

Other team members begin calling in and find out whom to contact. By the time the first Society Representative breaks contact with the scene, calls are coming to him and other Society Representatives can

be briefed. The first Society Representative serves as "Coordinator" in this instance (18.0), and other Society Representatives go to the scene.

9.3.2 Mode II: Call Received by Society Representative. The Society Representative answers the call to begin with. He obtains information and gives instructions (15.0, 17.0). He then calls another Society Representative and briefs him on the circumstances. The second Society Representative acts as "Coordinator" in this instance (18.0). He calls the answering service, leaves his name and number, and requests all team members be paged. The answering service pages ("beeps") all team members and provides the coordinator's name and phone number as they call in. All team members subsequently call the coordinator and are briefed as to the circumstances.

## 10.0 SELECTING APPROPRIATE COURSES OF ACTION

Upon learning that a Donor is dying, clinically dead (cardiopulmonary functions have ceased), or legally dead (death officially pronounced), the Society Representative must make decisions concerning what actions are required.

Different circumstances require different actions. A chart, provided in figure 10-1, refers the user to an appropriate set of instructions.

Society Representative Is At The Scene	Doctor Is At The Scene	Refer to Section Number
No	No	11.0
No	Yes	17.0
Yes	No	13.0
Yes	Yes	14.0

Figure 10-1

The circumstances represented in figure 10-1 are "initial conditions", in that they represent the conditions at the starting point of any episode, where the Society Representative learns that a Donor is dying, clinically dead, or legally dead.

In reviewing the sequence of events programmed for any specific circumstances, one point should be emphasized: Nothing is more important than sustaining life and potential viability in the Donor, so long as the actions required are lawful. Resuscitation and sustained cardiopulmonary assistance, where appropriate, are of primary importance. External cooling (36.0), where appropriate, should be begun *immediately*. Other activities, such as legal certification of death (33.0), avoidance of autopsy (34.0), maintenance of proper records (45.0), and medical tests (40.0) are essential, but sustained cardiopulmonary assistance (35.0) and external cooling (when appropriate) are of the greatest significance and importance.



# 11.0 SITUATION I

SOCIETY REPRESENTATIVE IS NOT AT SCENE --  
DOCTOR IS NOT AT SCENE

This circumstance is most likely to occur when the Society Representative is called by a friend or relative of the Donor, or by the Donor himself. The Donor may be critically ill, dying, or clinically dead. A doctor or ambulance may, or may not, have been called, but in any case no doctor is present at the scene.

The Society Representative must *get* detailed facts and *give* explicit instructions (15.0). In some circumstances, the Society Representative must take immediate action to secure medical assistance (16.0). He must provide for coordination (18.0). Following this, the Society Representative must go to the scene (19.0).

If it seems likely the Society Representative will reach the Donor before a doctor, refer to 13.0 for further instructions. If it seems likely that a doctor will be in attendance at the scene when the Society Representative arrives, refer to 14.0 for further instructions.

# 12.0 SITUATION II

SOCIETY REPRESENTATIVE IS NOT AT SCENE --  
DOCTOR IS AT SCENE

This circumstance is most likely to occur if the Donor is taken to a hospital without the knowledge of the Society Representative. Medical authorities may learn that their patient is an anatomical donor directly or through friends and relatives, and it is assumed in this case that the Society Representative is contacted by the physician in charge. If the first information does not come from the physician, the Society Representative should then contact the physician immediately.

In contacting the physician, the Society Representative must obtain specific information and request that certain procedures be carried out (17.0). He must also provide for coordination (18.0). Following this, a Society Representative must go to the scene (19.0).

The Society Representative may expect that a doctor will be in attendance at the scene when he arrives; he should therefore refer to 14.0 for further instructions.

# 13.0 SITUATION III

SOCIETY REPRESENTATIVE IS AT SCENE --  
DOCTOR IS NOT AT SCENE

This circumstance is most likely to occur where a Donor and a Society Representative are together and the life of the Donor is in danger. It may occur that both persons are both Donors and Society Representatives (friends, married couples). It should be clear that in these cases, the Donor's chances for successful resuscitation and immediate recovery are highest.

It is also possible that the Society Representative might have been called to the scene (Situation I, section 11.0) or that he arrived at the scene of a critically ill Donor without prior notice that anything was wrong. Therefore, it is possible that the Donor will be clinically dead or that resuscitation will already be underway. These possibilities are provided for in the following paragraphs.

13.1 Donor Alive or Being Resuscitated. If the Donor is alive or being resuscitated, the Society Representative should obtain medical assistance at once (16.0). Instead of doing this directly, he may alert another Society Representative who will act as a "Coordinator", sending medical assistance to the scene (18.0).

Once a physician is present, the course of action corresponds to that described in 14.0, for a doctor present and the Donor alive or being resuscitated.

13.2 Donor Clinically Dead Less Than Ten Minutes. If the Donor is clinically dead (20.0) and has been in that condition less than ten minutes, resuscitation should be initiated immediately (24.0). After resuscitation is begun, the Society Representative should obtain medical assistance at once (16.0). Instead of doing this directly, he may alert another Society Representative who will act as a "Coordinator", sending medical assistance to the scene (18.0).

Once a physician is present, the course of action corresponds to that described in 14.0, for a doctor present and the Donor alive or being resuscitated.

**13.3 Donor Clinically Dead More Than Ten Minutes.** The previous situations of this section were relatively simple. After initial steps, the reader was referred to section 14.0 for further instructions. This situation, however, is far more complex and may lead directly to the induction of solid state hypothermia. For purposes of greater clarity, the following narrative is charted (figure 13-1).

If the Donor is clinically dead and has apparently been in that condition without resuscitation for more than ten minutes, the Society Representative should quickly but carefully examine the Donor for signs of life (20.0). On that basis, and on the basis of whatever other facts that are available, the Society Representative should decide immediately whether or not resuscitation should be attempted. See 21.0 for further information concerning this decision.

If a decision to attempt resuscitation is made, and resuscitation is initiated, further reference to this narrative is not applicable. Refer instead to the previous cases of this section.

If the Society Representative decides not to attempt resuscitation, specific alternative actions are appropriate (22.0). The decision not to attempt resuscitation and to proceed with other actions is accompanied by many legal risks (23.0).

The Society Representative must deal with the special circumstances created by his decision (30.0), prior to obtaining pronouncement and certification of death (31.0). Every effort must be made to avoid autopsy, but this may not be possible. See instructions on the avoidance of autopsy and measures to be taken if it cannot be avoided (34.0).

Following resolution of problems associated with legal death and the avoidance of autopsy, the Society Representative should immediately implement the most complete external cooling procedures possible (36.0) and provide for any medical tests that may be appropriate (40.0). Complete records must be maintained (45.0), and standard procedures for the induction of SSH must be carried out (50.0).

Situation	PERSONNEL PRESENT:	CONDITION OF DONOR:
		Doctor <i>is not</i> at scene -- Society Representative <i>is</i> at scene
Courses of Action	ACTION	SECTION
	Examine Donor for Signs of Life	20.0
	Decision as to Resuscitation	21.0
	Action if Resuscitation Not Indicated	22.0
	Special Circumstances	30.0
	Legal Death	31.0
	Avoidance of Autopsy	34.0
	Immediate External Cooling	36.0
	Medical Tests After Legal Death	40.0
	Maintain Records	45.0
Standard Procedures for Induction of SSH	50.0	

Figure 13-1

# 14.0 SITUATION IV

SOCIETY REPRESENTATIVE IS AT SCENE --  
DOCTOR IS AT SCENE

This situation will most often occur at a hospital. Initial circumstances leading to the situation span almost all possibilities. The Society Representative may have been called by a doctor at the hospital, or by the Donor's relatives or friends as the Donor is taken away in an ambulance. The Donor may have been brought to the hospital by the Society Representative himself. The Society Representative may be on standby at the hospital while the Donor undergoes a critical operation. Figure 14-1 charts the sequence of actions required, depending on the circumstances.

14.1 Columns A and B. If the Donor is alive or being resuscitated, the Society Representative should assure that all necessary tests for complete documentation of the Donor's condition are performed (28.0). Heparin should be injected if death appears imminent (29.0). If the case exists that restoration of normal physiological functions are hopeless, the supervising physician should be persuaded to pronounce and certify death *without cessation of cardiopulmonary assist* (31.0). Autopsy must be avoided (34.0), cardiopulmonary assistance must be maintained (35.0), and external cooling must be initiated without delay (36.0). Complete records must be maintained (45.0), and standard procedures for the induction of SSH must be carried out (53.0).

14.2 Column C. If the Donor is clinically dead, and has been in that condition less than ten minutes with a doctor present, the Society Representative should insist that resuscitation be initiated and maintained until the Donor's condition is improved or is clearly hopeless (24.0). With rare exception, this procedure should be followed even if the Donor has been pronounced dead. All necessary medical tests to document the Donor's condition should be carried out while resuscitation is in progress (28.0). Heparin should be injected, unless the patient's prognosis is favorable (29.0). If the case exists that restoration of normal physiological functions is hopeless, the supervising physician should be persuaded to pro-

PERSONNEL PRESENT:		CONDITION OF DOWOR:			
Doctor and Society Representative	Alive	X			
	Resuscitation in Progress		X		
	CDWNR <sup>1</sup> , Less than 10 minutes			X	
	CDWNR <sup>1</sup> , More than 10 minutes				X
Action	Section	A	B	C	D
Resuscitation	24.0			•	
Medical Tests	28.0	•	•	•	
Heparin Injection	29.0	•	•	•	
Legal Death	31.0	•	•	•	•
Avoidance of Autopsy	34.0	•	•	•	•
Cardiopulmonary Assistance	35.0	•	•	•	
Immediate External Cooling	36.0	•	•	•	•
Medical Tests After Legal Death	40.0				•
Maintain Records	45.0	•	•	•	•
Std. Procedures for Induction of SSH	50.0	•	•	•	•

1. "CDWNR" means: clinically dead with no resuscitation attempted during the period of time indicated.

Figure 14-1

nounce and certify death *without cessation of cardiopulmonary assist* (31.0). Autopsy must be avoided (34.0), cardiopulmonary assistance must be maintained (35.0), and external cooling must be initiated without delay (36.0). Complete records must be maintained (45.0) and standard procedures for the induction of SSH carried out (50.0).

14.3 Column D. If the Donor has been clinically dead for more than ten minutes, with a doctor present, it is nearly certain that the physician will have pronounced the Donor dead (otherwise, resuscitation would have been in progress). Assuming that the state of clinical death under well defined conditions (20.0, 21.0) existed for longer than ten minutes, the chances of successful resuscitation are extraordinarily slim. Further, cardiopulmonary assistance in conjunction with the induction of SSH may not be appropriate (21.0).

The Society Representative should first assure himself that the finding of clinical death is valid. Should doubt exist, the appropriate course of action would be defined by Column C. Assuming that the Society Representative has no doubts, he should proceed with obtaining certification of death (31.0), avoidance of autopsy (34.0) and immediate external cooling (36.0). Medical tests as necessary to more completely document the Donor's condition should be performed (40.0). Complete records must be maintained (45.0), and standard procedures for the induction of SSH must be carried out (50.0).



# 15.0

## GETTING THE FACTS AND GIVING INSTRUCTIONS (NO DOCTOR PRESENT)

### 15.1 FIND OUT:

- (1) Name of Donor? Is he carrying a "Uniform Donor Card"? What does it say? (Be sure Donor is an individual protected by your society or by an affiliated society with formal reciprocal arrangements. Section 23.0 contains additional information.)
- (2) Condition of the Donor? (What is wrong? Is he conscious? Breathing? Does he have a pulse? If no breathing or pulse, how long has he been that way?).
- (3) Is help on the way? (Has a doctor or ambulance been called? What is the doctor's name? Name of the ambulance company? Which hospital will the Donor be taken to?).
- (4) Who is on the phone? Where is the Donor (exactly)? Can the caller render emergency assistance (resuscitation and first aid) as needed?

### 15.2 GIVE INSTRUCTIONS:

- (1) Help the Donor (resuscitation and first aid as appropriate).
- (2) Call for medical assistance, if this has not been done (give the caller the names of preferred ambulance companies, preferred physicians, preferred hospitals, and all applicable phone numbers (16.0) If this cannot be done by caller (caller too busy resuscitating Donor, etc.) Society Representative does it.

# 16.0 SOURCES OF MEDICAL ASSISTANCE

16.1 A telephone call will bring medical assistance. It is suggested that appropriate organizations and individuals be evaluated and listed below.

## 16.1.1 Ambulance Services

Name	Telephone	Category
_____	_____	_____
_____	_____	_____
_____	_____	_____

## 16.1.2 Physicians

_____	_____	_____
_____	_____	_____
_____	_____	_____

## 16.1.3 Preferred Hospitals

_____	_____	_____
_____	_____	_____
_____	_____	_____

16.2 How to Classify Ambulance Services. Ambulance services may be grouped into three categories as follows:

16.2.1 Category "A". Rescue and Medical Transportation Services only. Personnel highly trained and equipped. (Advanced Red Cross First Aid qualification or equivalent. Equipment includes heart-lung resuscitator.)

16.2.2 Category "B". Rescue and Medical Transportation Services only. Personnel highly trained (Advanced Red Cross First Aid qualification or equivalent).

16.2.3 Category "C". All others.

16.3 How to Classify Physicians. Physicians may also be grouped into categories, as follows:

16.3.1 Category "A". The Donor's own personal physician. Should be knowledgeable about the Donor's wishes concerning SSH and willing to cooperate. Skilled (not merely well informed) in resuscitation. As the Donor's personal physician, this doctor will be most able to assure that certification of death can be accomplished without autopsy or cessation of cardiopulmonary assistance.

16.3.2 Category "B". Skilled (not merely well informed) in resuscitation. Understands about anatomical donations for SSH through discussions with Society Representative. Not necessarily sympathetic to SSH objectives, but willing to do what is possible to effect certification of death without autopsy or cessation of cardiopulmonary assistance in the event of the Donor's death.

16.3.3 Category "C". All others.

16.4 How to Classify Hospitals. An evaluator should visit the emergency room of the candidate hospital, sit down, and observe what happens. He should try to judge if patients are promptly cared for, and whether or not proper resuscitation equipment is available. The evaluator can tell the person in charge that he is studying emergency room procedures for an incorporated, non-profit research society (assuming that the evaluator is a Society Representative). He may ask if the emergency room has, immediately available, the following equipment:

a heart-lung resuscitator,  
an electro-cardiograph, and  
a defibrillator.

The following general outline may be helpful in classifying hospitals:

16.4.1 Category "A". Preferred by Category "A" physician. Has a heart-lung resuscitator, an electro-cardiograph, and a defibrillator immediately available. All patients appearing to have serious complaints are promptly cared for by efficient, concerned personnel who seem competent. (This last evaluation is particularly difficult to make. If the person on duty is a fully qualified physician, rather than an intern or a nurse, the chances of getting competent medical care are greater, but this is still no guarantee.)

16.4.2 Category "B". Has a heart-lung resuscitator, an Electro-cardiograph, and a defibrillator, immediately available. All patients appearing to have serious complaints are promptly cared for by efficient, concerned personnel who seem competent. (This last evaluation is particularly difficult to make. If the person on duty is a fully qualified physician, rather than an intern or nurse, the chances of getting competent medical care are greater. But this is still no guarantee.)

16.4.3 Category "C". All patients appearing to have serious complaints are promptly cared for by efficient, concerned personnel who seem competent. (This last evaluation is particularly difficult to make. See above for suggestion.)

16.4.4 Category "D". All others.

## 17.0

GETTING THE FACTS  
AND GIVING INSTRUCTIONS  
( DOCTOR PRESENT )

17.1 Talk to the Physician in Charge. Ask to whom you are speaking. If it is not the physician in charge, find out who *is* the physician in charge (and his telephone number). Also, ask the caller the Donor's name, condition and (exact) location.

17.2 Ask the Physician in Charge:

17.2.1 Donor's name, condition, and exact location (even if you got it from a previous caller). Be sure the Donor is an individual protected by your society or by an affiliated society with formal reciprocal arrangements (23.0).

17.2.2 Specifically, learn the following things about the Donor's condition: Is he alive or being resuscitated? If so, what is the prognosis (how long is he expected to live)? If the Donor is clinically dead, how long has this condition existed (in minutes)?

17.3 Explain to the Physician:

17.3.1 Patient is an anatomical donor. The whole-body donation applies *only* to his society. He is *not* to be considered a donor for organ transplantation of *any* kind.

17.3.2 Autopsy would be very destructive to the value of the anatomical gift, and should be avoided. Integrity of the whole organism is of crucial importance to the research objective.

17.3.3 Continued potential viability of the Donor's tissues is of paramount significance. Since cooling can prolong the period of tissue survival in the absence of vital functions, surface cooling *must* be initiated after death is pronounced.

17.4 Depending on Circumstances, Request Action (One of the Following):

17.4.1 (Donor Clinically Dead, Less Than Ten Minutes). *Regardless* of whether or not death has been "pronounced", request that the physician begin resuscitation *immediately*, restoring respiratory and circulatory functions by artificial means (also request the physician proceed per paragraph 17.4.2 below).

17.4.2 (Donor Alive or Being Resuscitated). Request that the Physician:

17.4.2.1 Provide cardiopulmonary assistance without cessation should clinical death occur, augmented by any fluid therapy that is appropriate. Even if restoration of normal physiological functions seems hopeless, *do not discontinue* cardiopulmonary assistance. Maintain respiratory and circulatory functions by artificial means until an authorized Society Representative arrives.

17.4.2.2 Clearly establish the nature of the Donor's disorder, by any means necessary (such as further examination or tests) to assure that no autopsy will be required if the patient dies.

17.4.2.3 Unless contraindicated by Donor's condition, inject heparin (29.0) and repeat these injections every four hours until the Donor's condition improves or until the Society Representative arrives.

17.4.3 (Donor Clinically Death -- More Than Ten Minutes). First, verify that death has been "pronounced". Then request that the physician:

17.4.3.1 Externally cool the Donor by application of crushed ice, particularly to the head.

17.4.3.2 Postpone any autopsy or other post-mortem examination until an authorized Society Representative arrives.

17.5 Tell the Physician: That an authorized Society Representative will be on the scene as soon as possible.

## 18.0 GENERAL COORDINATION PROCEDURES

When an emergency arises and a Donor's life is in danger, a Society Representative must go to the scene at once. Meanwhile, a second Society Representative should be coordinating, by telephone, all who will be participating in the induction of SSH (if this is required). The Society Representative doing the coordinating, henceforth referred to as the "Coordinator", will:

18.1 Alert the SSH Induction Team. Assuming that the society has a well organized SSH induction team (8.0), these individuals must be located and notified of the condition of the Donor. A radio paging system in conjunction with an answering service may be used for this purpose (9.0).

18.2 Get Medical Assistance to the Scene. The Coordinator should refer to section 16.0 in deciding which physician should be contacted.

18.3 Arrange for Transportation of the Donor in the Event of Death. Prior arrangements for this transportation should be made if it seems likely that the Donor will die. See 44.0 for further information.

18.4 Keep Track of the Condition and Location of the Donor. The Coordinator should phone persons at the scene frequently, until a Society Representative has arrived, to keep abreast of changes in the Donor's condition, and to coordinate or be advised of any plans to move the Donor, from home to hospital, from one hospital to another, etc. The Coordinator should continue to call the scene even after the Society Representative arrives, to check on developments at the scene. The Society Representative might find it difficult to contact the Coordinator, whose phone is constantly busy as he carries out other coordination operations.

18.5 Alert Organizations Providing SSH Services or Facilities. The society may have formal arrangements with morticians, non-SSH rescue teams, or other organizations for use of equipment or facilities (8.0). Appropriate notification to these organizations involved should be provided in anticipation of need for the support of these organizations.

# 19.0 GOING TO THE SCENE

In going to the scene, a Society Representative must necessarily use that transportation which happens to be available. It is assumed that the Society Representative has access to a motor vehicle. Two factors should be considered:

- (A) How urgent is the situation?
- (B) What equipment must be taken?

19.1 Factor (A): Urgency. If no persons on the scene are skilled in resuscitation, and the Donor appears to be dying or has just entered a state of clinical death, it is very urgent that someone skilled in resuscitation arrive at the scene as quickly as possible. If the Society Representative can reach the scene very quickly, he may choose to leave immediately. If another Society Representative is closer to the scene, he should be called and sent. If police or fire department personnel are skilled in resuscitation (the latter usually are), or if a competent ambulance service (16.0) is located close to the scene, these organizations should be called.

The single most important factor is getting a person skilled in resuscitation to the Donor as quickly as possible, if the Donor is dying or clinically dead. If circumstances are less urgent, if there is time for several minutes of preparation and travel to the scene at lawful speeds, then consideration should be given to taking equipment and carrying out other actions before leaving for the scene.

19.2 Factor (B): Equipment. The Society Representative should take with him a heart-lung resuscitator (26.1) and/or any other resuscitation equipment (26.2, 26.3) he possesses. He should also take at least the minimum equipment needed for those steps which are required prior to arrival at a perfusion facility, in the event that the induction of SSH becomes necessary.



## 20.0 SIGNS OF CLINICAL DEATH

Clinical death is the cessation of respiration and blood circulation; breathing stops and the heart stops. This condition can often be reversed and life "restored" if resuscitation (24.0) is instituted quickly enough.

Asphyxiation is the stoppage of breathing alone; the heart continues to beat. This condition progresses into clinical death within a few minutes if respiration is not restored.

There is no inverse to asphyxiation, where the heart stops but breathing goes on. In cardiac arrest (heart stoppage), breathing stops when the heart stops.

In clinical death<sup>1</sup> no pulse or other sign of heartbeat can be found. No physical movements of breathing can be detected. The general color of the skin becomes pale or "blue". The pupils of the eyes dilate and will not contract on exposure to bright light. If a finger nail is sharply pressed, the color will disappear and will not reappear rapidly when the pressure is released.

Electrical activity of the brain will fall to very low levels shortly after clinical death, and blood gases will depart from normal levels (oxygen is lower, carbon dioxide is elevated). These signs can not be observed without sensitive instruments.

Two clearly distinguished levels of examination are appropriate, depending on the circumstances, in ascertaining the existence of clinical death:

20.1 Circumstance A. The Donor may have been clinically dead for only a short time. The Society Representative will probably attempt resuscitation immediately (24.0). He spends a few seconds as follows:

- (1) Listens for signs of respiration (20.1.1).
- (2) Checks for pulse or other sign of heartbeat (20.1.2)
- (3) Checks to see if pupils of the eyes are dilated, and tests for responsivity with a light if available (20.1.3).
- (4) Observes skin color (20.1.4).
- (5) Pinches a finger to test color response (20.1.5).

20.1.1 Listening for Signs of Respiration. One's ear is placed on the Donor's chest or immediately next to his mouth, and any sounds of air passage are noted. At the same time, any body motions in the chest or throat corresponding to breathing are sought. The Donor *may* be *attempting* to breath, without success, breathing being prevented by an obstruction in his throat or a throat injury of some kind. Only a definite in-rushing and out-rushing of air constitutes successful breathing. Unsuccessful attempts to breath on the part of the Donor call for immediate use of artificial respiration (24.0).

20.1.2 Detection of Pulse or Other Signs of Heartbeat. One's fingertips are pressed gently into the underside of the wrist, on the thumb side, or into the throat immediately beside the voice box (adam's apple). A distinct pulse should be felt in these locations if a normal heartbeat exists. Finding a pulse may be practiced on one's self. Placing an ear on the Donor's chest may reveal some indication of heartbeat. Even with the most careful examination, complete cessation of heartbeat (cardiac arrest) is not always easily determined. In some instances, sporadic spasms may continue to circulate blood which is oxygenated to some degree. This activity may go unnoticed, and yet it might sustain the necessary pre-conditions for successful resuscitation for periods greater than ten minutes.

20.1.3 Dilated Pupils, Unresponsive to Light. Several minutes after the onset of clinical death, the pupils of the eyes dilate (open up) and will not contract upon exposure to bright lights (as do most normal eyes). A small flashlight may be used to test this reflex. An exception to this phenomena is found where certain drugs have been taken (pupils do not dilate and remain constricted even after clinical death). Conversely, the sign of dilated pupils may continue for some time after resuscitation is begun. Instances have been cited where pupils remained fixed and dilated throughout resuscitation episodes lasting over three hours, where the patient made a full recovery with no evidence of neurologic (brain) damage<sup>2</sup>.

20.1.4 Observation of Skin Color. When blood circulation stops, oxygen is quickly consumed, and the blood loses its characteristic red color. This produces a color change in the skin, so that it appears pale, "dusky", or even "blue". One known exception to this exists, in that carbon monoxide poisoning may produce an abnormally pink skin coloration.

*OR CLAY RED*

20.1.5 The Fingernail Pinch Test. When the heart stops, the arterial-venous pressure which forces blood through the capillaries

(smallest blood vessels) drops, and blood squeezed out of tissues returns more slowly. In a person with normal circulation, a fingernail that is pinched turns to a lighter color quickly as blood is squeezed from the tissue directly underneath, and the normal color returns quickly when the pressure is released. In a case of cardiac arrest, color under a pinched fingernail returns noticeably more slowly than in a person with normal circulation.

20.2 Circumstance B. The Donor, according to the reports of bystanders or physician, has been clinically dead for well over ten minutes, and chances for successful resuscitation are extremely remote. The Society Representative feels inclined to accept this evaluation (otherwise he would be following Circumstance A), but he intends to confirm clinical death by his own examination. He will, at most, spend several minutes in this. If any signs of life are evident, he will begin resuscitation immediately. The Society Representative first performs the examination described previously under Circumstance A. He then proceeds as indicated below.

20.2.1 A mirror (preferably cooled with faucet water and then dried) may be placed beside the Donor's mouth. If any breathing function exists, water vapor will condense on the mirror.

20.2.2 A shallow dish of water may be placed on the Donor's chest. Any bodily movements or sporadic heartbeat will be evidenced by movements on the surface of the water (rippling).

20.2.3 Consideration must be given to any circumstances which would increase the probability of survival after extended periods of clinical death. Since these circumstances are not "signs" observed in the Donor, they are discussed elsewhere (21.3).

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1. In addition to the discussions in section 20.0 concerning signs of clinical death, the introductory paragraphs of "Heart-Lung Resuscitation", by A.S. Gordon, M.D., describe the integrated examination for clinical death and the initiation of resuscitation. "Heart-Lung Resuscitation" is an attachment to this manual, and is located in section 25.0.

2. "What Do 'Fixed, Dilated Pupils' Mean?", by G. G. Gauger, letter to the Journal of the American Medical Association, vol. 284, p. 1105, 1971.

# 21.0 THE DECISION NOT TO ATTEMPT RESUSCITATION

21.1 The Basic Issue. If the Donor is clinically dead (20.0) and has been in that condition under normal circumstances for more than ten minutes, the probability of successful resuscitation is extraordinarily slim. In this event, the Society Representative may decide not to attempt resuscitation and may decide to proceed with alternative actions (22.0).

In reaching this decision, the Society Representative must be certain that the condition of death is truly irreversible. He must understand that he must be prepared to defend the correctness of his decision and accept responsibility for the consequences of his actions. The Society Representative must take into account unusual circumstances (21.3) which may greatly extend the ten minute criterion, and any other factors (such as bystanders' probable ignorance of what constitutes clinical death) that may be applicable.

21.1.1 The Ten Minute Criterion. Figure 21-1 shows the curve from an American National Red Cross brochure titled "Artificial Respiration". As one can see, chances for recovery by means of current medical practice becomes very slight at eight minutes and vanishingly small at ten. Ann Cutler's book<sup>1</sup> cites slightly less optimistic figures, indicating

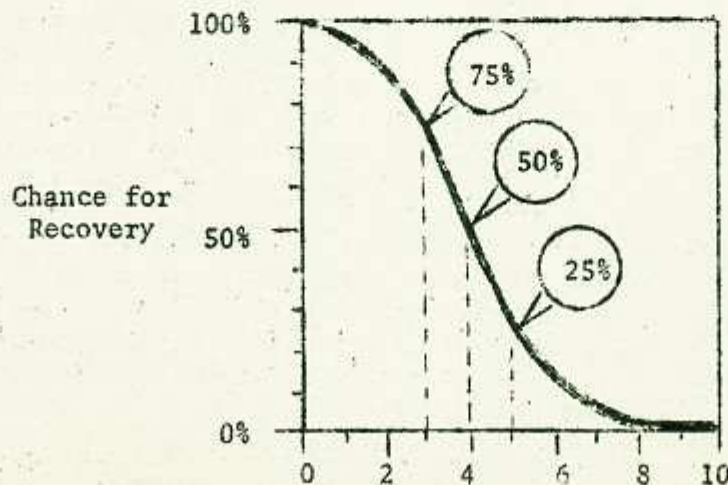


Figure 21-1

Time in minutes after  
Cessation of Breathing

- (1) Obtain "Advanced" Red Cross First Aid qualifications (27.0)
- (2) Study section 25.0 and its cited references. Formalize this study by maintenance of a notebook with dated, signed, and witnessed entries.
- (3) Consider all legal hazards (23.0) and possible interpretations of the circumstances (30.0).

21.3 Unusual Circumstances. Clinical death for periods exceeding ten minutes does not necessarily mean that resuscitation is hopeless. In certain circumstances, total arrest of circulation for far more than ten minutes will *not* result in permanent damage. In other circumstances, the imperceptible continuance of circulation and other physiological functions may sustain cellular metabolism to a partial extent, so that successful resuscitation is possible long after superficial signs of vital functions have vanished. These cases, while rare, occur too frequently to be ignored. The below treatment of unusual circumstances is not comprehensive, and is to be regarded only as an initial outline. Subsequent revisions of this section will be more detailed.

21.3.1 Acute Drug Intoxication. In cases of acute drug intoxication certain signs of life may be absent. In particular, a silent electroencephalograph may be observed. Since such patients frequently recover with little or no evidence of neurological damage, extended attempts at resuscitation are warranted, if required.

21.3.2 Hypothermia. In hypothermia, as in acute drug intoxication, a silent electroencephalograph may be observed, and this does not necessarily signify "brain death". Controversy exists as to the best approach to be followed in withdrawing patients from hypothermia<sup>5</sup>.

21.3.3 Drowning. Victims of drowning are often resuscitatable after greater periods than persons passing into a state of clinical death from other causes. Partially, this may be due to the hypothermic effects of contact with cold water. Also, if the drowning takes place in water of significant depth, the residual gas in the lungs has higher partial pressures of oxygen than would be the case in asphyxiation at normal atmospheric pressures.

## REFERENCES

1. "Four Minutes to Life", by Ann Cutler, Cowles, 1970, p. 19.
2. "Effect of Duration of Complete Circulatory Arrest on Recovery of Bodily Functions During Resuscitation with the Heart-Lung Apparatus", by A. A. Bozhev, Bulletin of Experimental Biology and Medicine, USSR, 70-747, 1970.
3. "Recovery of Neuronal Function after Prolonged Cerebral Ischemia", K. A. Hossman and K. Sato, Science, 168-376, 17 April 1970.
4. "Survival Following Extreme Hypoxemia", by F. D. Gray and G. J. Horner, The Journal of the American Medical Association, 211-1815, 16 March 1970.
5. "Rapid Active External Rewarming in Accidental Hypothermia", by J. P. Fernandez, et al, The Journal of the American Medical Association, 212-153, 6 April 1970.

# 22.0

## ALTERNATIVE ACTIONS IN THE EVENT OF A DECISION NOT TO ATTEMPT RESUSCITATION

In the event a Donor is in a state of clinical death where successful resuscitation appears hopeless, the Society Representative may decide not to attempt resuscitation. This decision is discussed in 21.0, and legal dangers are warned of in section 23.0. The alternative to resuscitation is as follows:

Immediate external cooling (36.0) should be initiated. The Donor's head, in particular, should be cooled as quickly as possible. No time should be lost in bringing the Donor (while being cooled) to proper medical authorities for certification of death. The manner in which this is done may be crucially important (30.0).

There is little else that can be said as to "alternative actions" at this time. The Society Representative may never encounter these circumstances, but he should be ready for them. In almost every case, hopefully, the Society Representative may decide to initiate resuscitation immediately. In those rare cases where this action is not appropriate, the alternative action *must* be immediate external cooling. Failure to undertake one of these two courses of action would be inconsistent with the objective of sustaining maximum potential viability in the Donor, and this objective must not be compromised. Either resuscitation or immediate external cooling must be undertaken if the Donor is clinically dead. There is no acceptable third alternative.

## 23.0 LEGAL PROBLEMS

The Society Representative faces legal problems regardless of his choice of actions. Laws vary from state to state. Although the Society Representative will not always meet adverse circumstances, a knowledge of possible problems is advisable.

If the Society Representative is called to the scene and finds the "Donor" is in fact not a donor at all, leaving the scene without taking action could lead to a suit for failure to render assistance. The probability is that the suit would fail, but a good deal of time might be expended in court proceedings.

If the Society Representative decides the Donor's case is hopeless and initiates external cooling, he could conceivably be tried for charges as serious as manslaughter. If the Society Representative successfully revives the Donor, but there is some residual injury or damage, he might be sued for these damages. If the Society Representative's resuscitation of the Donor is totally successful but involves measures traditionally used only by licensed physicians, he might be indicted for practicing medicine without a license.

23.1 Verifying the Donor's Identity. Upon first notification that a Donor is in danger, and later upon arriving at the scene, the Society Representative should verify the identity of the Donor. The Donor's "Uniform Donor Card" should show an anatomical gift to the Society Representative's own society, or to a society with which reciprocal arrangements exist (8.3). The Society Representative may possess up-dated lists identifying all Donors, so that unwarranted alerts are avoided and the legal dangers of rescuing a non-donor are not incurred. Some societies may provide for Donors signing releases, releasing authorized Society Representatives from liability in connection with attempts to save their lives through resuscitation or other measures (8.1.3).

23.2 The Decision Not To Attempt Resuscitation. If the Donor is alive, no resuscitation is called for. If the Donor is clinically dead for less than ten minutes, resuscitation is initiated as a matter of course. If the Donor has been clinically dead longer than ten minutes, a decision is required (21.0).



At the time of this writing (Jan 1972) it is unlikely that there would be suits or criminal charges filed simply as a result of "failure to resuscitate", if there were good reason to believe the Donor had been clinically dead longer than ten minutes. (In future years, should the induction of SSH become widespread practice, a Society Representative might be sued for "unwarranted resuscitation" where such would be held to be of greater damage to the Donor than immediate external cooling. This situation does not presently exist.) On the other hand, if a Society Representative decides "not to resuscitate", it will be incumbent on him to immediately pursue an alternative course of action (22.0). It is this consequence of "not resuscitating" that holds the greatest legal dangers at this time.

23.3 The Decision to Take Alternative Actions. If the Society Representative decides "not to resuscitate" and follows "alternative actions" involving immediate external cooling of the Donor, he may be subject to both civil and criminal action. There are no safeguards against these consequences known to the authors of this manual, but a review of possible circumstances and the courses of action that might be appropriate is provided in section 30.0.

## 24.0 RESUSCITATION

Resuscitation is the artificial restoration of breathing and circulatory (heart) functions when these are absent. The necessary effects can be produced entirely without equipment for short periods of time (25.0). More effective resuscitation can be carried out, and sustained for extended periods of time, if special equipment is available (26.0). Resuscitation administered by a lay person is only one aspect of first aid (27.0) and may be useless if other vital aspects of first aid are ignored. For example, serious loss of blood should be halted before resuscitation is started. Resuscitation is useless if the Donor is meanwhile permitted to bleed to death.

Assuming that the Donor is in a state of asphyxiation or has been clinically dead for a short period, resuscitation must be initiated at once (25.0) aided by special equipment if available (26.0). Once resuscitation is begun, it should be tenaciously continued until either the Donor's normal physiological functions are restored or until it is proper that cardiopulmonary assist be discontinued at the proper stage of SSH induction (35.0).

## 25.0 RESUSCITATION MEASURES

Proficiency in resuscitation cannot be simply grasped from brief instructions in this manual. The reader can learn the basic aspects of mouth-to-mouth resuscitation at a practical level in a first aid course (27.0). Depending on local policies of organizations such as the American Heart Association, training in cardiopulmonary resuscitation may be obtained. Other sources of knowledge are available, such as programmed learning courses from the Medical Supply Company (see Appendix A).

A pamphlet titled "Heart-Lung Resuscitation" is attached. A careful study of this will provide an introductory understanding of the mechanical actions required in manual cardiopulmonary resuscitation. An excellent book by Ann Cutler<sup>1</sup> is available which dramatically presents the importance of resuscitation, its history, current methods, and the enormous improvements that are needed in emergency medical services.

Other publications on resuscitation<sup>2,3,4,5</sup> are available. The reader will find that in addition to these, the medical literature is filled with applicable information, only a few of which are cited here<sup>6,7,8,9,10</sup>.

It is possible that resuscitation will become a medical specialty in the not too distant future. There is so much to be known that this would certainly be appropriate. Even more to the point, since resuscitative measures must be applied without pause for consideration and study, the individual responsible must be ready at any moment. One of Ann Cutler's<sup>1</sup> examples is so significant that a short excerpt is provided:

"Dr. Smith gave the case history of an eighteen-year-old football player who died while having a broken nose set by two general practitioners. The doctors used tetracycline-soaked wedges in the smashed bony ledges within the nose to provide local anesthesia. There was nothing complicated about the break and since the boy was a husky specimen whose health had always been excellent, there seemed little reason for apprehension.

"But the anesthetic was so rapidly absorbed that it produced vascular collapse that quickly progressed to cardiac arrest. The football player

who had come in with a broken nose was clinically dead. Neither of the physicians knew how to use modern methods of resuscitation to restore breathing. In desperation they called the fire department. But by the time the firemen arrived the boy was beyond recall."

One cannot know too much about resuscitation. The medical profession has many programs being vigorously pursued to prevent recurrence of such instances as the football player death cited above, but no one "knows it all" and each should learn as much as possible.

It is strongly recommended that the reader study thoroughly the information provided in and cited by this section. A detailed notebook formalizing this study with dated, signed, and witnessed entries might be valuable in the event that circumstances occur where resuscitation is not appropriate (21.0). It is recommended that courses available through the American National Red Cross and the local chapters of the American Heart Association be taken if these are available.

One last recommendation: Read "Four Minutes to Life" by Ann Cutler. The last chapter, 17 pages in length, is particularly interesting. Its title is "Cryonics--The Ultimate in Resuscitation".

#### REFERENCES

1. "Four Minutes to Life", by Ann Cutler, Cowles, 1970.
2. "Emergency Measures in Cardiopulmonary Resuscitation", The American Heart Association, 44 E. 23rd St., New York, N.Y., 10010.
3. "Cardiopulmonary Resuscitation; A Manual for Instructors", The American Heart Association, 44 E. 23rd St., New York, N. Y., 10010.
4. "Fundamentals of Cardiopulmonary Resuscitation", by J. R. Jude and J. O. Elam, Davis.
5. "Cardiac Arrest and Resuscitation", by H. E. Stephenson, Mosby, 1969.
6. "Closed Chest Cardiac Resuscitation: Problems in Training of Laymen in Volunteer Rescue Squads", by P. Braun et al, New England Journal of Medicine, 272:1-6, 1965.
7. "Mouth to Mouth Resuscitation", by B. R. Brown, Journal of the American Medical Association, 203: 156, 1968.
8. "A Year's Experience with a Mobile Coronary Resuscitation Unit", by H. A. Dewar et al, British Medical Journal, 4-226, 1969.

9. "Resuscitation and Survival in Motor Vehicle Accidents", C. F. Frey et al, Journal of Trauma, 9-292, 1969.
10. "Simplified Airway Management During Anesthesia or Resuscitation - A Binasal Pharyngeal System", Anesthesia and Analgesia, 48-307, 1969.
11. "Hazardous Complications of Closed Chest Cardiopulmonary Resuscitation", G. F. Fletcher, American Heart Journal, 77-431, 1969.

# 26.0

## RESUSCITATION EQUIPMENT

Primarily, resuscitation equipment serves to restore respiration and circulation by mechanical means. Heart-lung resuscitators function to assist both respiration and circulation, while more simple apparatus is available which is limited to restoration of respiration. Devices which keep the air passages to the lungs open are also available. Training manikins are available for resuscitation demonstration and practice.

**26.1 Heart-Lung Resuscitators.** Heart-lung resuscitators (HLR) or cardio-pulmonary resuscitators, as they are sometimes called, are the most elaborate and costly of mechanical aids to resuscitation. These devices provide for both artificial respiration and external cardiac compression (25.0) in a highly controlled and synchronized way. Two widely used units (see below) each weigh about 25 pounds (without oxygen bottles) and cost approximately \$1300.00. Both are fully powered by compressed oxygen, which drives the cardiac compression piston and also repetitively fills the patient's lungs. Users may write to the manufacturers of this equipment (26.1.2) to obtain detailed illustrated brochures providing specifications, descriptions of how the equipment is used, and operating instructions. One type of HLR is illustrated in figure 26-1.

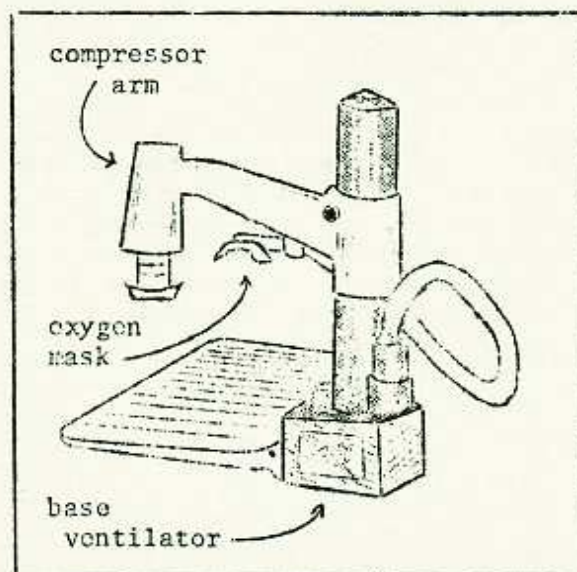


Figure 26-1  
Portable HLR

26.1.1 The Need for HLR's. The combined use of mouth-to-mouth resuscitation and external cardiac compression (25.0) makes it possible for trained rescuers to restore cardiopulmonary functions immediately in the clinically dead patient. The importance of beginning resuscitation at once makes the manual techniques particularly valuable.

In most cases, however, the final outcome will not be determined for some time. Normal cardiac function is frequently not restored by resuscitation alone, and up to several hours of resuscitation may be required before definitive therapy including electrical defibrillation and the administration of appropriate drugs can produce the desired effects. During this time, persons using manual resuscitation techniques will become exhausted. The manual methods, not optimum to begin with, will become even more imperfect as fatigue sets in. The effectiveness of resuscitation will be reduced, and along with it the chances of an ultimately successful recovery.

Mechanical aids to resuscitation, in cases where prolonged resuscitation is required, can make the difference between life and death.

26.1.2 Available Equipment. The Brunswick Manufacturing Co., Inc., makes the Model HLR 50/90 Heart-Lung Resuscitator, distributed by Travenol Laboratories, Inc. Michigan Instruments, Inc., manufactures and distributes the Model 1003 (MIICPR) Cardiopulmonary Resuscitator. The addresses of distributors are provided in Appendix A.

26.2 Oxygen Respirators. Oxygen respirators, functionally, fulfill the general requirements of that portion of the heart-lung resuscitator which provides artificial respiration. When using an oxygen respirator, external cardiac compression (when required) must be performed manually (25.0). Some oxygen respirators are highly automatic, while others require the continuous attention and control of a human operator. A broad selection of this type apparatus is distributed by the Kelco Supply Company (see Appendix A).

26.3 Airways. Airways are devices that may be fitted into the throat of persons needing artificial respiration, which provide an open path into the lungs. The "Brook" airway, shown in Figure 26-2 is a sophisticated device of this category with one way valves for blowing air into the patient's lungs, separate exhaust path, etc. Most airways are deeply inserted into the throat. They are not easy to insert, and can cause damage to the patient if insertion is not correctly performed.

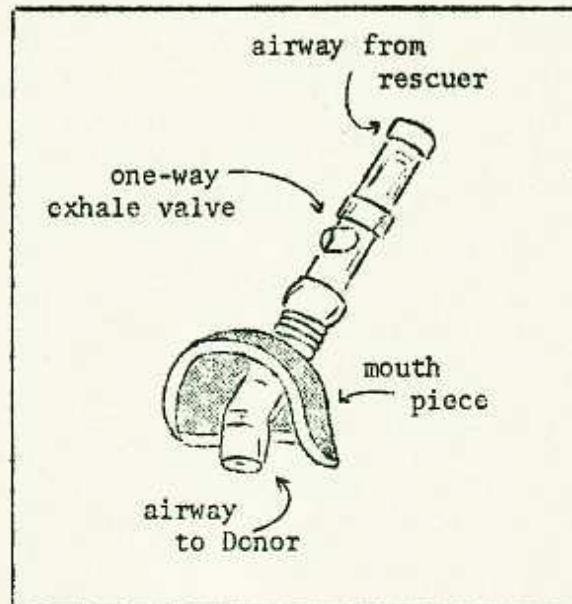


Figure 26-2: Brook Airway

26.4 Resuscitation Manikins. Devices are available which replicate the upper chest and head of a human being, and may be used for practice of manual resuscitation or the demonstration and testing of resuscitation equipment. Some of these devices have such features as artificial hearts to be compressed in the practice of external cardiac compression, inflatable lungs, points at which a pulse may be felt, etc. Several models of such equipment are available through the Kelco Supply Company (see Appendix A).



## 27.0 FIRST AID

A Donor who has been successfully resuscitated must be placed under the care of a physician as quickly as possible. The same applies to the Donor who is critically ill but still alive. In the event that delays occur in obtaining professional medical assistance, the best lay level care possible must be given. This constitutes "first aid".

Throughout this manual will be found recommendations that each reader obtain "Advanced" level first aid training from the American National Red Cross or other organizations offering first aid courses. It is also recommended that some Society Representative in each area continue in this training until an "Instructor" level of qualification is obtained. So qualified, he can then proceed to teach important life-saving principles to the others in a formal context.

One book is recommended in connection with first aid. It is written for situations where medical aid is remote, and the treatment is very comprehensive when compared with other lay-level texts on emergency medical care. The book is "Medicine for Mountaineering", edited by James A. Wilkerson, M.D. It is carried by many sporting goods stores stocking mountaineering supplies as well as all book stores.

Although this section is in the operational section of the instruction manual, no treatment of the actual subject matter is given. A comprehensive treatment would be impossible in the available space, and a simplified treatment might be more dangerous than helpful. Every user of this manual should know first aid, and should acquire this knowledge in advance of emergencies through participation in appropriate training programs.

## 28.0

MEDICAL TESTS ;  
DONOR ALIVE OR  
BEING RESUSCITATED

Assuming that a doctor is with the Donor, the doctor should be asked to summarize the Donor's condition. Then the doctor should be asked "if the Donor dies, is your knowledge of the case sufficiently complete so that no autopsy will be required to determine the cause of death?" If the doctor's knowledge is not sufficient to eliminate a possible autopsy, every effort should be made to persuade the doctor to conduct any additional examinations or tests that he needs while the patient is alive or while attempts at resuscitation continue.

Once it is clearly established that the danger of autopsy does not exist, the question of additional tests to further define the Donor's condition for record purposes should be raised. Perhaps the Donor has been under observation in a hospital for weeks, and extensive data may be available, in which case further tests would be totally unnecessary. It is also possible that the Donor has been brought to an emergency room with an "apparent heart attack", and nothing whatever of his condition is medically documented. In this event, any appropriate tests would appear to be well justified.

Some physicians may question the need for additional testing in the case of a Donor whose case seems hopeless. They are used to the practice of routine autopsy for obtaining complete information, and circumstances where additional information is desired without autopsy may seem contradictory to them. The Society Representative must explain the reasons for avoiding autopsy (17.3), and point out that the Society receiving the anatomical gift needs complete records of the Donor's condition for its future research.

The Society Representative should record the names of all physicians attending the Donor. Later, these doctors can be interviewed for supplementary information. The Society Representative should record a description of any tests or examinations performed, and determine where the applicable medical records are filed, so that copies of these records can later be requested from the proper office.

## 29.0 ADMINISTRATION OF HEPARIN

Heparin is a pharmaceutical preparation which can prevent the coagulation of blood constituents, but it cannot break up clots which have already formed. To be effective, heparin must be distributed throughout the circulatory system, so that introduction of heparin after circulation has stopped will do little.

Heparin should be introduced into the circulatory system while the Donor is alive or being resuscitated. If this procedure proves impossible, heparin should not be administered until Phase I perfusion (60.0) is initiated.

Heparin should NOT be administered by lay persons. A doctor's judgement is essential to this treatment.

**29.1 Contraindications.** If there is a reasonable chance the Donor will recover, and the use of heparin might be detrimental to his chances, it should not be administered, and no insistence as to its administration would be appropriate. If, for example, the Donor has been in a bad automobile accident, the injection of heparin would greatly aggravate any internal bleeding that exists and might cause the Donor to die very quickly.

**29.2 Doses.** If there are no contraindications, heparin should be administered according to the below chart. If the Donor is being resuscitated or dying quickly, an initial dose of double the hourly level may be appropriate. If the Donor's condition is stable, although critical, the hourly amounts shown should be adequate.

Donor Body Weight (lb.)	Initial Injection International Units (I.U.)	Hourly Injections International Units (.I.U.)
50	500	250
100	1000	500
150	1500	750
200	2000	1000
250	2500	1250

## 30.0 SPECIAL CIRCUMSTANCE

The special circumstance is one where the Society Representative finds the Donor in a state of clinical death from which he cannot be recalled with techniques of resuscitation available to the Society Representative. External cooling needs to be initiated immediately, but circumstances prohibit the pronouncement of death for an extended and indeterminate period (this situation could possibly occur if the Donor resides in a remote area far from medical assistance). In such a situation the Society Representative *may* decide to proceed in the best interests of the Donor despite possible legal repercussions. *Removal  
use of*

It is assumed that the Society Representative has decided not to attempt resuscitation (21.0). He has proceeded with alternative actions (22.0) which are filled with legal risks (23.0). The Society Representative has begun external cooling, and now must select an appropriate course of continued action. This may be most difficult. The best choices of action will be governed by the specific circumstances, and no fixed guidelines can be given. The following is a *discussion only* of this issue. The reader must judge for himself if all, or any, of the following are valid or useful.

30.1 The "Remote Possibility of Survival" Premise. The Donor has been clinically dead for some time, under circumstances which to the Society Representative indicate that the possibility of recovery through Resuscitation is nil. The Society Representative, however, unless he is a physician, cannot make a final determination; he cannot "pronounce" legal death. Since the Society Representative has induced a state of hypothermia by direct means (external cooling), he has established conditions more favorable for the Donor's recovery than had he taken no action at all. In principle, it is possible that some factor unknown to the Society Representative exists which would still predispose a physician to attempt saving the Donor's life.

In consideration of this, a logical course of action would be to transport the Donor to the largest, most competently staffed hospital in the area. There, the Society Representative can review all pertinent facts with the resident physicians. If any chance of recovery exists, they will be able to make the best possible attempt to withdraw the Donor from his hypothermic state and effect a successful resuscitation.

On the "Remote Possibility of Survival" premise, then, the Society Representative should call an ambulance (16.0) or provide other transportation for the Donor to the appropriate hospital.

Once committed to this course of action, the Society Representative should steadfastly hold to the premise that a final answer can only be reached by physicians skilled in the use of hypothermia and resuscitation, under operating room conditions. The Society Representative should persuade anyone he encounters, including police, Health Department officials, etc., that the only valid destination for the Donor is a hospital. He should insist that any interference with this or with the process of external cooling compromises what little chance there is that the Donor might be saved.

30.2 The "Call the Police" Choice. In some instances the Donor's condition of death may be so clear cut and well witnessed that no basis for the "Remote Possibility of Survival" premise exists. In this event, after proceeding with alternative actions (22.0) the Society Representative should inform the Police. He should simultaneously inform every other Society Representative he can reach that grave danger exists; the Donor may be summarily autopsied and legal entanglements may forestall proper induction of SSH for extended periods of time.

The first Society Representative's actions, in instituting external cooling may be represented in a manner consistent with the interests of the law. External cooling is accompanied by measurement of body core temperature (46.0), and the initial measurements will furnish police precise information from which the time of death may be estimated (which information, of course, will also be of great value to the Society Representative.) Also, by reducing the rate of biological deterioration and chemical processes within the body, the best interests of later laboratory evaluation are served (traces of poisons, drugs, etc. may be more easily detected if chemical reactions are halted early).

## 31.0

LEGAL DEATH  
AND IMMEDIATE CONSEQUENCES

When a Donor is "pronounced" dead by an authorized individual (32.0), this signifies a turning point in the course of events. Occasionally (17.4.1, 32.1) there will be exceptions, but in most cases, the Society Representative is committed to carry out the induction of SSH in the most effective way possible.

Usually, following the pronouncement of death, the physician or a coroner's representative will execute a death certificate (33.0). Sometimes an autopsy (34.0) is called for as a means of determining the cause of death. The dead person may, or may not, be placed in a cooled container during this period. In many circumstances, embalming is carried out as a matter of course.

Obviously, the usual course of events is totally antithetical to the objective of sustaining viability in a SSH Donor. A totally different procedure is called for, and the Society Representative must prevail over tradition, customary procedures, and all other obstacles.

31.1 Maintaining Cardiopulmonary Functions. If cardiopulmonary resuscitation is in progress, and restoration of normal physiological functions is deemed impossible by the physician in charge, he may be disposed to pronounce death, but he may be very reluctant to do so unless the resuscitation equipment is turned off and all vital signs are permitted to slowly ebb away. This would be disastrous, and must be prevented. See section 35.0 for further information on this subject.

31.2 Avoidance of Autopsy. Once death is pronounced, certification of death (33.0) may be effected readily, if the cause of death is well understood. If it is not, there will be some chance of an autopsy, which must be prevented (34.0). If cardiopulmonary functions are being maintained after legal death (35.0), it may be possible to persuade the physicians to substitute an examination for an autopsy. Perhaps an exploratory operation is required, but this would be far preferable to an autopsy. Surgical procedures would be employed, blood volume being maintained by fluid therapy, etc.

## 32.0      P R O N O U N C E M E N T   O F   D E A T H

The pronouncement of death is a legal act by a properly authorized individual, indicating in effect that no further efforts for the purpose of restoring normal physiological processes are warranted, in consequence of the case being hopeless. Licensed physicians and other individuals (in some cases, policemen and firemen, acting as deputy coroners) may "pronounce" death.

This act signifies a termination of these individuals' attempt to save life, which otherwise they would be bound to pursue. Most well trained individuals authorized to pronounce death will pursue a course of attempting resuscitation if any chance at all exists of saving life. If a Donor is pronounced dead by one of these individuals, the chance of recovery is probably nil.

In certain circumstances, the Society Representative may find that death has been pronounced by a properly authorized individual whose understanding of the signs of clinical death or the techniques of resuscitation is faulty. While these cases may be rare, they are still possible. The Society Representative may find that the conditions indicate good chances for a successful resuscitation, but the official involved, (physician, policeman, etc.) finds the situation instead to be a threat to his position and reputation. It is possible the official may put his reputation and position ahead of the Donor's welfare, and the Society Representative may have to use all his ingenuity to assure that resuscitation is promptly initiated.

Further discussion related to this subject, with references, is provided in Section 35.0.

# 33.0

## CERTIFICATION OF DEATH

Certification of death follows pronouncement of death (32.0) and represents legally sanctioned findings concerning the cause of death. The Donor's personal physician may certify the cause of death if he has examined the Donor within some period of time preceding death (10 days, in Los Angeles County, California), and has findings that are relevant to the cause of death. Otherwise, cause of death is determined by a public official (usually the coroner). Depending on the circumstances, the Coroner may determine that an autopsy is necessary (34.0).

33.1 Blank Death Certificate. The availability of blank death certificates may help expedite certification of death. The Society Representative should have a supply of these. They can be obtained from most morticians and from the County Health Department or Coroner's Office.

33.2 Pre-filled In Death Certificate. If death is anticipated, such as in the case of a Donor with a terminal illness, it is advisable to have a death certificate partially filled in, so that the physician need only add such information as the place, date, time, and cause of death. If the physician (or other official) must acquire the death certificate himself, an extended delay with serious compromise of the Donor's potential viability might result.



## 34.0 AVOIDANCE OF AUTOPSY

The information in this section needs to be specific, so as to be most useable. At the same time, laws regarding autopsy vary from state to state and even from locality to locality. The principal part of the information that follows will be applicable in most places within the United States, but users of the manual should verify this, becoming intimately familiar with local and state regulations, practices, and procedures. General information on autopsy is provided first (34.1), followed by specific recommendations as to its avoidance, and as to measures which can be attempted in the event avoidance is not possible.

34.1 Autopsy; General Information. Autopsy is an external and internal (surgical) examination performed to establish the cause of death. In a "complete" autopsy, "the internal contents of the thorax (chest), abdomen, pelvis and head are examined, usually in that order".<sup>1</sup> The examination is catastrophic to the integrity of the body. Organs are "removed" for examination. The brain is taken from the cranium and "sectioned". After autopsy, organs are returned to their original locations in plastic bags. A "partial" autopsy is restricted to specific locations, in search of specific information.

34.1.1 Basis for Autopsy. Autopsies are either "medico-legal" or "pathological". A medico-legal autopsy is solely for determining whether death resulted from (1) suicide, (2) homicide, or (3) natural causes. In the pathological autopsy, a more detailed determination of the exact and specific cause of death is sought.

34.1.2 Authorization for Autopsy. Medico-legal autopsies are performed in the event that doubt exists as to whether death was from suicide, homicide, or natural causes, as authorized by the Coroner or Medical Examiner of the City or County in which death occurred. Some times, this power of authorization includes the Attorney General, Justices of the Peace, Judges, and Public Health Officials. *Other than for medico-legal purposes (WHEN NO DOCTOR WILL SIGN A DEATH CERTIFICATE) autopsies cannot be performed without consent, usually in written form, of the next of kin or other person entitled under law to possess the body for burial or other purposes.* In the case of an anatomical donor, then,

permission (for other than a medico-legal autopsy) would have to be obtained from the society receiving the anatomical gift. Naturally, such permission would be refused.

34.1.3 Double Indemnity Insurance. Some insurance provides that an increased payment will be made if death is "accidental", and reserves the right of autopsy to the insurance company, as proof of accidental death, if such payment is to be made. In the event that a donor dies and has a double indemnity policy, and an insurance company requests an autopsy, permission could be refused, and the company then would be initially obligated to pay only the face value of the policy. If the request for autopsy was not warranted (Donor died on an operating table from massive injuries sustained in an automobile accident), the matter could perhaps later be taken to court and the additional amount recovered.

## 34.2 Avoidance of Autopsy.

34.2.1 Delay. The first step in avoiding an autopsy is to delay it. The Society Representative should use any valid pretext to delay an autopsy, and at the same time secure permission to begin external cooling (36.0).

34.2.2 Obtain Injunctions. A court can issue injunctions restraining the actions of officials in the administrative branch of the government. This includes the coroner or other individual who may be insisting on an autopsy. If a medico-legal autopsy is *not warranted*, the case should be taken to a court with the authority to suspend or eliminate the requirement for autopsy. The court could also be asked to restrain governmental officials from interference with external cooling and Phase I/Phase II perfusion. It should be clear to the reader that such measures as these will most likely be effective if advanced preparations have been made. The judge contacted should have been briefed concerning SSH well in advance of emergencies in which his judgement is sought.

34.3 If Autopsy Cannot Be Avoided. In some cases autopsy will be unavoidable. The Society Representative should then attempt to do the following:

34.3.1 Protect and Cool the Head. The Donor's brain is the seat of his intellect and personality. The Society Representative should exercise every effort to (1) exclude the head from autopsy and (2) obtain permission to externally cool the head during autopsy. The measures prescribed for external cooling of whole persons (36.0) may be adapted to this end.

34.3.2 Further Restrict the Extent and Duration of Autopsy to the Greatest Degree Possible. The Society Representative should attempt in *every way* to limit the duration and consequences of autopsy. He should argue against "removal" of organs or severing of major vessels and nerves. He should endeavor to prevent the heart, lungs, or other major organs from being dissected. It should be evident that each individual case will be different. The degrees of pressure the Society Representative can bring to bear will vary with the circumstances. He must do whatever is possible within the law and/or within the limitations of what is possible in the greatest long term interest of preserving potential viability in the Donor's person.

#### Reference

1. "Mortuary Science", by Frederick C. Gale, published by Charles C. Thomas, Springfield, Illinois, 1961.

# 35.0

## MAINTAINING CARDIOPULMONARY FUNCTIONS AFTER LEGAL DEATH

During resuscitation or in the course of long term cardiopulmonary assistance, the physician may decide that normal physiological functions cannot be restored by the means available. He may be ready to "give up". His inclination may be to turn off all mechanical resuscitation aids, wait until no signs of life exist, and then pronounce death.

This would be an unfortunate course of events. Assuming that the Donor's brain cells have remain oxygenated, the best chance of avoiding the currently irreversible damages of prolonged ischemia would require that the Donor be externally cooled as resuscitation was continued. Ideally, the physician in charge can be persuaded to pronounce death and certify it without cessation of cardiopulmonary assistance.

35.1 Proof of "Brain Death". A physician requested to pronounce death without turning off resuscitation equipment might argue that this is only permissible if brain death is proven by a prolonged silent EEG (1-7). This alternative would be as unfortunate or worse than cessation of cardiopulmonary assistance for pronouncement of death. A simple EEG has been restored in a cat's brain after one hour of ischemia (8); by crude parallel, the prolonged wait (perhaps several hours) for "brain death" would be more damaging than the 10-20 minutes of ischemia that perhaps could be involved with the more usual approach to pronouncement of death.

35.2 Options. The physician must, of course, have some evidence that normal physiological functions have really ceased before he pronounces death. Two options are described which may provide this basis for pronouncement of death with minimal detriment to the objectives of inducing SSH.

35.2.1 Temporary Cessation of Resuscitation. During the process of resuscitation itself, cardiopulmonary assistance may be periodically stopped for periods of five seconds (9) so that the Donor's spontaneous functions and physiological state may be assessed. A brief pause of cardiopulmonary assistance such as this may be all that the physician requires for pronouncement of death.

35.2.2 Induction of Hypothermia. Monkeys which are cooled to body temperatures of 21°C experience ventricular fibrillation and subsequent (below 20°C) cardiac arrest (10). If a pause of more than five seconds is required by the physician to pronounce death, he may be persuaded to permit the Donor's body core temperature to be lowered to approximately 25°C by external cooling (36.0) before cardiopulmonary assistance is discontinued (11). After this, as external cooling is continued for a period of 10 to 15 minutes, normal physiological responses would be demonstrated by ventricular fibrillation.

If these are not evidenced, then presumably death will be pronounced by the physician. By initiating external cooling and reducing body core temperature by 10° to 15°C before terminating cardiopulmonary assistance, the potential damage due to ischemia is greatly reduced. After 10 to 15 minutes (assuming that death has been pronounced) cardiopulmonary assistance can be recontinued.

35.3 After Pronouncement of Death. After pronouncement of death, assuming that external cooling and cardiopulmonary assistance are continued (and that there is no requirement for autopsy) body core temperatures are permitted to fall until a critical value is reached (36.3), at which time cardiopulmonary assistance is discontinued. External cooling is maintained until standard procedures for the induction of SSH are initiated.

35.4 Procedures for Maintenance of Cardiopulmonary Assistance. Once resuscitation (25.0) is begun, using mechanical aids (26.0), procedures do not change except as necessary to provide for external cooling. This change is covered under the part of section 37.0 (External Cooling Procedures) which deals with the case of "Donor Legally Dead; Cardiopulmonary Assist Sustained" (37.1).

#### References

1. "Legal and Medical Death -- Kansas Takes the First Step", by W. J. Curran, The New England Journal of Medicine, 284:260, 4 February, 1971.
2. "Criteria of Brain Death; Selection of Donors for Transplantation", by Dr. Palle Juul-Jenson, Munksgaard (Copenhagen), 1970 (Reviewed in the New England Journal of Medicine, 284:163, 21 January 1971).
3. "EEG Prediction of Outcome After Resuscitation from Cardiac or Respiratory Arrest", by C. D. Binnie, Electroencephalography and Clinical Neurophysiology, 29:105, 1970.
4. "Electroencephalographic Prediction of Fatal Anoxic Brain Damage After Resuscitation from Cardiac Arrest", by C. D. Binnie, British Medical Journal, 4:265, 1970.

# 36.0

## EXTERNAL COOLING

Next to sustained cardiopulmonary assist (35.0), external cooling is the most fundamental principle employed to sustain viability in the Donor at a cellular level. In all circumstances where restoration of the Donor's normal physiological processes is infeasible, external cooling is applied as soon as and as effectively as possible.

36.1 There are possibilities of injury to the Donor associated with external cooling (36.3), and these must be taken into consideration. Monitoring of body core temperatures is important (46.0). Specific equipment may be useful in external cooling (38.0).

36.2 Varying circumstances dictate different actions in regard to external cooling. The simultaneous use of cardiopulmonary assist and external cooling (37.1) requires different steps than the use of external cooling with no cardiopulmonary assist under conditions where no ice is available (37.5). A chart, below (Figure 36-1), indicates a number of possible circumstances. In all except the "no ice available" case, it is assumed that ice is obtainable. The user should choose circumstances from the chart which fit the particular situation he faces, and then refer to the cited paragraph in section 37.0 for further information.

Figure 36-1

Circumstances		Reference
Donor	Cardiopulmonary Assist Sustained	37.1
Legally	No Cardiopulmonary Assist; No Autopsy	37.2
Dead	No Cardiopulmonary Assist; Autopsy	37.3
Donor Not Legally Dead; Continues From Section 22		37.4
What To Do If No Ice Available		37.5

36.3 Possibilities of Injury from External Cooling with Cardiopulmonary Assist. Tissue damage<sup>1</sup> and fat necrosis<sup>2</sup> have been reported in connection with the direct application of ice in the induction of hypothermia. Risk of such damage may be slight when compared with the risks of methods which protect outer tissue layers, but greatly slow the reduction of body core temperatures. A more serious danger is that tissues of the thorax (chest) will become less flexible as the body cools, and that external cardiac compression could cause damage which would not occur at normal body temperatures.

Frequency of cardiac compression might be reduced, along with stroke length, as body core temperatures reach lower levels, but nothing is known about the degree of danger, or the efficacy of preventing damage by these measures. Until further information is available, it is recommended that cardiopulmonary assistance be discontinued when body core temperature has fallen to 10°C.

36.4 Physical Build vs. Cooling Rates. Following complete cessation of active surface cooling, the body core temperature will continue to drift downward<sup>3</sup>. This after-drop can be related to physical build, as shown in Figure 36-2.

Patient	Cooling Rate	After-drop
Obese Patient	Slowly	Considerable
Thin Patient	Quickly	Little
Infant	Rapidly	Considerable

Figure 36-2

#### References

1. "Techniques for Inducing Hypothermia", by G. S. Spurr, Vol. 3, Part 3, pp. 495-510 of "Temperature; Its Measurement and Control in Science and Industry", Reinhold, 1963.
2. "The Occurrence of Subcutaneous Fat Necrosis in an Infant Following an Adjuvant in Cardiac Surgery", by H. A. Collins, et al, Ann. Surg. 138:880, 1953.
3. Same as No. 1.

## 37.0 EXTERNAL COOLING PROCEDURES

This section covers those explicit procedures which apply to specific circumstances. Section 36.0 is a more general treatment of external cooling which refers readers to the below subparagraphs by means of a chart (Figure 36-1). A description of equipment for use in external cooling is provided in 38.0, supplementing the procedures described below.

### 37.1 Donor Legally Dead; Cardiopulmonary Assist Sustained.

37.1.1 Preparatory Steps. As cardiopulmonary assist continues, remove the clothing, insert a remote thermometer probe, described in 59.0 (rectal only, since artificial respiration prevents insertion of an oral probe). Cover an external cooling stretcher (38.1) with an 8 ft. x 10 ft. plastic sheet (38.2).

37.1.2 Transfer of Donor to the External Cooling Stretcher. It is assumed that a Michigan Instruments Heart-Lung Resuscitator (Figure 26-1) or similar equipment is in use as some models would not permit the following to be carried out.

The heart-lung resuscitator (HLR) is turned off and removed from the Donor, and the HLR base plate is slid *underneath* the plastic sheet on the external cooling stretcher. The Donor is transferred to the external cooling stretcher, and positioned (*on top* of the plastic sheet) appropriately with respect to the HLR. The total period without cardiopulmonary assist should not exceed three minutes. Manual resuscitation techniques (25.0) should be used if this time limit is exceeded. Use of the HLR is resumed at the earliest possible moment.

37.1.3 Application of Crushed Ice. With cardiopulmonary assist continuing, apply crushed ice directly to the Donor. The ice and the water it produces in melting are contained by the plastic sheet, so that the HLR base plate and all its associated apparatus remains dry. Insert ice beneath the Donor, to a thickness of several inches. *Do not permit ice to come between the Donor's back and the area of contact with the HLR base plate.*



Surround the Donor with crushed ice, *maintaining a clear area around the points of contact of the HLR's piston pad with the Donor's sternum and the HLR respirator mask with the Donor's face.* Critical points of ice application are the armpits, the groin, and the left side of the chest (tissues of these areas are in intimate contact with large blood vessels). The edges of a plastic sheet may be laced together across the top of the Donor so that ice can be held in contact with the Donor's upper body surfaces.

#### 37.1.4 Continuation of External Cooling with Cardiopulmonary Assist.

Body core temperatures will drop quickly and should be recorded no less frequently than every five minutes (46.0). Periodically, the thickness of ice beneath the Donor is checked and more ice is inserted as needed to maintain a layer several inches thick. Ice in contact with the Donor's side and upper body surfaces is supplemented as it melts. Particular attention must be given to the adequacy of ice in the Donor's armpits, groin, and on the left side of the chest since this melts most quickly.

Ice should be agitated frequently (HLR action may be sufficient) so as to promote the closest contact with the crushed ice and the greatest degree of heat transfer. Water accumulating in the plastic sheet should be drained as its depth increases, so that the depth remains less than six, but more than three, inches. A stretcher drain may be used for this (38.3).

Cardiopulmonary assist should be discontinued at lower body core temperatures (36.3). When cardiopulmonary assist is discontinued, the HLR should be removed. An oral probe for the remote thermometer should then be inserted, and the Donor's eyes, nose and mouth should then be covered with a small plastic sheet, and ice added as necessary to cover the Donor's face and chest surfaces. Particular care should be taken to assure thorough cooling of the head and neck.

37.2 Donor Legally Dead; No Autopsy. Remove the clothing, and insert remote thermometer probes (59.0). Cover an external cooling stretcher (38.1) with an 8 ft. x 10 ft. plastic sheet (38.2). Spread a layer of crushed ice several inches thick over the plastic sheet on the bottom of the external cooling stretcher and place the Donor on top of this. Cover Donor's eyes, nose and mouth with a small plastic sheet. Surround the Donor with crushed ice and draw the larger plastic sheet across the top of the Donor, covering him with crushed ice.

Frequently, agitate the Donor so as to promote the closest contact with the crushed ice. Periodically check to see that the thickness of the crushed ice bed beneath the Donor is maintained. Drain the water from within the

plastic sheet, using a "stretcher drain" (38.3) whenever it becomes approximately 6 inches deep. Leave a depth of approximately 3 inches, since this promotes a greater cooling effect beneath the Donor.

37.3 Donor Legally Dead; Autopsy. During autopsy, hopefully, external cooling of at least the Donor's head will have been permitted (34.2.1) More favorably, whole body cooling will have been permitted and the autopsy abbreviated (and expedited) to the maximum degree. Remote thermometer probes may have already been inserted. Aside from the fact that external cooling may already have been started, the desired procedure is as described in 37.2.

37.4 Donor Not Legally Dead; But Clinically Dead Longer Than Ten Minutes.

The basic procedure described in 37.2 is most favorable, but circumstances of the kind examined in section 30.0 may call for certain changes. Esophageal insertion of a remote thermometer probe may not be wise. Removal of clothing may not be appropriate. In some cases, cooling of the Donor's head *only* may be judged to be the only measure that can be exercised. The Society Representative must weigh all facts and decide how far toward full implementation of 37.2 it is possible to go.

37.5 What To Do If No Ice Is Available. The recommendations given below are speculative in that they are not the product of comparative studies or other formal investigation. The reader may have other, perhaps superior, ideas.

37.5.1 Use of a Refrigerator or Freezer. External cooling by cold-air contact is very slow, particularly when there is little or no circulation of the air. "Warm pockets" form around the body unless the air is circulated. If ice is available within an hours drive, other solutions (discussed below) are probably more effective.

If no alternative is available, place Donor in refrigerator or freezer, and hang numerous wet cloths in the free air space left. Periodically open the door and wrap these around the Donor's head. Make sure these rags are water saturated, and include ice cubes in the wrapping if any are available. Place a small electric fan in the refrigerator or freezer to circulate air. Remember that the motor will create heat, so do not run the fan continuously. Plug in the fan for ten seconds, unplug it for thirty seconds, etc.

Opening the door more frequently than once every 10-15 minutes is not advantageous. The door should be opened for the briefest periods possible. Tape a plastic or even a fabric sheet across the opening to the freezer or refrigerator and slit it so that hands may be inserted to change the position of the cloths, etc. This will help minimize mixing of warm air outside with cold air inside. Trim away excess fabric and close door over top of this except when opening it for access is necessary.

37.5.2 Use of Direct Evaporation. Cloths may be loosely wrapped in a thin layer around the Donor's head and body, and then saturated with water. A forced draft of air will produce an evaporative effect, cooling the Donor. Water should be added, very slowly but almost continuously, so that cloths remain saturated with water. The forced draft may be produced by use of fans or by transporting the Donor at highway speeds in a motor vehicle with all the windows open. In the later case, the vehicle should be driven to the nearest source of ice.

37.5.3 Other Possibilities. In winter time, driving at highway speeds with all windows open may be far more effective than exposure to the inside of a freezer. At lower air temperatures, direct exposure of the Donor's body surface to the forced draft may be more effective as a cooling method than the use of water-saturated cloths. Immersion in a running stream of water would be an ideal way of external cooling if the stream temperature were close to the freezing point of water.

## 38.0 EXTERNAL COOLING EQUIPMENT AND APPARATUS FOR PHASE I

This section describes equipment which for the most part has not been thoroughly tested or evaluated in other ways.

38.1 External Cooling Stretcher. The external cooling stretcher is a plywood platform seven feet long by thirty inches wide, with a rim one foot high and handles extending six inches at each end.

38.2 Plastic Sheet. The plastic sheet is a waterproof (impermeable) rectangle of material that can be used to prevent contact between crushed ice and other objects. The best material is polyethylene, since it is more chemically resistant and less subject to cracking at low temperatures than any other readily available material. Grommets should be spaced along the edge of the sheet, so that it may be laced into any desired position.

38.3 Stretcher Drain. The stretcher drain is a four to six foot length of surgical tubing with a clamp, used to drain water from within the external cooling stretcher when the continued melting of ice makes this necessary. All but one end of the tube is submerged in the liquid. The clamp is attached to the end left above the surface. Beginning with this end, the tube is then pulled out of the water, leaving the far end submerged, and it is led over the rim of the stretcher and down below stretcher level. Release of the clamp while the tube is in this position permits siphon action to drain the stretcher of excess water.

38.4 Ice. Crushed ice is preferable to cubed or block ice -- chips must be loose and easily arranged. Automatic vending machines usually have a better quality of crushed ice (less compacted) than grocery stores. Alternate sources of crushed ice are motels and truck stops. Yellow pages of telephone directories usually list sources of "ice".

# 39.0

## TRANSPORTATION OF THE LEGALLY DEAD DONOR

Following pronouncement and certification of death, it will usually be necessary to transport the Donor to a society operated facility for Phase I procedures (51.0). External cooling (36.0) and sustained cardiopulmonary assistance (35.0), as appropriate, will be continued during movement of the Donor. The point of departure will usually be a hospital, although some instances will occur in which death is pronounced and certified elsewhere.

39.1 Continued Assistance by the Hospital. It would be fortunate if hospital assistance were available for all procedures involved in the induction of SSH. Clearly, the participation of physicians and the use of medical facilities would represent a preferred set of circumstances. As matters currently stand, this cooperation will seldom be extended. Assistance with the temporary maintenance of cardiopulmonary functions and the initiation of external cooling is the most that can be expected. The hospital should be persuaded to assist in this until transportation for the Donor can be arranged. The Society Representative must then provide for those continued actions that are appropriate.

39.2 Legal Aspects of Transportation. All 50 states have health department regulations regarding the manner in which legally dead persons are to be transported. Society Representatives should familiarize themselves with all applicable state and local regulations. In general, the following restrictions will exist:

39.2.1 Removal Permits. In order that the Department of Vital Statistics can know at all times the location of all deceased persons, a "removal permit" is required for transportation from one district to another. For example, an initial removal permit might be required to transport a Donor from a hospital to an SSH perfusion facility, and a second permit might be required for subsequent movement to a storage facility. It is possible that verbal permission could first be obtained (so that delays are avoided) and that a formal permit could be executed later. In some states regulations pertaining to anatomical donors are different from regulations pertaining to other deceased persons.

### 39.2.2 Types of Vehicles that May Be Employed.

39.2.2.1 Common Carriers. Common carriers are public transportation vehicles such as taxis, busses, trains, and airplanes. They are almost always limited to transporting those legally dead bodies which have been embalmed by a licensed embalmer. For movement of a Donor from the point of death to an SSH induction facility, then, common carriers are effectively excluded.

39.2.2.2 Ambulances. Most states have laws which state or imply that "conveyances used for transporting unembalmed bodies should be kept solely for that use"<sup>1</sup>. Laws may vary from state to state, however, and may not be stringently enforced. Under circumstances where a Donor is being transported from a hospital to an SSH induction facility in a large metropolitan area, little difficulty in securing ambulance service should be experienced.

39.2.2.3 Private Vehicles. In connection with the laws cited in section 39.2.2.2, it may be contrary to law to transport an "unembalmed" body in a private vehicle. If the society provided for a special purpose vehicle no legal problems would be expected to exist.

39.3 Obtaining the Cooperation of an Ambulance Service. It is thought that ambulance service companies will best respond to requests for transportation if the Donor's status as an anatomical gift is stressed. The destination need only be identified as the "research facility of a non-profit organization". If the Donor is receiving sustained cardiopulmonary assistance, the ambulance company should be informed of this. If an extended trip of several hours is required, the ambulance company should be requested to provide additional oxygen as needed. If the society has its own heart-lung resuscitator (HLR) equipment, supplementary oxygen will probably be all that is required. If the society does not own an HLR, then an ambulance company having such equipment should be sought and asked to provide it during the period of transportation.

#### Reference

1. "Mortuary Science", by Frederick C. Gale, published by Charles C. Thomas, Springfield, Illinois, 1961.

## 40.0

MEDICAL TESTS AND PROCEDURES  
FOLLOWING LEGAL DEATH

The research objectives of SSH societies are oriented towards improvements of knowledge concerning the preservation of cellular viability in human beings by SSH. This implies that data and samples will be taken during the induction of SSH which will be of significance in the solution of general problems in the development of efficacious, proven methods. It follows also that this research can be most effective if it is coordinated in an efficient way.

40.1 Types of Data and Samples to be Acquired. The table of contents of the initial issue of this instruction manual shows sections following 40.0 which deal with (41.0) Fluid Samples, (42.0) Tissue Samples, (43.0) Radiographic Tests, and (44.0) Mechanical Tests. It is not clear that these categories are inclusive of all testing that may be desirable, so they represent only a starting point on which to build.

40.2 Detailed Development of Sections 41.0 through 44.0. Recommendations are desired, as to those standardized types of tests which will contribute in every instance to the store of knowledge which must be developed in support of research objectives. These can be included within the referenced sections so that data of primary importance can be acquired in all instances where SSH induction occurs. Advice from many sources will be sought in this area, so that the test procedures recommended will be:

40.2.1 Of fundamental Importance.

40.2.2 Of a kind which will not delay or impede the effective induction of SSH.

40.2.3 Of a kind which will not detract from the long term potential viability of the Donor. In particular, biopsy and fluid sampling tests will be carefully reviewed to assure that damage to important organs is avoided. Radiographic and mechanical tests will strictly be limited to those which do not have structural or cellular consequences of a serious kind.

# 45.0

## MAINTAINING PROPER RECORDS

The careful documentation of all significant events and procedures followed in the induction of SSH is of great importance. Quantitative data is essential, and the use of charts facilitate its being recorded. Check lists are helpful in assuring that essential steps are not overlooked.

In subsequent revisions, this manual will provide the user with a complete set of forms, charts, and check lists to guide proper record keeping practices. Individual sections (46.0 through 49.0, see Table of Contents for titles) have been allocated for this purpose. For the present, a brief discussion will be provided, stressing the importance of certain kinds of information and the need for recording it.

45.1 Monitoring Body Temperatures. Body core temperature is a parameter of critical importance, and should be measured frequently. The time of clinical death should be determined as accurately as possible, and the times of all temperature measurements recorded so that temperature vs. time after death is accurately known. Ordinary oral or rectal thermometers are ineffectual, as they only measure temperatures close to normal body temperatures. Wide range thermometers (59.0) can be purchased from laboratory supply houses such as Cole Parmer (see Appendix A) for this purpose.

45.2 Log Keeping Procedures. At the moment a Society Representative learns something is wrong, he should start making records, indicating the time of each event. If forms are not available, anything that can be obtained should be used as writing materials. This can later be transformed into a smooth time-log of events. If these notes are not made *as the events occur*, they will not exist. Approximation of times later will be a futile exercise by comparison.

Particularly important times are those that correlate with the occurrence of clinical death, the beginning of resuscitation, the initiation of external cooling, etc. Log keeping should continue until all time correlated events are being recorded on standard forms and the standard procedures for the induction of SSH are well underway.



45.3 Perfusion Records. In perfusion, records of all quantitatively known parameters should be made. A chart illustrating some of the types of data which are important is shown in Figure 45-1. Note that every measurement is associated with a specific time. Without time correlation, quantitative data of the kind indicated is nearly useless.

45.4 Overall Donor SSH Induction History. This record, compiled after SSH induction is completed, includes all the records described above and many others. All relevant documentation including medical records from hospitals, statements of physicians, etc., would be included.



## 50.0 STANDARD PROCEDURES FOR THE INDUCTION OF SSH

The remainder of these instructions are devoted to the standard procedures involved in and with the induction of solid state hypothermia in humans. From this point on three assumptions are made.

First it is assumed that either resuscitation attempts failed to restore the Donor's normal physiological functions, or resuscitation was considered inappropriate under the circumstances and was not attempted in the first place.

The second assumption is that the death certificate has been executed, autopsy has been avoided or completed, and all legal obstacles have been dealt with.

The third assumption is that external cooling has been initiated and the induction of solid state hypothermia is the objective of all remaining procedures.

The induction of SSH may be divided into three phases. Phase I includes all procedures above 0°C, Phase II includes all steps below 0°C in a liquid state, and Phase III includes the transition from liquid to solid states plus any subsequent lowering of temperature that is effected.

Phase I, in its broadest interpretation, includes everything preceding this section as well as many sections that follow. The administration of heparin (29.0) and external cooling (36.0) are fundamental to Phase I protocol. In a more restricted sense, Phase I procedures include cannulation, Phase I perfusion, and certain concurrent procedures relating to purging of the digestive and excretory tracts. A more detailed description of Phase I is provided in its introductory section (51.0).

Phase II includes those liquid state operations which are carried out below 0°C. The primary objective of Phase II is to maintain a liquid cellular state as temperature is lowered until this is no longer possible. Among the measures employed are the introduction of substances into the Donor's body which prevent crystallization of water. The introductory section of Phase II (65.0) describes the currently recommended procedures in more detail.

Phase III includes the lowering of temperature wherein liquid components of the body cells solidify, in crystalline or vitreous form. Sections concerning Phase III and other phases which follow it are in the process of being formulated. A brief overview of this activity is presented in section 72.0.

## 51.0 STANDARD PROCEDURES: PHASE I

As noted in section 50.0, Phase I in its broadest sense covers all activities preceding Phase II. Emergency rescue procedures, including resuscitation, and such activities as avoidance of autopsy fall within the scope of Phase I. Steps such as administration of heparin and external cooling are more directly related to the induction of SSH, and so they seem more properly a part of Phase I, but there are circumstances where heparin is *not* administered and many variations in external cooling measures are possible or appropriate.

With this section, a point is reached after which procedures are "standard". The same actions are basically applicable to all circumstances. It is assumed that no further efforts at immediate resuscitation have any chance of success. Legal death of the Donor is a fact, and autopsy procedures have either been avoided or have been completed. The Donor has been transported to a facility where the induction of SSH may be carried out at least through the completion of Phase I. External cooling is in progress, and cardiopulmonary assistance may or may not be continuing at this point. In any case, the remaining standard procedures for Phase I may be undertaken.

The primary objectives of the standard procedures for Phase I are (1) to cool the Donor rapidly, lowering the body core temperature from ambient to 0°C, (2) to remove blood constituents from the vascular system, replacing these and intercellular body fluids with an intracellular solution. The sections covering Phase I standard procedures are charted below (Figure 51-1).

In all parts of these procedures, teamwork is necessary. Only under the most difficult circumstances would a single individual be expected to carry out all the required activities. A distribution of team activities should be preplanned and followed. Section 52.0 provides further information.

Cannulation is the first step towards Phase I perfusion (53.0). Sterile techniques are advisable in preparation for cannulation (54.0), after which cannulation is carried out through specific procedures (55.0). Specialized instruments are employed (56.0).

During cannulation, the perfusate and perfusion equipment should be made ready for use (57.0). Perfusate is brought to a liquid, chilled state (58.0) and perfusion equipment (59.0) is assembled and checked out as necessary.

Sections Covering Phase I Procedures	Section Number
Division of Activities within Phase I Team	52.0
Cannulation	53.0
Preparation for Cannulation	54.0
Cannulation Techniques and Procedures	55.0
Cannulation Instruments	56.0
Preparatory Steps Prior to Phase I Perfusion	57.0
Preparation of Perfusate for Phase I	58.0
Equipment for Phase I Perfusion	59.0
Procedures for Phase I Perfusion	60.0
Concurrent Phase I Procedures	61.0
Post Phase I Procedures	62.0

Figure 51-1

With the Donor cannulated and the perfusion equipment fully ready, the equipment is connected to the cannulas and perfusion is begun (60.0). As perfusion is performed, other procedures should be effected, in which the digestive and excretive systems are purged (61.0)

As already noted, certain transition activities are required prior to the beginning of Phase II (62.0). Circumstances may frequently make it more advantageous to carry out Phase I perfusion at some location near the scene, rather than at the main facility where Phase II measures must ultimately be executed. Once Phase I procedures have been completed, a delay in starting Phase II perfusion (up to, but not exceeding 24 hours) can be tolerated without jeopardizing the condition of the Donor as long as external cooling is maintained. During this period, the Donor may be transported to an appropriate facility for Phase II perfusion. Sections 63.0 and 64.0 are concerned with this trans-phase Donor movement.

Information on the standard procedures for Phase II begins with section 65.0.

Sections Covering Phase I Procedures	Section Number
Division of Activities within Phase I Team	52.0
Cannulation	53.0
Preparation for Cannulation	54.0
Cannulation Techniques and Procedures	55.0
Cannulation Instruments	56.0
Preparatory Steps Prior to Phase I Perfusion	57.0
Preparation of Perfusate for Phase I	58.0
Equipment for Phase I Perfusion	59.0
Procedures for Phase I Perfusion	60.0
Concurrent Phase I Procedures	61.0
Post Phase I Procedures	62.0

Figure 51-1

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Information on the standard procedures for Phase II begins with section 65.0.

## 52.0 DIVISION OF ACTIVITIES WITHIN THE PHASE I TEAM

Phase I operations might possibly be carried out in their entirety by a single well equipped Society Representative. It is considered that this approach would result in many undesirable delays, inconveniences, and short-cuts, however. A minimum two man team is advised (a third team member would undoubtedly be valuable).

While team members will assist each other throughout the entire procedure, individual areas of responsibility should be predefined, and team members should attempt to develop the most complete levels of competence with regard to their individual areas of responsibility.

Future revisions of this section will contain a recommended team organization and division of responsibilities, including those areas of skill in which individuals should concentrate their attention.

## 53.0 CANNULATION

Cannulation is the opening of veins and arteries in the Donor's circulatory system and the insertion of tubes (cannulas) prior to (and for use in) perfusion procedures.

Preparation for cannulation (54.0) involves the use of sterile procedures and the appropriate preparation of the incision site. Specific procedures are appropriate for cannulation, with important differences based on the chosen cannulation site (55.0). Specialized equipment is required for cannulation, and must be acquired and prepared for use before needs for it arise (56.0).

53.1 Ideal Circumstances. Under ideal circumstances, it would be desirable to arrange for cannulation to be performed by a surgeon under operating room conditions. Particularly in the case where cardiopulmonary assist continues during cannulation (55.1), the working conditions approximate surgery on a patient with normal physiological functions. Blood vessels must be clamped, sponges and/or an aspirator may be necessary to clear the incision of fluids, etc. While the desirability of physician participation is clear, such participation will frequently be unobtainable. Alternate approaches will be necessary.

53.2 Arrangements with Morticians. Next to the physician-surgeon, morticians are most proficient in the cannulation of human beings. They, more than anyone lacking equivalent training, are capable of performing a cannulation effectively and with minimum damage to the tissue and nerves surrounding the vessels to be cannulated. Morticians should be sought who can understand the goals of SSH objectively and who will assist with cannulation should the induction of SSH be necessary.

53.3 The Third Alternative. If neither the services of a physician nor those of a mortician can be obtained, and these circumstances sooner or later will arise, the Society Representative must perform the cannulation procedures himself. It is assumed that the Society Representative will have at least witnessed one or more cannulations, since to attempt a cannulation without such prior exposure would be extraordinarily difficult. Notwithstanding this, details concerning cannulation procedures are included in section 55.0, both as a guide to learning and for purposes of aiding recollection.



## 54.0 PREPARATIONS FOR CANNULATION

When possible, sterile or semi-sterile techniques should be employed in cannulation. If time and circumstances force a choice between rapid cooling and the sterility of techniques, it should be remembered that infections are not as serious as the damage resulting from delays in cooling. The following procedures should be followed whenever possible.

54.1 Room Preparation. The room to be used should be scrubbed from ceiling to floor immediately before perfusion is begun. Use of an antibacterial agent (such as Lysol) is recommended. If scrubbing of the room is impractical, a spray disinfectant (such as Lysol spray) should be used on all exposed surfaces.

54.2 Preparation of the SSH Team Members. The hands of all members of the team are subject to vigorous cleansing. Particular care should be directed toward the crevices under the nails and cuticles.

54.2.1 Scrubbing should include the entire arm to the elbow. Warm water should be used as it promotes sweating. Sweating brings embedded bacteria to the surface. Scrubbing with warm water and an antibacterial soap containing hexachloraphine (e.g. phisohex) for ten minutes with a stiff brush affords greater sterilization than the use of antiseptics.

54.2.2 Rinsing should be done without contact except that of water over cleansed area, allowing water to drain off the elbows. *DO NOT touch the faucet or turn it off.* This should be done by a person who will not be involved in the cannulation. Dry hands and arms with a sterile towel. Hands and arms should then be bathed in alcohol and dried again.

# 55.0 CANNULATION TECHNIQUES AND PROCEDURES

55.1 General Discussion. In the actual procedures of cannulation, a surface incision is made, and blunt instruments are used to open the tissue, exposing the appropriate vessels (veins and arteries). All attached tissue is cleared away, and the vessels are lifted so that they are fully exposed. Openings are made in the vessels; tubes of the proper size (cannulas) are inserted and tied (ligated) in place.

External cooling (36.0), already in progress, must be continued during cannulation to the greatest degree possible. If cardiopulmonary assist is in progress (35.0), either (1) cannulation will be accompanied by the types of surgical problems encountered when a living person is operated upon, or (2) use of cardiopulmonary assist must be discontinued. Unless body core temperature is below  $10^{\circ}\text{E}$ , it is recommended that cardiopulmonary assistance *not* be discontinued during cannulation.

In the detailed discussion which follows, proper medical terminology is used for all anatomical parts. The reader is advised to familiarize himself with this terminology by carefully noting the more common terms shown in parenthesis, and by reference to the figures provided.

Certain precautions are so fundamental to the procedures of cannulation that they are given at this point. These instructions will allow the cannulation to be more easily accomplished and more efficient.

- 55.1.1 Use a sharp pair of scissors to open a vessel. Never use a scalpel. Scissors make a cleaner cut through the three layers of the arterial wall.
- 55.1.2 Never force a cannula into a vessel. When difficulty is experienced while attempting to insert a cannula, apply petroleum jelly to the tip of the cannula before inserting into vessel.

- 55.1.3 Be sure to use the correct size cannula for any specific application. When in doubt, always use a smaller size.
- 55.1.4 Care should be exercised to see that nerves are not raised with or ligated (tied) to vessels.

Because relatively little nerve and muscle damage is done while dissecting for the femoral artery and vein, the ilio-femoral region (upper thigh) is the preferred and recommended site of cannulation in the usual circumstance. When dissecting for cannulation in either the carotid (neck) or axillary (armpit) regions, more extensive damage is probable. Another advantage to using the ilio-femoral region is the large size of the vessels in this area, permitting a good perfusion flow rate.

Circumstances do not, however, always make it advisable, or even possible, to cannulate the femoral vessels. Some situations (severe cases, i.e., autopsy performed, advanced arteriosclerosis, major abdominal surgery having been performed, or severed body parts) may even call for perfusion to be performed in all three areas simultaneously. For this reason, the anatomy (both surface and gross) of each region, and corresponding cannulation procedures for each area will be discussed.

The ilio-femoral region (the preferred and recommended site of cannulation) is discussed in more detail than either the carotid or the axillary regions. The discussion of these latter two has been shortened by eliminating redundant details only. If more information is desired while reading the later sections, refer back to the description under the ilio-femoral region.

## 55.2 ILIO-FEMORAL APPROACH

**5.2.1 Anatomy of the Ilio-femoral Region.** Figure 55-1 shows the surface anatomy of the ilio-femoral region. The recommended site of amputation is approximately 3 inches below the inguinal ligament, in the femoral triangle. Except in obese individuals, the femoral triangle, which centers between the sartorius and adductor-longus muscles, can be felt with the fingers. The course of the femoral vein and artery can be traced by an imaginary line pointing to the knee, drawn midway between the anterior superior spine of the hip bone and the symphysis pubis, as shown.

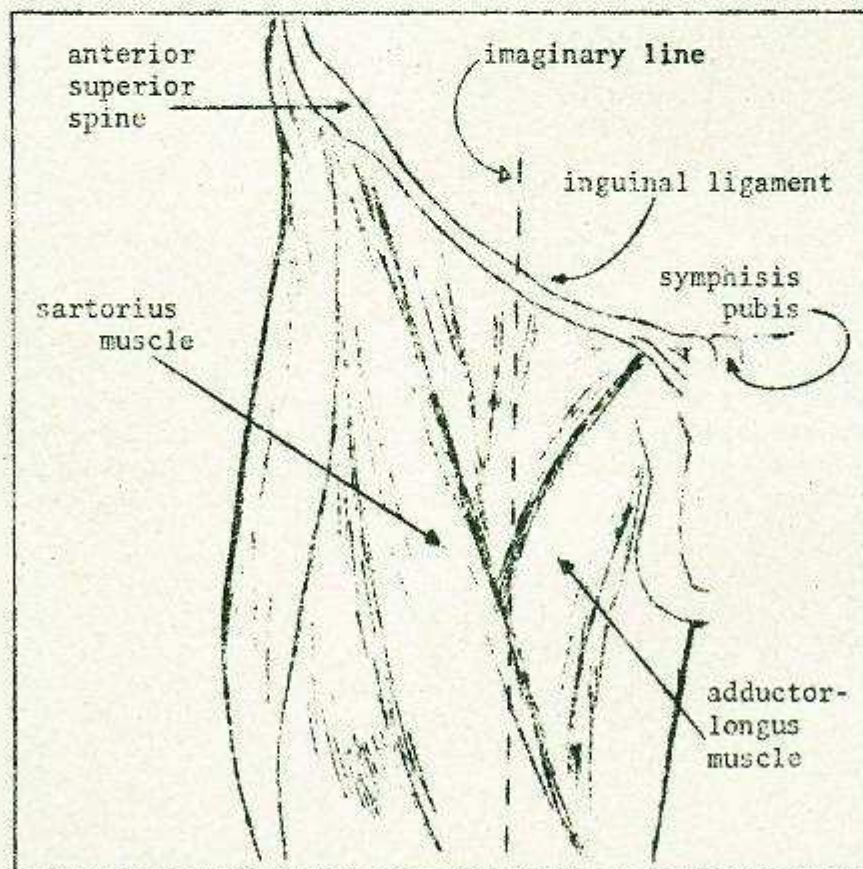


Figure 55-1: Surface Anatomy of the Ilio-femoral

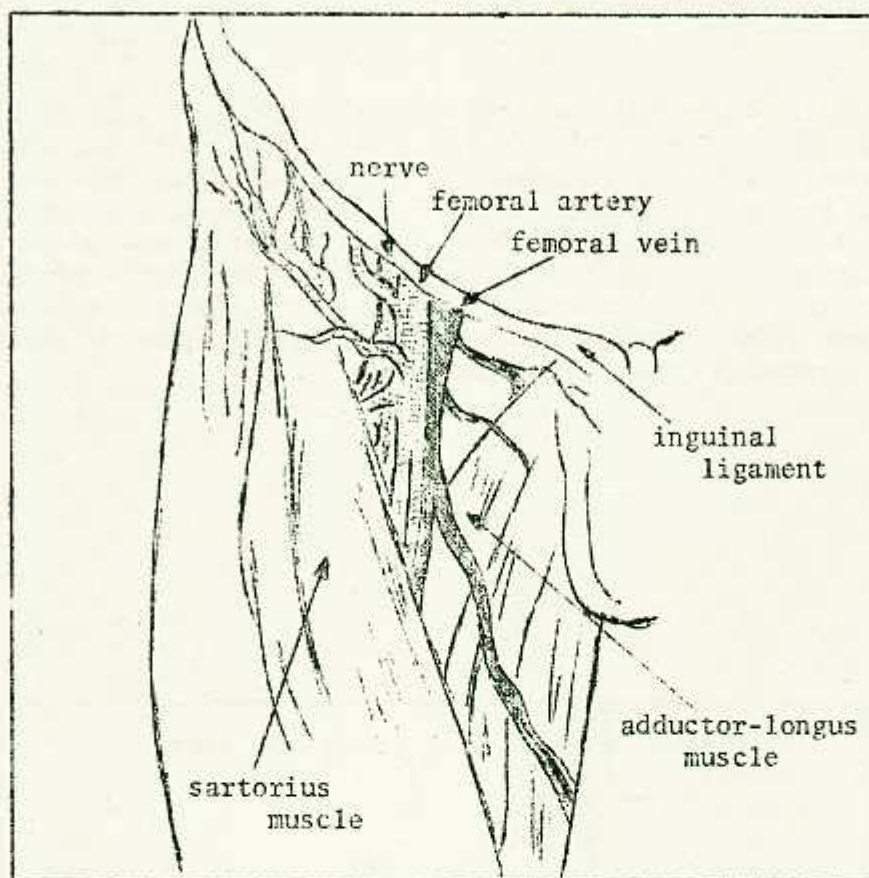


Figure 55-2: Gross Anatomy of the Ilio-femoral

The femoral artery lies on top of the femoral vein (Figure 55-2). The artery can be felt deep in the triangle. It will feel like a cord about the size of a little finger. The artery passes vertically downwards from the middle of the base of the femoral triangle to the point where the adductor-longus and the sartorius meet. At this point, the femoral artery passes over the adductor-longus and under the sartorius.

**55.2.2.3 Raising the Vessels.** The artery should be raised to the surface of the skin incision by an aneurism hook (Figure 55-5) and held in this position by running the handle of the aneurism hook under the vessel (Figure 55-6). Four pieces of 4-ply thread, approximately 8 in. long, are then drawn under the vessels (Figure 55-7). The thread is used to ligate the vessel around the cannula (after cannula is inserted). It is also helpful in quick relocation of a vessel which has been "dropped".

The same procedure should be followed with the vein. The structure of the wall of the vein is more delicate than the artery wall. For this reason, more caution should be exercised while raising the vein.

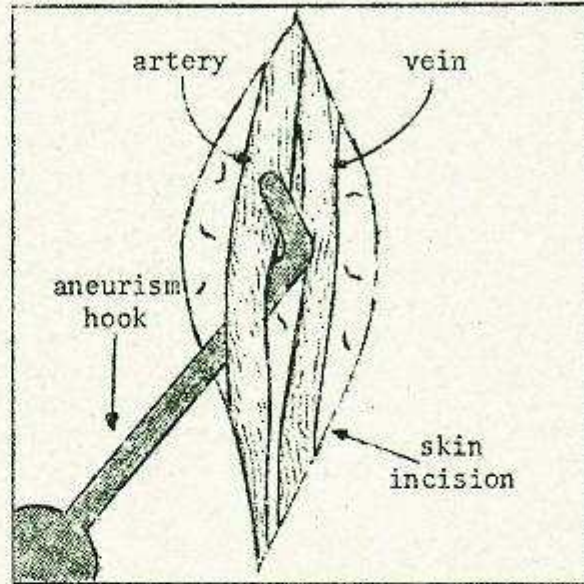


Figure 55-5: Raising Vessels

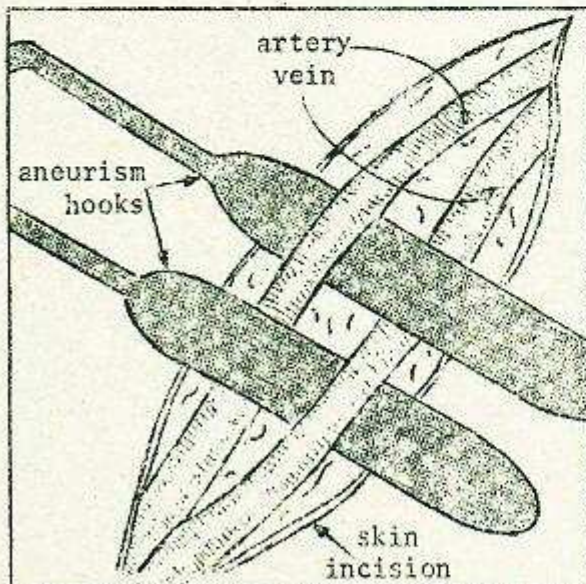


Figure 55-6: Holding Raised Vessels

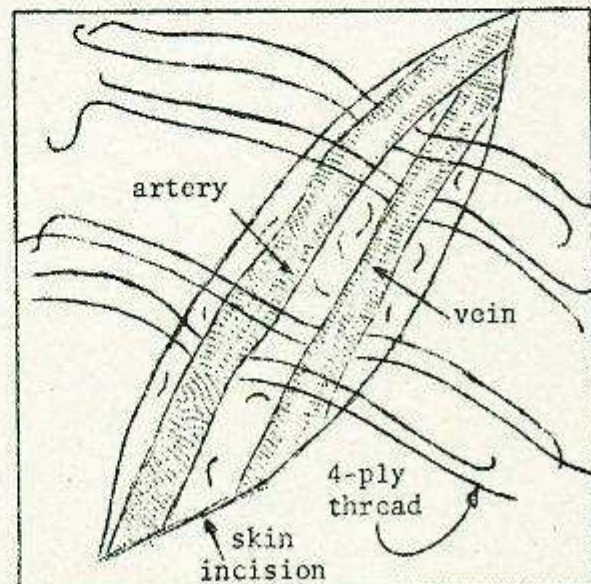


Figure 55-7: Placement of Ligatures (For greater simplicity and clarity, the aneurism hooks are not shown).

### 55.2.2 Cannulation of the Ilio-femoral Region

**55.2.2.1 Initial Incision.** Using a scalpel, make the skin incision along the midway point on the inguinal ligament, extending downward for 3 inches, terminating at the junction of the sartorius muscle and the adductor-longus muscle (Figure 55-3). The incision should be 1/4 in. to 3/8 in. deep in thin subjects. Obese subjects will require a deeper incision. The amount of fat on any particular individual will determine the depth of the artery.

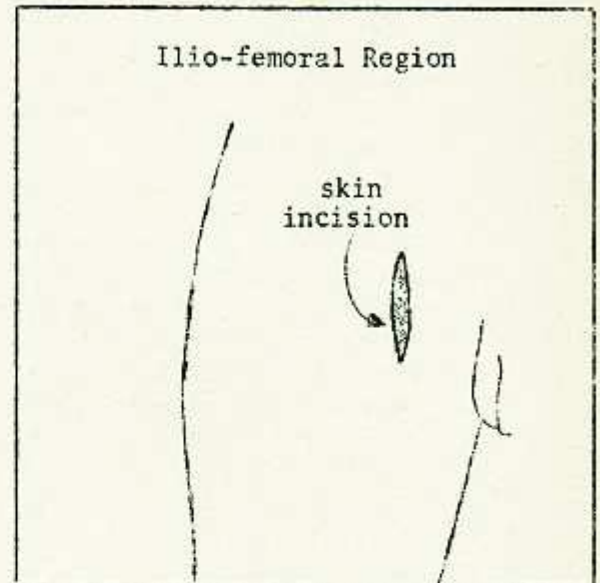


Figure 55-3: The Initial Incision

**55.2.2.2 Location of Vessels.** After the skin incision has been made, the fascia (fibrous tissue) should be carefully dissected (spread) using aneurism hooks to expose the femoral sheath (Figure 55-4). The sheath contains the femoral artery, vein and nerve. Special care should be taken to clean the vessels of connective tissue without damaging the nerve which is in such close proximity. Blunt dissection (i.e., spreading or separating the fibrous tissues from around the vessels by means of blunt instruments, usually aneurism hooks, rather than instruments with blades or sharp, pointed surfaces) should be used for this part of the procedure. Care should be exercised to properly separate the femoral nerve from the adjacent vessels.

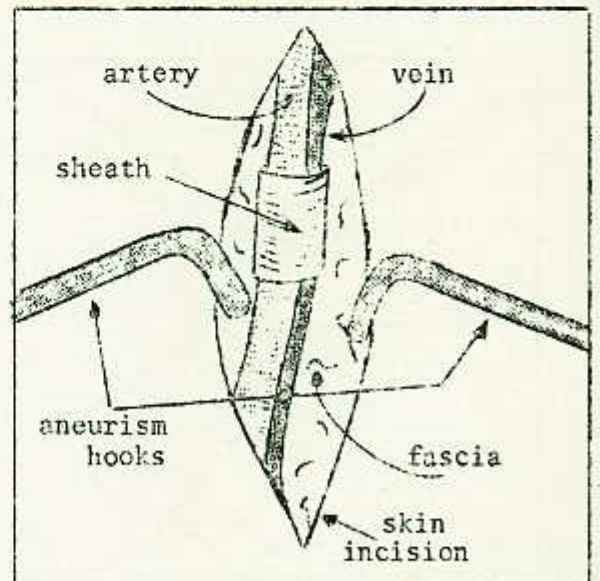


Figure 55-4: Locating the Vessels

55.2.2.4 Incisions in Vessels and Insertion-Securing of "T-shaped" Cannulas. If arterial pressure exists (heart-lung resuscitator still in use) apply hemostats to both sides of the vessel prior to incision. Make one horizontal (parallel) incision in the top of the femoral artery with a pair of scissors. *(Do NOT make an incision in the vein at this point).* The incision should be no longer than necessary to insert the cannula. An excessively long incision will not permit a good seal to be made by ligating the vessel to the cannula.

Holding the incision open with hemostats, insert "T-shaped" cannula (Figure 55-8). The vessel should then be ligated securely around the cannula, using the 4-ply threads previously drawn under the vessels (Figure 55-9). Repeat this procedure with the vein. Remove aneurism hooks after both vessels have been cannulated.

See 55.2.2.6 for cases in which extensive post-mortem clotting has occurred.

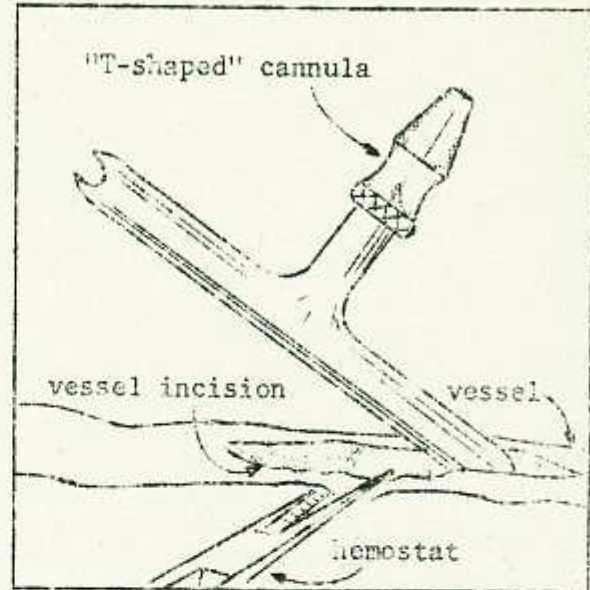


Figure 55-8: Inserting "T-Shaped" Cannulas

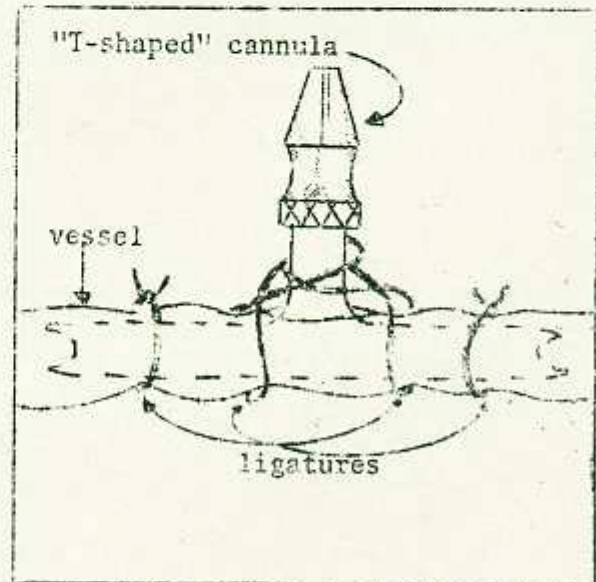


Figure 55-9: Ligating "T-Shaped" Cannulas



**55.2.2.5 Incisions in Vessels and Insertion-Securing of Conventional Cannulas.** If arterial pressure exists (heart-lung resuscitator still in use) apply hemostats to both sides of the vessel prior to incision. Using scissors, make two transverse (crosswise) incisions in the femoral artery (Figure 55-10). These incisions should be approximately one inch apart. (Do NOT make an incision in the femoral vein at this point). Using hemostats to hold the incision open, insert one arterial cannula in each incision (Figure 55-11).

Securely ligate the vessel around the cannula using the thread previously drawn under the vessels. The recommended method for ligating vessels is shown in Figure 55-12. Because there is a distinct tendency for cannulas to work free during perfusion, it is important to double tie the cannula and the vessel. The ligatures should be tied as tight as possible, using two separate strings and looping one string a-

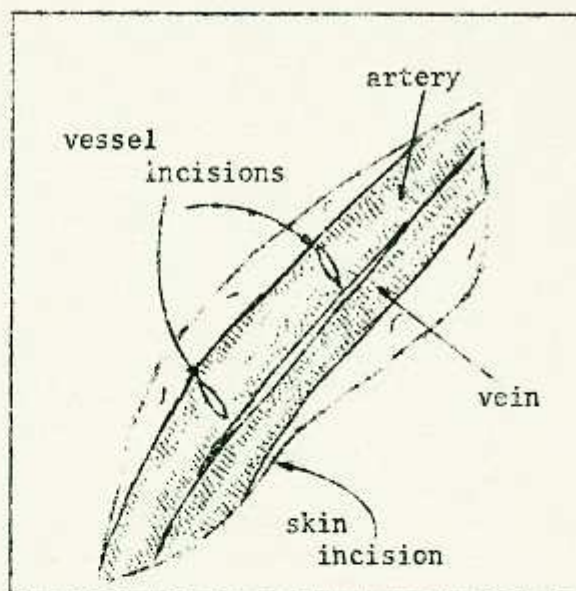


Figure 55-10: Incisions in Artery

round the cannula as shown. Follow the same procedures with the vein. Remove aneurism hooks after both vessels have been cannulated.

See 55.2.2.6 for cases in which post mortem clotting has occurred.

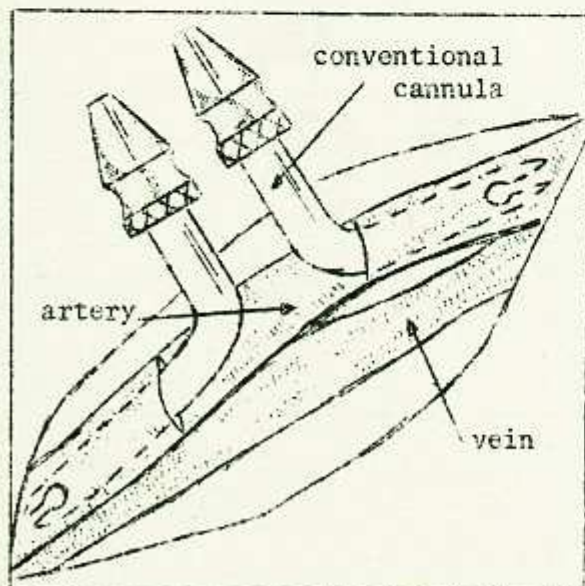


Figure 55-11: Inserting Cannulas (Conventional Type)

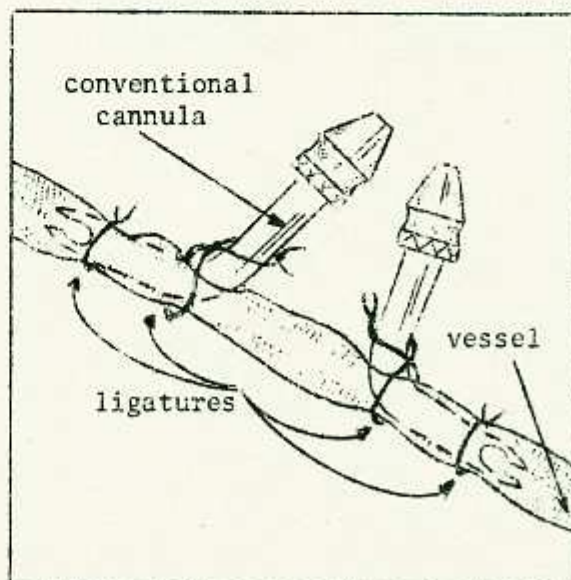


Figure 55-12: Ligating Cannulas (Conventional Type)

55.2.2.6 Procedures for Venous Cannulation in Cases Where Extensive Post Mortem Clotting Has Occurred. When circumstances result in external cooling of a Donor without cardiopulmonary assist, particularly where heparin has not been administered, extensive coagulation of blood constituents may occur before perfusion is begun. In these cases, a "drainage tube" (Figure 56-2) is inserted in the veins, instead of a "T-shaped" or arterial cannula. After the initial period of perfusion is finished, during which coagulated blood constituents have been removed, "T-shaped" or arterial cannulas will replace the drainage tubes by insertion in the manner previously described. Drainage tubes are described in section 56.0, and their use is explained in conjunction with perfusion operations (60.0).

### 55.3 THE CAROTID APPROACH

#### 55.3.1 Anatomy of the Carotid Region.

When the Donor is placed in a supine (face up) position, the sternocleido-mastoid muscle can be seen and felt running upward from the sternoclavicular joint and terminating behind the ear lobe at the mastoid bone (Figure 55-13). The internal jugular vein parallels the sternocleido-mastoid muscle the whole distance.

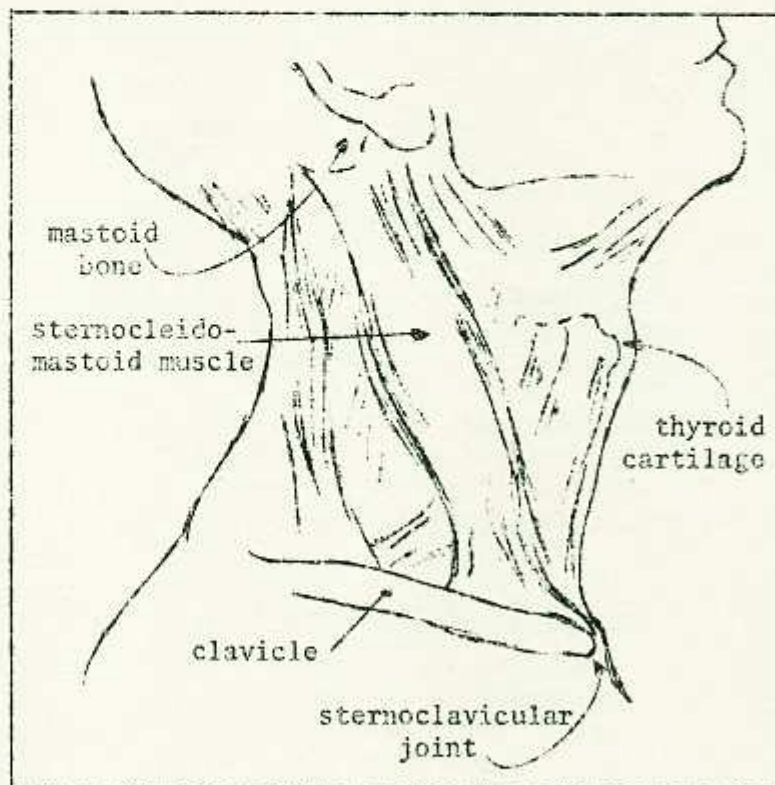


Figure 55-13: Surface Anatomy, Carotid Region

The right carotid artery is a branch of the innominate artery and begins at the sterno-clavicular joint. The carotid artery is below the jugular vein and follows the same route as the jugular vein. The vagus nerve parallels the carotid artery and is contained, with the vein and the artery, in the carotid sheath (Figure 55-14).

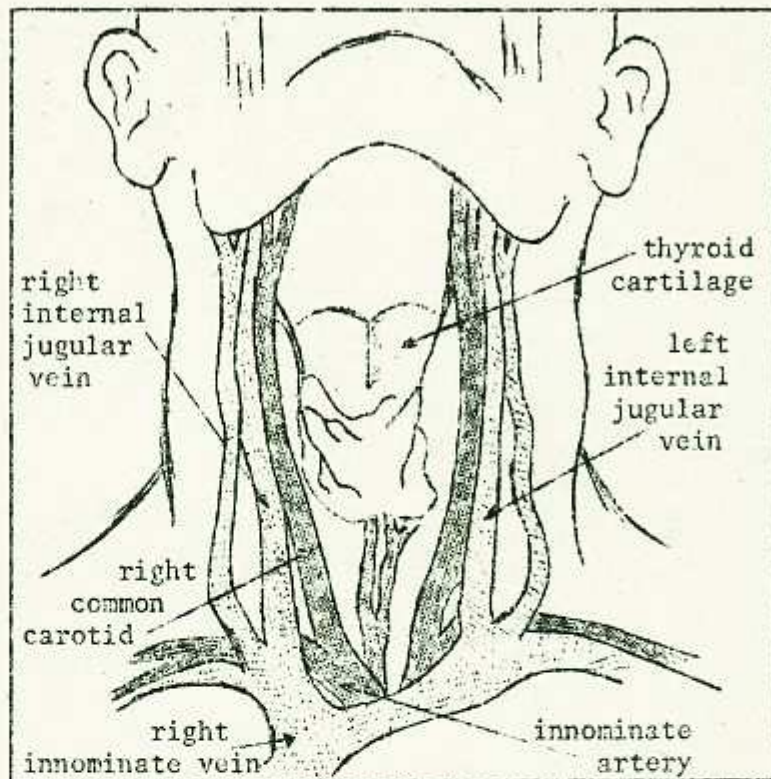


Figure 55-14: Gross Anatomy, Carotid Region

**55.3.2 Cannulation of the Carotid Region.** While cannulating into the carotid region, particular care must be exercised to do as little damage as possible to the dissected muscles and the vagus nerve.

Adequate drainage is not always possible from the left side. For this reason, whenever possible, cannulate the carotid region on the right side. The head should not be supported. Let the head rest directly on the table with the face turned away from the side to be cannulated.

#### 55.3.2.1 Initial Incision.

Using a scalpel, make a skin incision, two and one half inches long, upwards from the clavicle along the anterior (forward) border of the sterno-cleido-mastoid muscle (Figure 55-15). Readily, this muscle will be seen beneath the incision. Use blunt aneurism hooks to very carefully separate this muscle along a straight line.

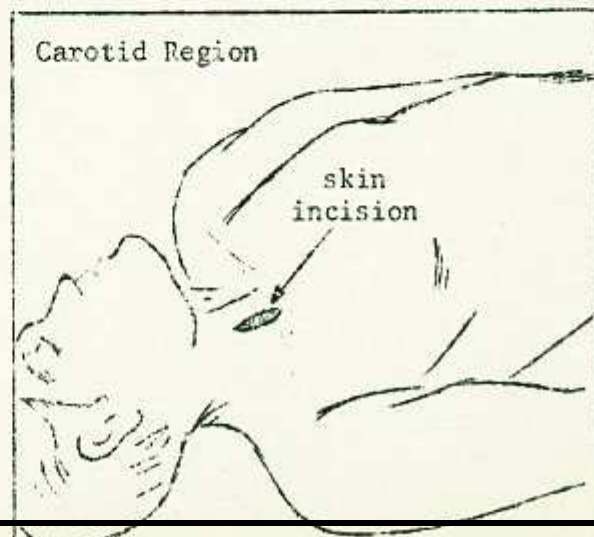


Figure 55-15: Initial Incision

55.3.3.2 Location of Vessels. The carotid sheath will be exposed as the fascia, or fibrous tissue, is separated (Figure 55-4). The carotid artery and jugular vein should be freed from the carotid sheath by blunt dissection (rather than cutting away the tissues). This procedure affords greater protection to the vessels.

55.3.3.3 Raising the Vessels. Using aneurism hooks, raise the common carotid artery and the internal jugular vein to the surface of the skin incision (Figure 55-5). Hold the vessels at the surface by sliding aneurism hooks under the vessels (Figure 55-6), while four pieces of 4-ply thread, approximately 8 inches long, are drawn under each vessel (Figure 55-7).

55.3.3.4 Incisions in Vessels and Insertion-Securing of Cannulas. If arterial pressure exists (heart-lung resuscitator still in use) apply hemostats to both sides of vessels prior to making incisions. The incisions made in the vessels depend on the type of cannulas used.

If "T-shaped" cannulas are used, make one horizontal (parallel) incision in the top of the carotid artery with a pair of scissors and insert the "T-shaped" cannula (Figure 55-8). The incision should be no longer than necessary for insertion. The vessel should be securely ligated around the cannula using the thread previously drawn under the vessels. Refer to figure 55-9 for the recommended method of ligation. Follow the same procedures with the jugular vein.

If conventional cannulas are used, the operation is slightly different and more complex. Using scissors, make two transverse (crosswise) incisions in the top of the artery (Figure 55-10). These incisions should be approximately one inch apart. (*Do NOT make an incision in the jugular vein at this point*). Insert one arterial cannula in each incision (refer to Figure 55-11). The vessel should be securely ligated to and around the cannula, with threads previously drawn under the vessel (refer to Figure 55-12). Follow the same procedures with the jugular vein.

See 55.2.2.6 for cases in which extensive post-mortem clotting has occurred.

## 55.4 THE AXILLARY APPROACH

55.4.1 Anatomy of the Axillary Region. When the Donor is lying in a supine (face up) position, the arm should be extended to a 45 degree angle. The palm of the hand should be supinated (turned upward). The course of the axillary artery and vein can be traced by drawing an imaginary line down the middle of the inner arm from the clavicle to the elbow (Figure 55-16). This line will run between the point where the teres major (the major muscle running from the upper back) and the pectoralis major (the major muscle running from the upper chest) muscles terminate in the axillary space. This muscular anatomy can be easily seen and felt with the fingers, except in exceptionally obese subjects.

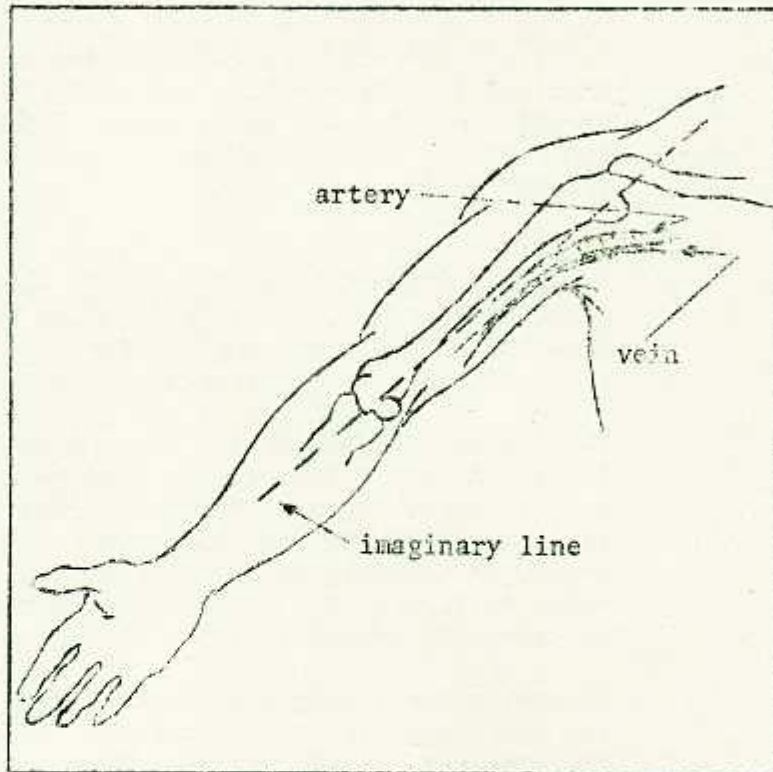


Figure 55-16: Surface Anatomy, Axillary

The axillary vein begins opposite the lower border of the top rib, at the top of the axillary space. The axillary vein then passes down the arm along the outer wall. The axillary artery lies beneath the axillary vein and follows the same course. The median nerve follows in close proximity to the axillary artery and vein and is contained with the vessels in the axillary sheath (Figure 55-17).

#### 55.4.2 Cannulation of the Axillary Region.

##### 55.4.2.1 Incision.

The arm is placed at a 45 degree angle with the palm pointing upward (the body is supine). The skin incision should be made with a scalpel along the groove made by the pectoralis major muscle and the teres major muscle. The incision should begin at the point where the coraco-brachialis muscle (the major muscle of the upper arm on this side) terminates at the axillary region and should extend approximately two inches toward the center of the armpit (Figure 55-18).

55.4.2.2 Location and Raising of Vessels. Blunt dissection with aneurism hocks will readily uncover the axillary sheath (Figure 55-4). The axillary artery and vein are then raised to the surface (Figure 55-5) of

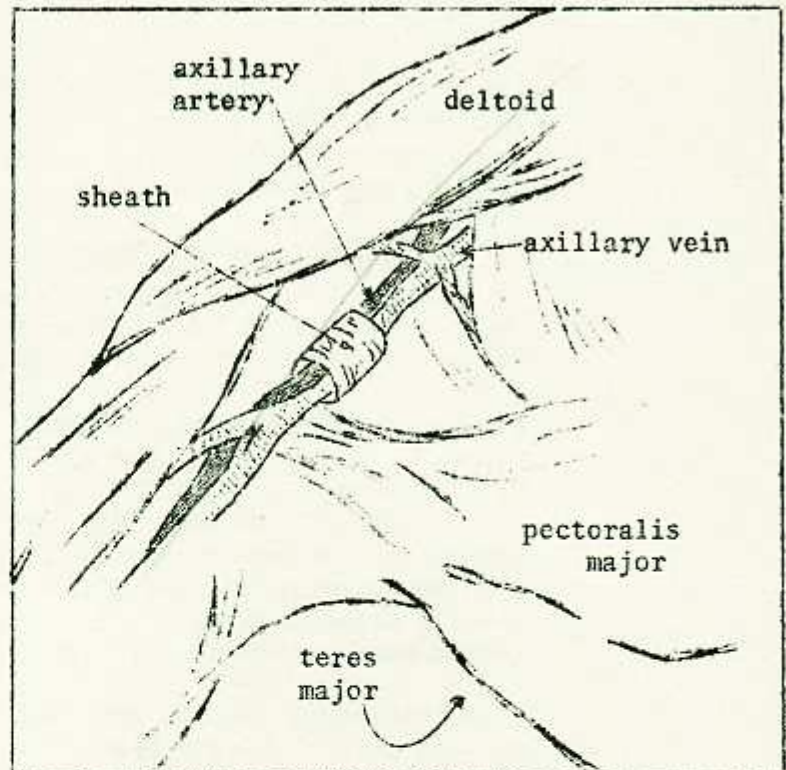


Figure 55-17: Gross Anatomy, Axillary Region

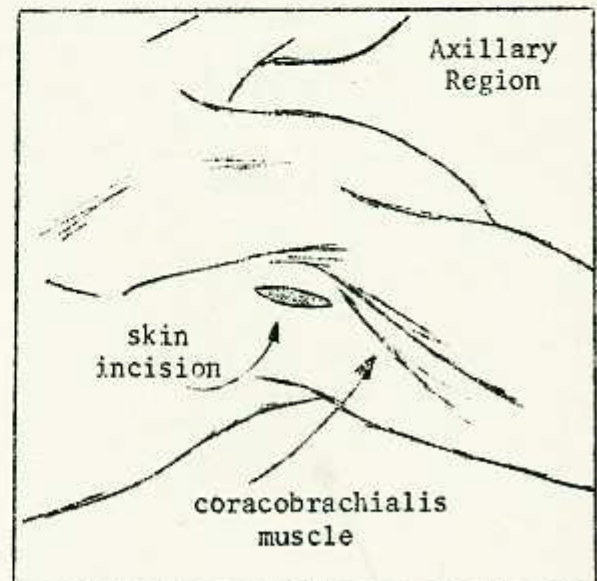


Figure 55-18: Initial Incision

the skin incision and held at the surface by sliding the handle of an aneurism hook under the vessel (Figure 55-6). Four pieces of 4-ply thread, approximately 8 inches long, are drawn under each vessel (refer to Figure 55-7).

#### 55.4.2.3. Incisions in Vessels and Insertion-Securing of Cannulas.

If arterial pressure exists (heart-lung resuscitator still in use) apply hemostats to both sides of vessels prior to incision. The incisions made in the vessels depend on the type of cannulas used.

If "T-shaped" cannulas are used, make one horizontal (parallel) incision in the top of the axillary artery with a pair of scissors. The incision should be no longer than necessary for insertion of the "T-shaped" cannula. Holding the incision open with a hemostat, insert the "T-shaped" cannula (Figure 55-8). The vessel should then be securely ligated around the cannula using the thread previously drawn under the vessels. Refer to Figure 55-9 for the recommended method of ligation.

If conventional cannula are used, the operation is slightly different and more complex. Using scissors, make two transverse (cross-wise) incisions in the top of the artery (refer to Figure 55-10). These incisions should be approximately one inch apart. (*DO NOT make an incision in the axillary vein at this point*). Insert one arterial cannula in each incision (refer to Figure 55-11). The vessel should then be securely ligated around the cannula using threads previously drawn under the vessel (refer to Figure 55-12). Follow the same procedures with the axillary vein.

See 55.2.2.6 for cases in which extensive post-mortem clotting has occurred.

# 56.0

## CANNULATION INSTRUMENTS

Special instruments are required to carry out the recommended cannulation procedures as described in section 55.0). The instruments shown in Figures 56-1 and 56-2 are the minimum required for dissection and cannulation procedures. Figure 56-3 shows the suggested minimum quantity of each item which should be kept on hand. Cannulas may not be removed after Phase II perfusion is completed. They must be replaced as they are expended. The chart also shows the approximate unit price of each item as of December, 1971. A brief discussion of the use of each of these instruments follows.

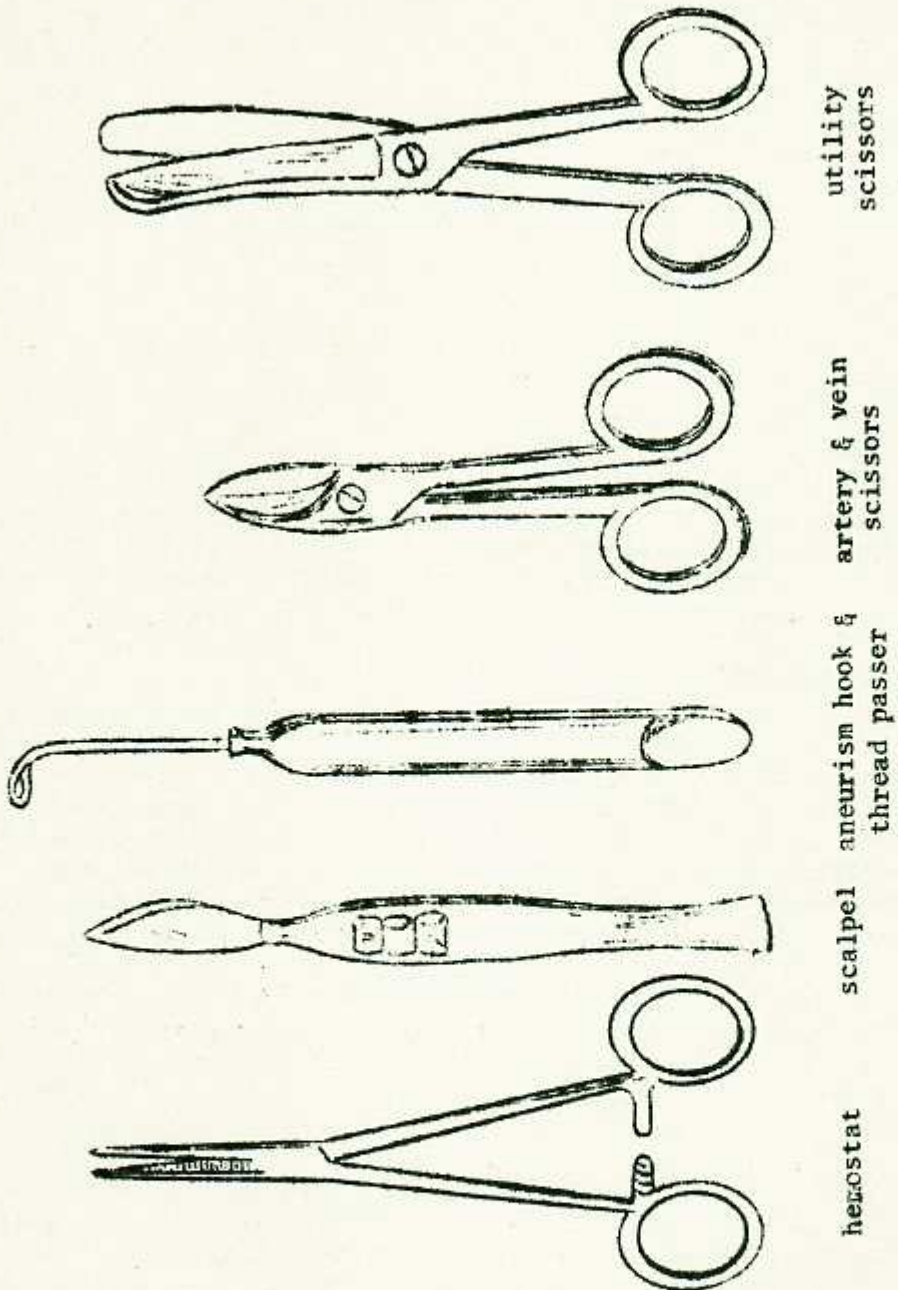
56.1 Hemostats. Hemostats resemble scissors, but do not have a cutting edge. They are used for clamping and holding. The lock found beside the finger grips allows the hemostats to be locked closed without having to be held. The hemostats are used in clamping blood vessels closed, and are also helpful in holding vessel incisions open while inserting cannulas, etc.

56.2 Scalpel. A scalpel is a knife used for making incisions in soft tissue. Scalpels are also available with detachable, disposable and interchangeable blades. A scalpel is seldom used for more than the initial skin incision unless available instruments are limited.

56.3 Aneurism Hook. The aneurism hook is one of the most useful of the instruments shown. It is used for dissecting (spreading) fibrous tissues, raising vessels, and holding vessels at the surface of the skin incision (Figures 55-4, 55-5, and 55-6). The aneurism hook is also available (from most suppliers) in a configuration which adds the capability of a thread passer, i.e., a thread eye is provided in the end of the hook (this type of instrument is pictured in Figure 56-1).

56.4 Artery and Vein Scissors. Artery and vein scissors have one jaw serrated for better holding while cutting. A sharp pair of the correct scissors should always be used for making incisions in arteries and veins (rather than using a scalpel).





utility  
scissors

artery & vein  
scissors

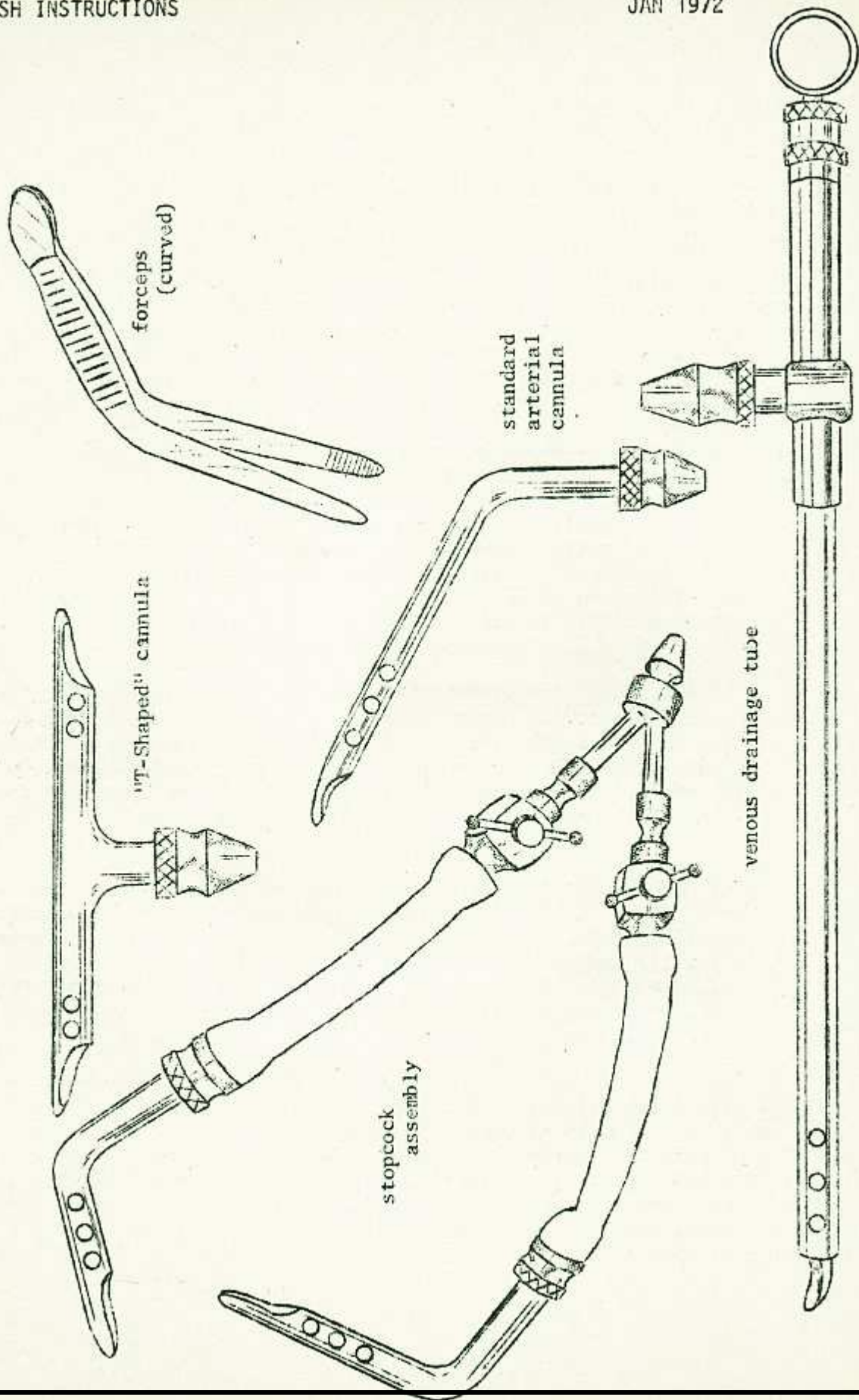
aneurism hook &  
thread passer

scalpel

herostat

Figure 56-1: Cannulation Instruments

Figure 56-2: Cannulation Instruments



56.5 Utility Scissors. Utility scissors should be used for general purpose tasks such as cutting ligatures (4-ply thread used for ligating or tying vessels). This will help preserve the cutting edges on the artery and vein scissors. Utility scissors are available in a large variety of configurations.

56.6 Cannulas. Cannulas are tubes which are inserted into an artery or vein for the purpose of injecting or extracting fluids.

56.6.1 Embalming Cannulas. Conventional embalming cannulas are available in varying sizes, from suppliers such as Kelco Supply Company (Appendix A). They can often be purchased in sets containing various sizes which would accommodate cannulation of all appropriate vessels in individuals of different body size.

The configuration of the opening at the tip of embalming cannulas may not allow optimum flow. Also, the materials used in these instruments (usually chrome plated brass) may not be the most suitable for use in the induction of SSH and its ultimate objectives. Stainless steel would probably be preferable.

56.6.2 Hemodialysis Cannulas. Hemodialysis (artificial kidney) cannulas are made of silicone rubber and other physiologically superior materials, but they may not be available in configurations and sizes appropriate for perfusion in the induction of SSH. One possible source of information about silicone rubber cannulation supplies is the Seattle Artificial Kidney Supply Company (Appendix A).

56.6.3 "T-Shaped" Cannulas. Although "T-shaped" cannulas as such are not known to be commercially available at this time, this configuration has been recommended. "T-shaped" cannulas would greatly simplify and enhance cannulation and perfusion procedures. "T-connectors" for flexible tubing are marketed by such suppliers as Van Waters and Rogers (Appendix A), and may be adapted to use as cannulas.

56.7 Stopcock Assembly. The stopcock assembly allows two-directional, double injection to be used when "T-shaped" cannulas are not available. The "Y-shaped" stopcock is connected to the cannulas with two inch to ten inch lengths of surgical tubing. The stopcock allows flow in either direction to be restricted or completely cut off. 4-way and 6-way stopcocks are also available for use in cases where injection must enter in several sites at once (55.1).

56.8 Drainage tubes. Drainage tubes are inserted temporarily into veins to aid in the removal of blood coagulation (55.2.2.6). Conventional embalming drainage tubes are available in varying sizes to accommodate different regions to be cannulated as well as different body sizes. The plunger aids in removing clots.

Description	Suggested Min. Qty.	Approx. Unit Cost	Approx. Ext. Cost
Hemostats	2	5.80	11.60
Scalpel	1	3.20	3.20
Aneurism Hooks	2	3.70	7.40
Artery and Vein Scissors	2	5.80	11.60
Utility Scissors	1	2.50	2.50
Thread Passer	1	8.00	8.00
Forceps (straight)	2	6.10	12.20
Forceps (curved)	2	6.10	12.20
"T-Shaped" Cannulas [Set of (3) Assorted Sizes]	2 sets	?	?
Arterial Cannulas [Set of (3) Assorted Sizes]	2 sets	5.30	10.60
"Y-Shaped" Stopcock Assembly	2	8.30	16.60
Total Approximate Extended Cost			95.90

Figure 56-3

1. Curved arterial cannulas and "Y-shaped" stopcock assembly are only needed if "T-shaped" cannulas are not available.
2. Prices shown are approximate prices as of December, 1971.
3. All instruments shown are made of stainless steel with the exception of the cannulas and stopcock assembly, which are of chrome plated brass.

One possible source of these instruments is Kelco Supply Company, (Appendix A).

## 57.0 PREPARATORY STEPS PRIOR TO PHASE I PERFUSION

As the Donor is being cannulated, it is necessary to prepare the perfusate and load it into perfusion equipment so that Phase I perfusion can begin promptly when cannulation is completed. These steps, of course, presuppose the ready availability of the required materials and apparatus.

The two sections which follow will give instructions for these preparations. They provide for activities which should begin months prior to an actual need for perfusion, and lead up to the very moment that apparatus is connected to the Donor.

This scope of treatment is necessary, since it is impossible to predict the state of readiness that will exist in any set of circumstances. A large well organized SSH society might have perfusate premixed and stored in refrigerators, with highly automatic perfusion equipment pre-sterilized and standing by. Instructions for preparation could be as simple as "turn on the perfusion apparatus and fill it with perfusate". Such will not always be the case. A small, new SSH society may have its perfusate stored in pre-measured dry chemical form, and its equipment might be a relatively unsophisticated set of components that would have to be assembled and checked out prior to use. For this reason, these instructions are intended to provide a comprehensive basis for preparation regardless of individual circumstances.

# 58.0

## PREPARATION OF PERFUSATE FOR PHASE I

The perfusate for Phase I is a liquid closely approximating the basic constituents interior to cells. It first replaces the blood and then, by diffusion, replaces the body's extracellular fluids (those fluids outside of and surrounding the cells). The Phase I perfusate is pre-chilled and then circulated by some type of perfusion apparatus so as to most rapidly lower the body's temperature. This section covers the Composition and Preparation of Phase I Perfusate (58.1), Practical Problems and Possible Solutions (58.2), and pH Measurement and Control (58.3).

58.1 Composition and Preparation of the Phase I Perfusate. The following instructions are derived directly from a recent paper by Art Quaife (1) concerning Phase I perfusates for the induction of solid state hypothermia.

58.1.1 Summary. Figure 58-1 provides fundamental guidance in preparing one liter of perfusate.

58.1.2 Accuracy of Weighing. In weighing components, it is required that a scale be used which is accurate to one hundredth part of the component weighed or better. This may be difficult in the case of components such as phenoxybenzamine, where an accuracy of 0.0025 grams or 0.0386 grains would be required. By forming the perfusate in amounts of four gallons (15.1 liters), the accuracy figure may be raised to 0.038 grams or 0.58 grains. These still are stringent requirements, but must be attained since mis-weighing of certain critical constituents (some of them are only present in small amounts) can significantly alter pH or osmolarity.

As a last resort, an accurately weighed amount of the component (weighed to an accuracy of better than one percent) can be thoroughly dissolved in deionized, distilled water. It finally becomes necessary to divide the liquid into smaller portions, containing the amounts of the component needed, in such a way that overall errors do not exceed one percent. In short, there is no simple substitute for an accurate scale.

I N S T R U C T I O N S   F O R   P R E P A R A T I O N  
O F   O N E   L I T E R   O F   P E R F U S A T E   Q - 2

<b>1</b>	<b>WEIGH OUT COMPONENTS</b>	<b>2</b>	<b>AFTER WEIGHING COMPONENTS</b>																
	<table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;"><u>COMPONENTS</u></th> <th style="text-align: left;"><u>GRAMS</u></th> </tr> </thead> <tbody> <tr> <td>KH<sub>2</sub>PO<sub>4</sub></td> <td>0.92</td> </tr> <tr> <td>K<sub>2</sub>HPO<sub>4</sub> · 3H<sub>2</sub>O</td> <td>4.37</td> </tr> <tr> <td>NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O</td> <td>1.15</td> </tr> <tr> <td>Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O</td> <td>6.12</td> </tr> <tr> <td>NaCl</td> <td>0.93</td> </tr> <tr> <td>NaHCO<sub>3</sub></td> <td>0.84</td> </tr> <tr> <td>Glucose *</td> <td>25.00</td> </tr> </tbody> </table>	<u>COMPONENTS</u>	<u>GRAMS</u>	KH <sub>2</sub> PO <sub>4</sub>	0.92	K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	4.37	NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	1.15	Na <sub>2</sub> HPO <sub>4</sub> · 7H <sub>2</sub> O	6.12	NaCl	0.93	NaHCO <sub>3</sub>	0.84	Glucose *	25.00		<p>TO THE COMPONENTS, ADD DISTILLED, DEIONIZED WATER TO FORM A FINAL QUANTITY OF ONE (1) LITER.</p> <p>THE SOLUTION IS STERILIZED BY AUTOCLAVING, STORED ON SHELF, AND REFRIGERATED BEFORE USING.</p>
<u>COMPONENTS</u>	<u>GRAMS</u>																		
KH <sub>2</sub> PO <sub>4</sub>	0.92																		
K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	4.37																		
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	1.15																		
Na <sub>2</sub> HPO <sub>4</sub> · 7H <sub>2</sub> O	6.12																		
NaCl	0.93																		
NaHCO <sub>3</sub>	0.84																		
Glucose *	25.00																		
<b>3</b>	<b>IMMEDIATELY BEFORE USE, ADD:</b>																		
	<table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;"><u>COMPONENTS</u></th> <th style="text-align: left;"><u>AMOUNTS</u></th> </tr> </thead> <tbody> <tr> <td>DEXTRAN 40</td> <td>50 Grams</td> </tr> <tr> <td>HEPARIN</td> <td>5000 Units</td> </tr> <tr> <td>PHENOXYBENZAMINE</td> <td>0.25 Grams</td> </tr> <tr> <td>MgSO<sub>4</sub> · 7H<sub>2</sub>O</td> <td>0.37 Grams</td> </tr> </tbody> </table>	<u>COMPONENTS</u>	<u>AMOUNTS</u>	DEXTRAN 40	50 Grams	HEPARIN	5000 Units	PHENOXYBENZAMINE	0.25 Grams	MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.37 Grams								
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<b>4</b>	<p>TO REMOVE ANY POSSIBLE (UNDETECTED) PRECIPITATE, THE SOLUTION IS NOW FILTERED THROUGH A 5 TO 10 MICRON PORE SIZE SINTERED GLASS FILTER.</p>																		

\* See 58.2.1

58.1.3 Mixing Quantities Greater Than One Liter. Quantities of components for mixing amounts of perfusate greater than one liter are shown in figure 58-2. Units are given in terms of both grams and grains, so that all appropriate scales may be utilized. Dilution of the first seven components to form the quantities indicated, sterilization, storage, refrigeration, addition of the last four components immediately before use, and filtration are performed as indicated in figure 58-1.

58.1.4 Water of Crystallization Corrections. Although one of the components shown is  $K_2HPO_4 \cdot 3H_2O$ , this chemical normally is sold without "water of crystallization", in powdered form ( $K_2HPO_4$ ). If purchased in this form, lesser quantities (weights) must be used to compensate for the absent water. The amounts shown below apply, rather than the amounts shown in figures 58-1 and 58-2, IF THERE IS NO WATER OF CRYSTALLIZATION (reduction factor is .724). No correction factors are provided for  $Na_2HPO_4 \cdot 7H_2O$  or  $MgSO_4 \cdot 7H_2O$ , since these chemicals are usually sold with the water of crystallization ( $H_2O$  factor) included.

	<u>GRAMS</u>	<u>GRAINS</u>
$K_2HPO_4$ for 1 liter	3.34	51.5
$K_2HPO_4$ for 1 gallon	12.6	195.0
$K_2HPO_4$ for 4 gallons	50.5	782.0

58.1.5 Filtration of Perfusate. The recommended procedure is for filtration through a sintered glass filter with 5-10 micron pore size. This recommendation stems from an article cited in reference (1). Millipore (see Appendix A) offers a glass disc "prefilter", consisting of pure glass fibers with an acrylic binder, .035 or .011 inches thick. No indication of particle size filtration is shown. The user is referred to a discussion of filters in the section on equipment for Phase I perfusion (59.0). There is also discussion of filtration for the purpose of sterilization in paragraph 58.2.3.3.

58.1.6 Storage Containers. Containers for Phase I perfusate (per figure 58-1) should be autoclavable bottles which can be emptied into the reservoir of a perfusion machine when needed. Linear polyethylene bottles such as those by Nalgene sold through Cole Parmer (see Appendix A) are probably suitable. Teflon-TFE thread-seal tape (also available through Cole Parmer) can be used to improve the seal at the containers' openings.



COMPONENT	AMOUNT OF PERFUSATE TO BE PREPARED					
	One Liter		One Gallon		Four Gallons	
	Grams	Grains	Grams	Grains	Grams	Grains
$KH_2PO_4$	0.92	14.2	3.48	53.8	13.9	215.0
$K_2HPO_4 \cdot 3H_2O$ *	4.37	67.5	16.5	256.0	66.3	1024.0
$Na_2HPO_4 \cdot H_2O$	1.15	17.7	4.35	67.2	17.4	269.0
$Na_2HPO_4 \cdot 7H_2O$ *	6.12	94.5	23.2	358.0	92.8	1432.0
NaCl	0.93	14.4	3.52	54.3	14.1	217.0
$NaHCO_3$	0.84	13.0	3.18	49.0	12.7	196.0
Glucose **	25.0	386.0	94.6	1460.0	379.0	5840.0
Dextran 40	50.0	772.0	189.2	2920.0	758.0	11,680.0
Heparin	5000 Units		18,920 Units		75,800 Units	
Phenoxybenzamine	0.25	3.86	0.946	14.6	3.79	58.4
$MgSO_4 \cdot 7H_2O$ *	0.37	5.97	1.4	21.6	5.61	86.4

Figure 58-2

\* See 58.1.4

\*\* See 58.2.1

58.1.7 Sources of Chemicals. In Appendix A, suppliers are listed by name and their addresses are given. Many of these will furnish detailed catalogs upon request. The MCB (Matheson, Coleman and Bell) Division of the Matheson Company publishes a "Laboratory Chemical Catalog" listing a wide range of supplies. Van Waters and Rogers distributes "Catalog 700, J. T. Baker Laboratory Chemicals and Products" which contains not only many different grades of chemicals, but highly detailed impurity analyses, reference tables, and other useful information. Heparin is most readily and inexpensively obtained from companies supplying users of artificial kidneys. Ries Biologicals, Inc. is one such firm. Phenoxybenzamine is sold under the trade name of "Dibenzaline" by Smith, Kline, and French Laboratories. Dextran 40 is available from Pharmachem Corporation.

58.1.8 Quantities of Perfusate to be Prepared. The quantities of perfusate to be prepared are dependent on the resources available. Ideally, open circuit (no recirculation) perfusion using chilled perfusate would be carried out until body core temperatures were near 0°C. In the case of a large adult, this could require either many hundred liters of perfusate or very slow perfusion with maximum attention to surface cooling. The latter procedure would lead to slower internal cooling, while the first procedure involving very large quantities of perfusate might be very expensive. It is recommended, at this time, that no less than 50 liters of perfusate be available, in the case of a 73 kg adult.

58.1.8.1 30 liters would be expended in an initial flush of the circulatory system, whereupon recirculation would begin.

58.1.8.2 After 30 minutes of recirculation, an additional 20 liters could be expended in flushing the system, following which recirculation would be resumed.

58.1.8.3 Many possible "recirculate and flush" schedules are possible and (besides the recommendation for continuous open circuit perfusion) no medical authority has addressed this question. When specific recommendations are available, they will be incorporated into this section.

58.1.9 Cooling the Perfusate. The preferred method of chilling the perfusate to the desired 0°C is by direct refrigeration. A second method involves rapid recirculation of the perfusate through heat exchanger coils immersed in a cooling medium of water and crushed ice. (In one experiment\* 3.7 gallons of perfusate was cooled from 90 F to 50 F in six minutes by this method, a heat removal rate of 11,800 BTU/hour.) The ice water should be agitated frequently to prevent "warm pockets" around the heat exchanger coil.

\* conducted by Manrise Corporation

The least desirable method of cooling the perfusate (adding ice directly to concentrated perfusate) is discussed in 58.2.3.2-3-4. This method poses many problems and is not recommended except in emergency situations where other means are not possible.

58.1.10 Perfusate Shelf Life. The useful "shelf" or storage lifetime of the perfusate in liquid form is not known. Typically, these solutions are used in experimental rather than clinical procedures, so no standard practice has been established. Since a primary constituent is glucose, a necessary element for bacteriological growth is available and liquid perfusates to be stored must be effectively sterilized.

58.2 Practical Problems and Possible Solutions. With access to unlimited resources, under conditions where all events are foreseen and no emergencies occur, the foregoing instructions are sufficient. Chemicals are mixed as directed in the required amounts, stored, refrigerated, and filtered prior to use.

Those with limited resources, and/or facing emergency circumstances may find it necessary to deviate from standard procedures. They will encounter problems which will prevent full adherence to the recommendations given previously. In such circumstances, compromises may be required, although it is clear that such compromises are detrimental to a greater or lesser degree and are not to be used except when the preferred alternative(s) is not possible. The following discussion of alternatives must be taken in that context.

58.2.1 Glucose. Glucose is a thick liquid consisting primarily of hydrolyzed dextrose (dextrose dissolved in water). In this liquid form, it is relatively expensive and difficult to handle. As an alternative, dextrose can be procured as a dry powder and combined with other components in forming the perfusate. It may be weighed more easily since there is no need to compensate for residue clinging to container walls or provide for flushing out this residue. Glucose is approximately 95 percent dextrose by weight, so subtracting 5 percent from the weight of glucose gives the required weight of powdered dextrose to be substituted.

58.2.2 Dry, Separate Storage of Components. As readers who have followed the evolution of basic perfusate composition are aware, changes have occurred frequently and still other changes may be required with the passage of time. The rebalancing of premixed liquid perfusate could be very cumbersome, so that entire stocks of perfusate might be obsoleted and required to be discarded again and again. This will not pose a problem for societies with ample funds and hired laboratory workers, but organizations with more limited resources will

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find this load untenable. The latter organizations may find it necessary to weigh out perfusate components in dry form and store them in separate containers until the need arises. If changes of quantities occur, the chemicals involved are simply reweighed individually.

58.2.2.1 Containers. Small polyethylene wide mouth bottles obtainable from laboratory supply firms such as Cole Parmer (see Appendix A) can be used for storage of dry chemicals. Alternatively, plastic containers may be purchased from pharmacists (be sure that such plastics are non-reactive with chemicals to be stored). Separate containers are required for each chemical in each "batch" (neglecting heparin, which comes in pre-measured containers). The side of each container should be labeled with the specific chemical contents and its exact weight.

58.2.2.2 "Batch" Size. Since several containers per batch are required, it is recommended that chemicals be weighed and stored for preparing large quantities. Four gallon units are convenient, and quantities for this size batch are provided in figure 58-2. Containers should be grouped in and stored as sets, so that direct addition of distilled, deionized water to form the appropriate volume can be carried out efficiently.

58.2.3 Mixing Perfusate which is Stored in Dry, Separate Containers. Given sufficient notice as to need, and provided with adequate autoclaving facilities, refrigeration space, etc., the dry, pre-weighed components can be combined in an essentially ideal way. In this event, the advantage of pre-weighing is that some time is saved, but the lengthy processes of autoclaving and chilling in refrigerators must still be accomplished. If the need is immediate, these preparatory functions may require too much time. An alternative (though it is certainly less desirable) approach is described below:

58.2.3.1 Mix Perfusate in Concentrated Form. Dissolve the dry chemicals necessary to form four gallons of perfusate (exactly four times that needed for one gallon solution) in just one gallon of distilled, deionized water. (When perfusate is to be used, an additional three gallons of water and/or ice will be added.) Use commercially available distilled, deionized water as possible. *Do not add heparin at this point.*

58.2.3.2 Add Ice and Water to Form Standard Strength Perfusate at a Temperature Between 0°C and 4°C. By adding the proper amounts and proportions of water and crushed ice, a fully liquid perfusate at proper concentration in the 0°C to 4°C temperature range may be produced. For a four gallon batch the total weight of water and ice added will be 24 pounds. At one pound per pint, this is 24 pints or three gallons. This will fully dilute the (gallon of 4:1) concentrated perfusate to the proper concentration. The detailed discussion which follows will guide the user in determining the proper proportions of water and ice to be added:

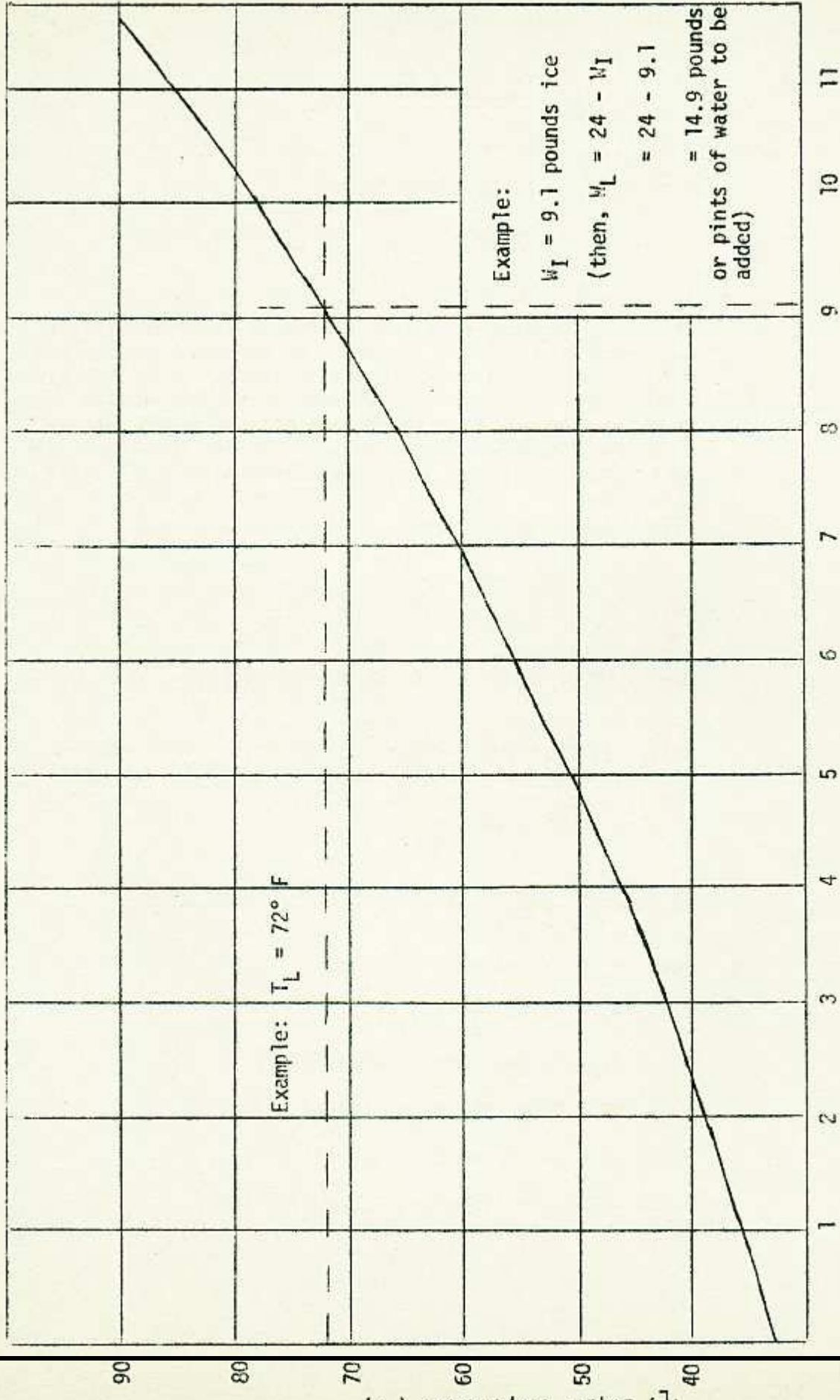
58.2.3.2.1 Measure Temperature of Concentrated Perfusate ( $T_C$ ) and Water ( $T_W$ ). Both of these should be approximately at room temperature and nearly equal. If this is the case, this temperature is the "Liquid Temperature" ( $T_L$ ) to be used in part 58.2.3.2.2 below. If the values are somewhat different, refer to paragraph 58.2.3.2.3.

58.2.3.2.2 Determine the Proportions of Ice and Water to be Added. In figure 58-3, draw a horizontal line at liquid temperature " $T_L$ ". (Example; 72°F). Directly beneath the intersection of the line and the curve, at the bottom of the graph, read the weight of ice " $W_I$ " in pounds (example; 9.1 pounds). Subtract  $W_I$  from 24 to obtain the weight of water to be added ( $W_L$ ) in pounds, or in pints; either is correct (example; 24.0 - 9.1 = 14.9 pounds or pints of water to be added). The mathematics used to determine the curve in figure 58-3 are provided at the end of the section in paragraph 58.2.3.2.4.\*

58.2.3.2.3 Determination of  $T_L$ . " $T_L$ " is an essential factor in determining the proportion of ice to be added. It is an undesirable situation, to begin with, where perfusate must be mixed in concentrated form and then be diluted with ice. Where the concentrated perfusate and other water of dilution do not match in temperature, this unfortunate situation is compounded.

If  $T_C$  and  $T_W$  are somewhat different but both close to room temperature (72°F),  $T_L$  may be obtained, approximately, with the below formula.\*

\* After determining  $W_I$  and  $W_L$ , add ice and water to concentrated perfusate and refer directly to paragraph 58.2.3.3 for further instructions.



Example:  $T_L = 72^\circ \text{F}$

Example:  
 $W_I = 9.1$  pounds ice  
 (then,  $W_L = 24 - W_I$   
 $= 24 - 9.1$   
 $= 14.9$  pounds  
 or pints of water to be  
 added)

Figure 58-3

$W_I$ : Weight of Ice (pounds)

$$T_L = \frac{T_C + 2 T_W}{3}$$

where:  $T_L$  = "Liquid Temperature"

$T_C$  = Temperature of Concentrated Perfusate

$T_W$  = Temperature of Water

If the temperature of liquid perfusate is substantially different from that of the water to be used in diluting the perfusate, and they are not "balanced" on either side of room temperature, then the process of calculating  $T_L$  is quite complex, and requires successive iterations in which (see 58.2.3.2.2) estimates of  $W_I$  are used to derive test values of  $T_L$  and  $W_L$ . A trial value of  $T_L$  ( $T_L'$ ) is calculated by:

$$T_L' = \frac{8T_C + W_L T_W}{8 + W_L}$$

where:  $W_L$  = Weight of water to be added (see 58.2.3.2.2)

If  $T_L'$  is less than  $T_L$  then the estimate of  $W_I$  must be adjusted and the procedure repeated until  $T_L'$  is approximately equal to  $T_L$  (within 2-3 degrees). Then the value of  $W_I$  estimated is correct.

#### 58.2.3.2.4 Derivation of Curve in Figure 58-3.

let:  $W_L$  = Weight of Liquid (lb)

$W_I$  = Weight of Ice (lb)

$T_L$  = Temperature of Liquid (°F)

32 = Total Weight of Liquid + Ice (lb)

111.5 = Latent Heat of Ice (BTU/lb)

H = Heat Transfer, Liquid-Ice (BTU)



$$W_L + W_I = 32$$

$$H = 111.5 W_I$$

$$W_L = 32 - W_I$$

$$H = (T_L - 32)(W_L)$$

$$111.5 W_I = (T_L - 32)(32 - W_I)$$

$$W_I = \frac{32 T_L - 1024}{T_L + 69.5}$$

Values of  $T_L$  were substituted and values of  $W_I$  were calculated. The curve of figure 58-3 is a plot of these values.

58.2.3.3 Filter Chilled Perfusate to Sterilize. The perfusate is pumped through a precision pore filter (such as those by Millipore or G.E.'s Nucleopore; see Appendix A) with pores of 0.5 micron or smaller. The perfusate emerges with all microscopic particles removed in an essentially sterile condition. If this procedure is to be of maximum benefit, the final end of the filter, the tubing leading to a collection container, and the container itself must have been pre-sterilized. Since the perfusate is filtered in a chilled state, it should be used immediately, or refrigerated. (Filtration cannot precede mixing with ice, and final dilution, unless the ice and water for dilution are both sterile and pre-filtered).

58.2.3.4 Heparin. Heparin, in the amount required, (figure 58-1) is added immediately prior to use.

58.3 pH; Its Measurement and Control. The primary reference of this section (1) stresses the importance of maintaining a pH of 7.0, rather than (as has previously been held desirable) a pH of 7.4. The proportions of constituents should result in a pH of 7.0, with essentially no rebalancing required.

Should perfusate be recirculated, as suggested in 58.1.8, pH changes could occur. The perfusate will absorb salts present in the extracellular fluids and rebalancing may be appropriate. For this reason, particularly during recirculation, pH should be monitored and controlled.

58.3.1 pH Measurement. Either pH sensitive tape (Figure 58-4) or a pH meter (Figure 58-5) may be used for pH measurement. This type apparatus is available from laboratory supply houses such as Cole Parmer and Van Waters and Rogers Scientific (see Appendix A).

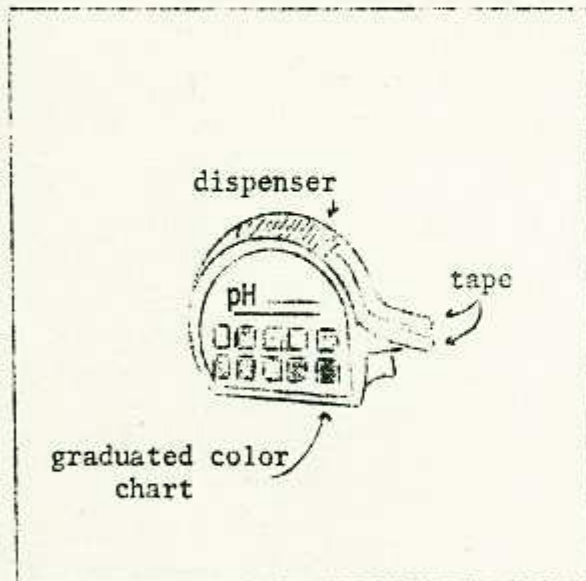


Figure 58-4: pH Tape

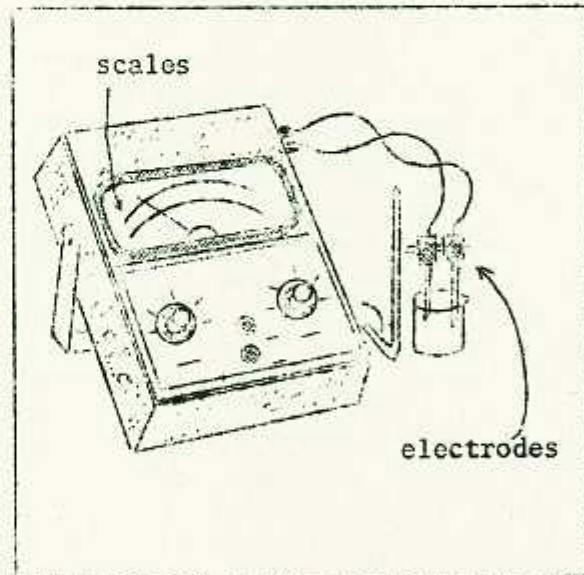


Figure 58-5: pH Meter

58.3.2 pH Control. pH may be raised by adding a sodium hydroxide solution (described below). Similarly, the pH may be lowered by adding a hydrochloric acid solution.

58.3.2.1 Preparation of the Sodium Hydroxide Solution. Add 14.0 grams of undiluted sodium hydroxide to a liter of distilled, deionized water.

58.3.2.2 Preparation of the Hydrochloric Acid Solution. Add 12.8 grams of undiluted hydrochloric acid to a liter of distilled, deionized water.

58.3.2.3 Amounts to be Added. The solutions used should be added in sufficient quantity to restore pH to the target value (7.0). Quantities less than one cubic centimeter (or one milliliter) will probably have little effect. It is recommended that quantities added be increased in steps of two (2, 4, 8, 16) until noticeable changes (alteration of pH by at least 0.1) are observed. Once noticeable changes can be produced, the same amounts should be repeatedly added until the pH is rebalanced. Between each treatment, the perfusate treated should be allowed to mix thoroughly and should be retested.

58.3.2.4 Effects of Rebalancing pH. The use of sodium hydroxide and hydrochloric acid for pH control will add sodium and/or chlorine to the perfusate. The perfusate (assuming it is being recirculated) will be picking up substantial amounts of salt (sodium chloride) from the tissues in any case, so increases of sodium and chlorine will occur even if pH is not rebalanced. Since the objective of perfusing with an intracellular fluid is to remove salt, the perfusate will ultimately become laden with salt and will be discarded, being replaced with low-salt fresh perfusate approximating intracellular fluid. The fresh perfusate will initially be of the correct pH, and as before, may have to be rebalanced for the proper pH as it is recirculated.

#### Reference

1. Quaife, A., *Manrise Technical Review*, 2:3-9, 1972.

## 59.0 EQUIPMENT FOR PHASE I PERFUSION

Phase I perfusion equipment basically provides for the injection of perfusate into the Donor's circulatory system. There are some very simple ways to accomplish this, which are not desirable because they are relatively ineffective, and there are more effective ways which are comparatively complicated. The more effective methods are more costly, and the equipment is not so easy to use. Also, the more effective equipment is usually more difficult to transport.

There is no "standard apparatus". A number of perfusion systems are described from which one may choose. This section includes general systems descriptions (59.1), specific systems descriptions (59.2), a discussion of components (59.3), and operational procedures (59.4).

The reader is cautioned that this treatment, however detailed it may appear, is only a preliminary survey. More detailed information can be obtained through direct contact with Manrise Corporation, if the need is immediate. More complete information will be contained in future revisions, although it is doubtful that this will answer all the questions of those who may decide to construct apparatus themselves.

59.1 General System Descriptions. Equipment for Phase I perfusion may be placed into two categories. Category "A" systems are embalming devices, adapted for Phase I perfusion. Category "B" is comprised of that equipment which is specifically designed for Phase I perfusion in the induction of SSH.

59.1.1 Category "A". Equipment in category "A" is commercially available, and systems can be assembled which provide for the minimum essential functions required in Phase I perfusion. The basic elements include a reservoir, containing perfusate, and a controllable means of developing injection pressure (usually a pump). Perfusate flows from the reservoir to the pressure producing apparatus, through flexible rubber or plastic tubing, and from there to the Donor. A means of measuring or determining perfusion pressure must be provided, and instrumentation for measuring perfusate temperature must be available. There must be a container for collecting the perfusate after it has

passed through the Donor's circulatory system. A functional block diagram of this basic system is shown in Figure 59-1.

59.1.2 Category "B". Equipment in category "B" is intended solely for use in conjunction with the induction of SSH. It embodies all functions of category "A" equipment, and provides such additional features as a flow meter and internal recirculation of perfusate through a heat exchanger so that temperature can be controlled (59.2.5).

59.2 Specific System Descriptions. The following descriptions provide the reader a working familiarity with specific perfusion systems. External appearances and general capabilities are indicated; advantages and disadvantages are cited. A brief evaluation is provided in each case.

59.2.1 System A-1. A relatively uncomplicated perfusion method involves the use of a hand pump (Figure 59-2). This device has an input and output nozzle, each containing a metal valve. Flexible tubing is attached to both nozzles. The pump's input tubing is connected to a container of some kind that is used as a reservoir. The pump's output tubing is connected to a cannula in the Donor. When the plunger is drawn out, perfusate from the reservoir is drawn into the pump. When the plunger is depressed, perfusate is injected into the Donor's circulatory system.

A pressure gauge (Figure 59-3) must be connected to the output tubing and carefully monitored as the plunger is depressed, so that physiological range pressures will not be exceeded. A thermometer (Figure 59-4) is used to monitor reservoir perfusate temperature levels. The fluid levels in the reservoir and in the output container must be measurable by means of a depth gauge of some kind, convertible to volumetric units so that the amounts of perfusate injected and perfusate collected from the Donor may be determined as a function of time.

59.2.1.1 Advantages. System A-1 is highly portable and inexpensive.

59.2.1.2 Disadvantages. The perfusion pressure is related to hand pressure on the plunger at every moment, and is difficult to control. It is possible to damage the Donor's circulatory system by excessive pressure, and constant input pressure is virtually impossible to attain (pressure goes to zero every time the pump is recharged by withdrawing the plunger). As perfusate travels to the pump and then to the Donor, it warms up, thus the temperature measured at the reservoir may be quite different from the temperature of the perfusate at the point of injection.

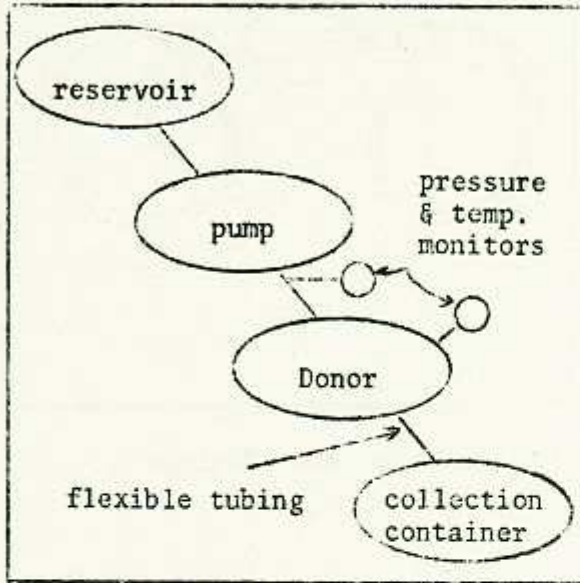


Figure 59-1: Phase I Perfusion

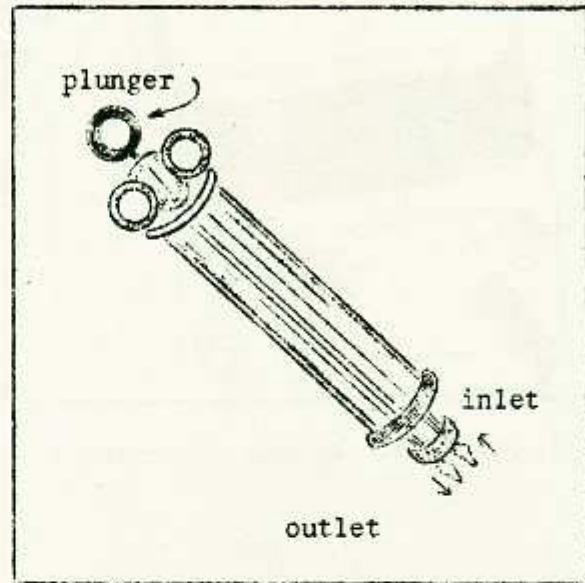


Figure 59-2: Hand Pump

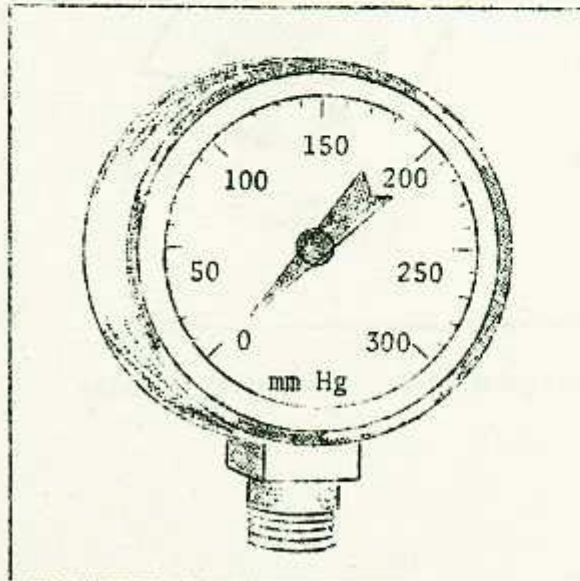


Figure 59-3: Pressure Gauge

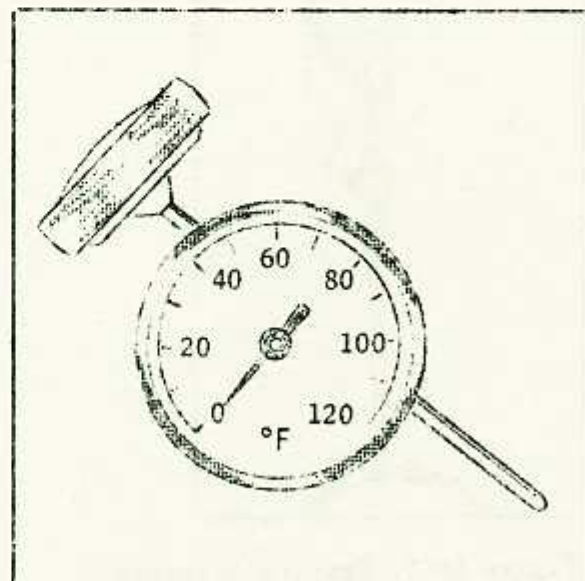


Figure 59-4: Thermometer

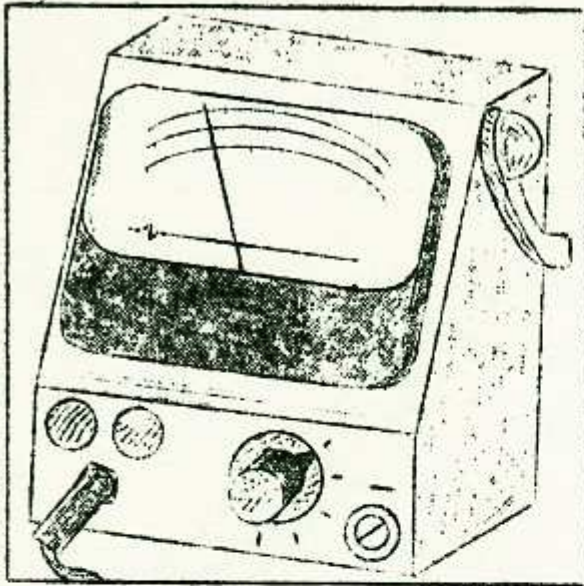


Figure 59-5: Remote Thermometer

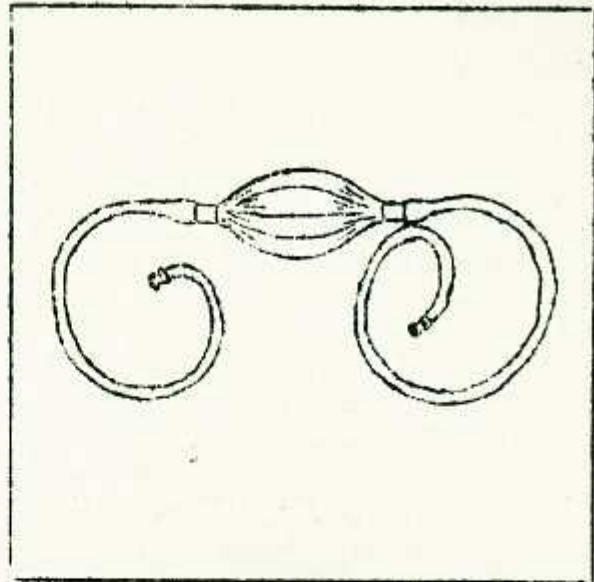


Figure 59-6: Bulb Syringe

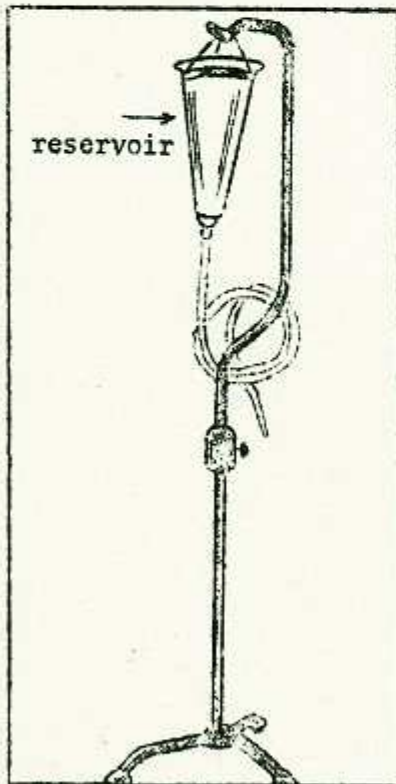


Figure 59-7: Gravity Injector

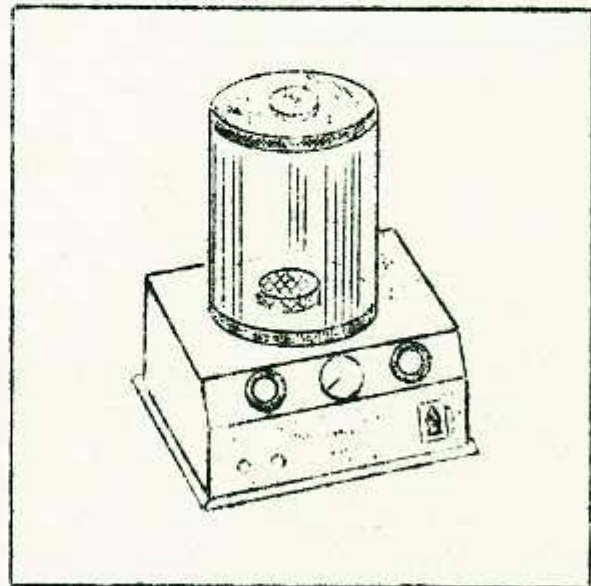


Figure 59-8: Embalming Machine

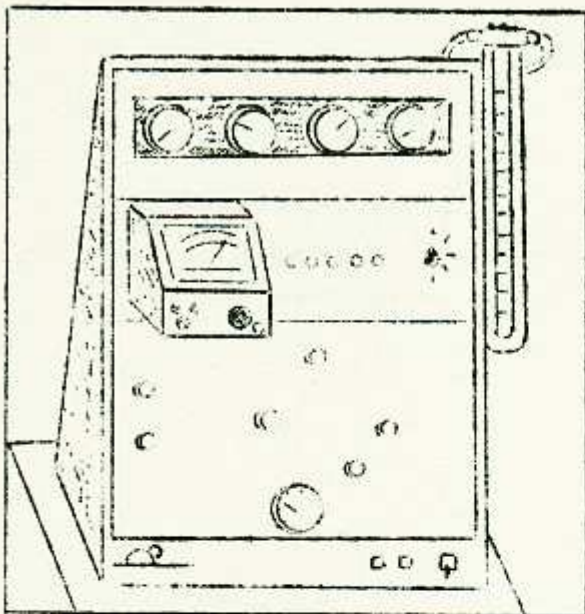


Figure 59-9: Special Perfusion Apparatus

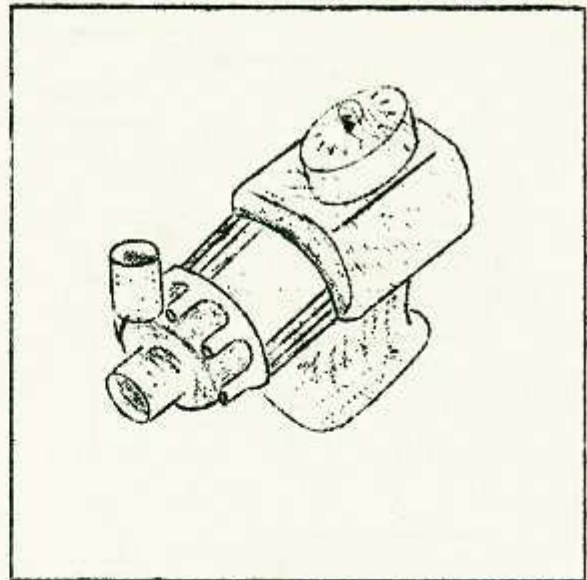


Figure 59-11: Centrifugal Pump

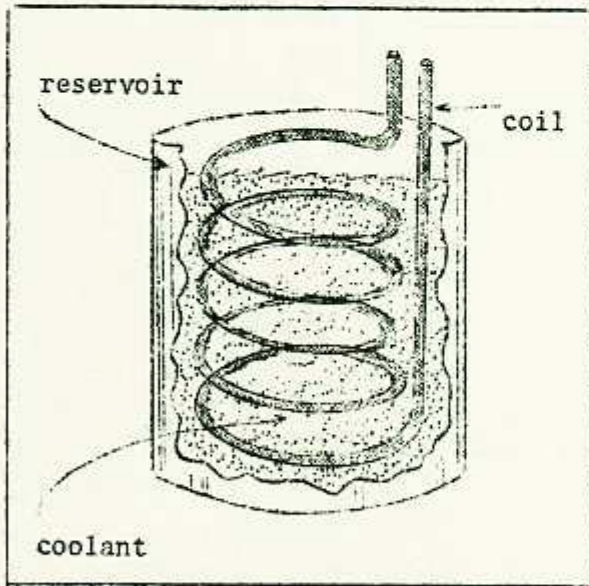


Figure 59-12: Coiled Tube Heat Exchanger

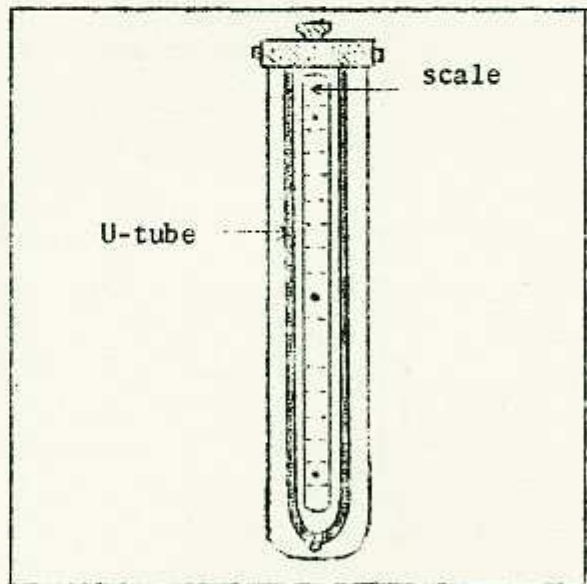


Figure 59-13: Manometer



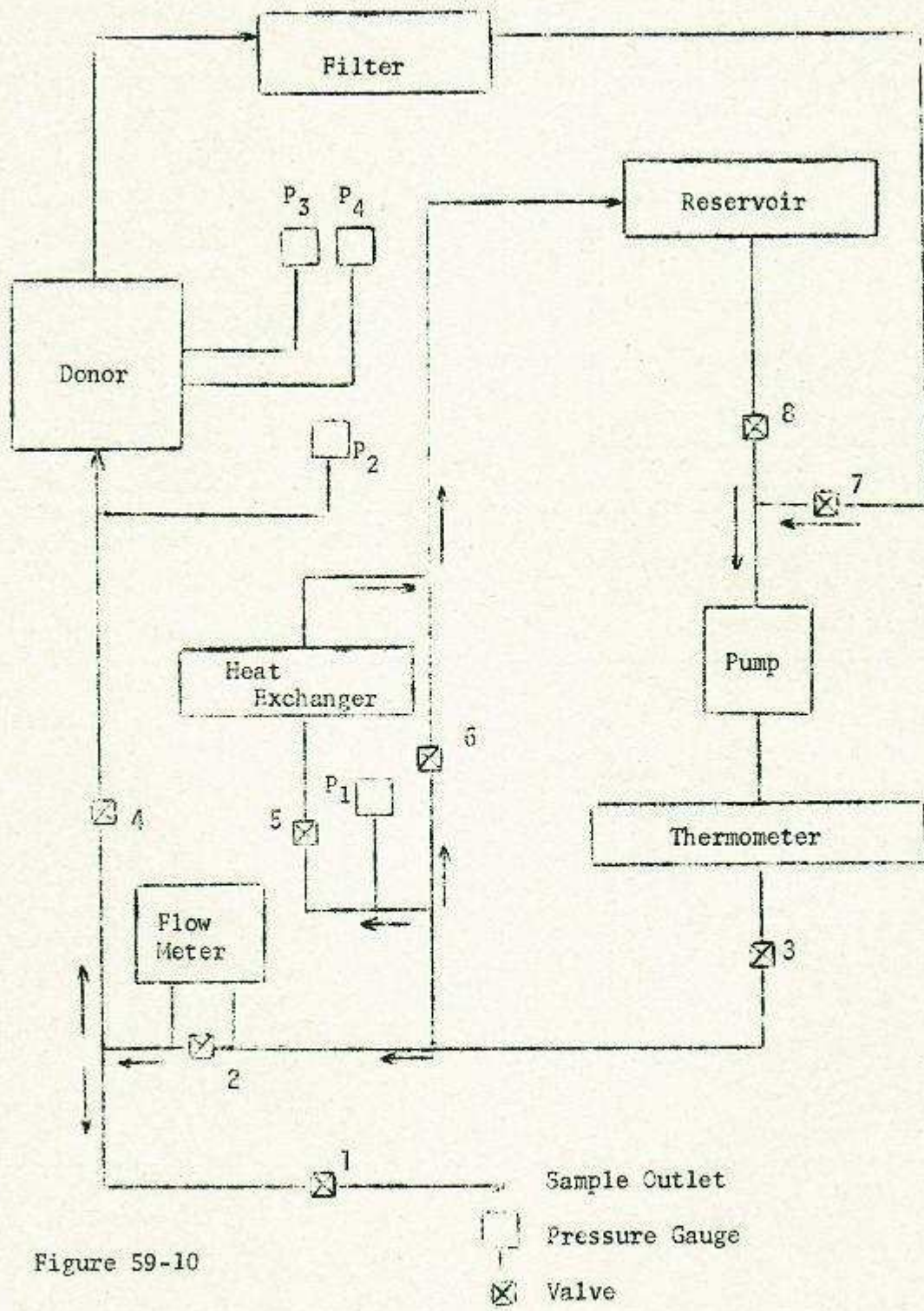


Figure 59-10

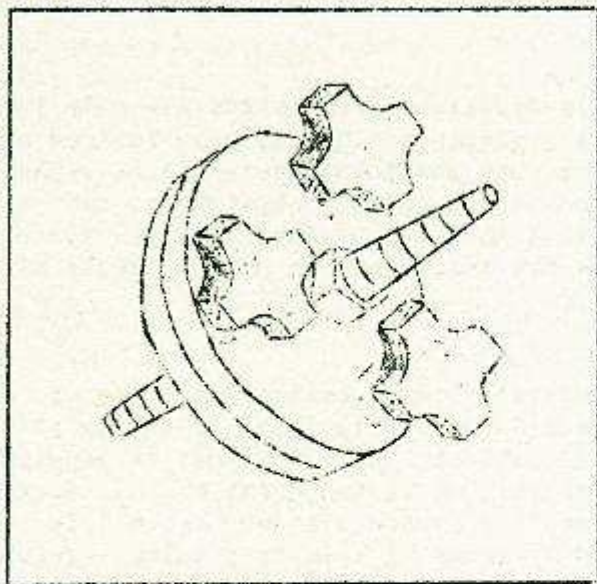


Figure 59-14: Millipore Filter Housing

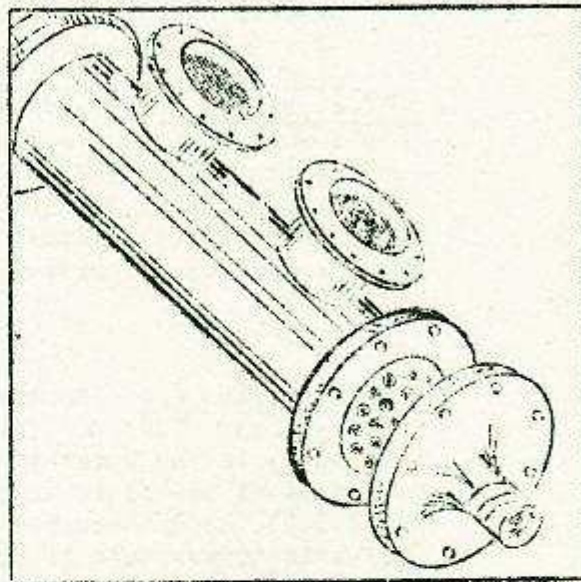


Figure 59-15: Shell and Tube Heat Exchanger

59.2.1.3 Evaluation. System A-1 is valuable only in those emergencies where nothing else is available. It is unsuitable as a primary means of perfusion.

59.2.2 System A-2. System A-2 is different from System A-1 only in that a bulb syringe (Figure 59-6) replaces the hand pump. Instead of pushing in the plunger of the hand pump and then drawing it out, the bulb syringe is first squeezed and then released. System A-1 and System A-2 are essentially identical in terms of function, advantages, and disadvantages. System A-2 is not suitable as a primary means of perfusion.

59.2.3 System A-3. Another relatively simple system makes use of gravity (Figure 59-7). Perfusate flows directly from the reservoir (as shown) to the Donor through flexible tubing. Pressure is regulated by height of the fluid in the reservoir above the point of injection (59.2.3.2), so measurement of injection pressure is not essential. Perfusate temperature is measured by means of inserting a thermometer (Figure 59-4) or the probe of a remote thermometer (Figure 59-5) into the reservoir. Levels of fluid in the reservoir and the output container must be measurable by a depth gauge of some kind, convertible to volumetric units, so that the perfusate injected and perfusate collected from the Donor may be determined as a function of time.

59.2.3.1 Advantages. System A-3 provides for constant and controlled perfusion pressure. It is relatively inexpensive, and is unparalleled in simplicity.

59.2.3.2 Disadvantages. Maximum pressure is limited by the vertical distance from the point of injection to the level to which the reservoir can be raised. (Each foot of elevation corresponds to 23 mm Hg. To perfuse at the maximum of 150 mm Hg, the difference of elevation needed is 6½ feet.) Some commercially available equipment includes a funnel shaped reservoir where conversion of depth measurements to volume measurements might be difficult.

59.2.3.3 Evaluation. System A-3 is an acceptable, portable means of Phase I perfusion, and should be considered as emergency back-up equipment for those circumstances where bringing the Donor and more sophisticated equipment together is not feasible. It is probably possible to select suitable elements from a laboratory catalog and assemble a more useful apparatus than can be purchased commercially.

59.2.4 System A-4. A standard commercial embalming machine (Figure 59-8) includes a reservoir, a pump, and a gauge for measurement of output pressure. The output of the embalming machine is connected directly to the Donor's input cannula. A thermometer (Figure 59-4) or the probe of a remote thermometer (Figure 59-5) is inserted in the reservoir to monitor perfusate temperature. Levels of the reservoir and the output container must be measurable by a depth gauge of some kind, convertible to volumetric units, so that the amounts of perfusate injected and perfusate collected from the Donor may be determined as a function of time.

59.2.4.1 Advantages. System A-4 is a compact unit providing for all essential functions except for temperature measurement. The reservoir is usually of a shape well suited for converting depth measurements into volume measurements. The length of tubing leading from the reservoir to the Donor is minimized, so that temperature measurements of perfusate in the reservoir correspond more closely to injection temperatures than in simpler systems.

59.2.4.2 Disadvantages. System A-4 is more costly than the more simple units.

59.2.4.3 Evaluation. System A-4 is a limited but satisfactory means of Phase I perfusion, when supplemented by means of measuring temperature. It is commercially available, as a standard product.

59.2.5 System B-1. System B-1 as described below is an early prototype of special purpose perfusion apparatus for use in the induction of SSH. Equipment in this configuration was demonstrated by Manrise Corporation at the 1971 cryonics conference in San Francisco. It is illustrated in Figure 59-9. (Improved equipment is now under development and is expected to be commercially available by January, 1973.)

59.2.5.1 Inner Loop Description. Refer to the block diagram (Figure 59-10). Perfusate is stored in a reservoir, and is drawn off through valve #8 by a centrifugal pump (Figure 59-11). Perfusate leaving the pump passes a thermometer and flows through valve #3. The major part of the perfusate flows upward toward valve #6, and the system pressure developed by the pump is monitored by a pressure gauge  $P_1$  (Figure 59-3). The perfusate that flows through valve #6 returns directly to the reservoir. Some portion of the perfusate flowing toward valve #6 is diverted and flows through valve #5, the heat exchanger (Figure 59-12), and then returns to the reservoir.

59.2.5.2 Outer Loop Description. All of the perfusate pumped does not return directly to the reservoir through valves #5 and #6. During perfusion, some of the perfusate passes a flow restriction (shown as "valve" 2) where an indication of flow is produced in a differential manometer (Figure 59-13). Perfusate may be sampled by opening valve #1. (Also, the rate of collection through valve #1 may be used to calibrate the flow meter.) Perfusate permitted to pass valve #4 is injected into the Donor's circulatory system. Perfusion pressure is monitored by pressure gauge  $P_2$  (Figure 59-3). Other pressure gauges ( $P_3$  and  $P_4$ ) are attached to hypodermic needles and measure physiological pressures in the Donor's circulatory system. Perfusate leaving the Donor can be lead to a collection container as in simpler systems (Figure 59-1) or it can be returned to the pump through a filter (Figure 59-14) and through valve #7.

59.2.5.3 Advantages. In addition to those functions provided for in system A-4, system B-1 provides continuous recooling of perfusate and the monitoring of physiological pressures. The two loop system provides improved perfusion pressure stabilization. Indication of flow is provided by the differential manometer so that even minor circulation blockages are detected immediately. Temperature measurement of perfusate is an incorporated feature and perfusate temperature may be manually regulated by adjusting flow through the heat exchanger. Multiple channel switching to facilitate use of a remote thermometer is provided.

59.2.5.4 Disadvantages. System B-1 is not yet commercially available as a standard product. It is less easily transportable than other units, more complex to operate, and its intrinsic component costs are higher.

59.2.5.5 Evaluation. Societies that have centrally located perfusion facilities may desire equipment providing for the basic functions incorporated in system B-1. The information here concerning system B-1 may be of value to these societies in establishing their requirements for such equipment.

59.3 Components and Auxiliary Instrumentation. Certain types of components are of fundamental importance in perfusion equipment and its associated instrumentation. A brief review of some of this equipment is provided below.

59.3.1 Pumps. The two basic types of pumps are those which produce "positive displacement" and "positive pressure". Positive displacement (gear, roller, and piston types, fall into this category) pumps tend to

force a fixed volume per minute out their outlets, up to maximum pressure. Output increases slightly as flow resistance decreases. Positive pressure (centrifugal and impeller types fall into this category) pumps have a maximum pressure under no-flow conditions. As flow resistance decreases, the volume of flow increases. At low pressure resistance, high rates of flow are produced. Corrosion-resistant low-temperature materials such as stainless steel and polyethylene are preferred. Ideally, the pump should be magnetically driven, so that no drive shaft comes in contact with the perfusate. A speed control feature permits the pump to be operated most efficiently. Pumps for category A equipment are available through Kelco Supply Company, while pumps for category B equipment may be obtained through Cole Parmer, Micropump Corporation, and others (see Appendix A).

59.3.2 Heat Exchangers. A heat exchanger permits perfusate as it is circulated to be cooled through contact with cold surfaces. The simplest approach to this is the use of a coiled tube in a container of coolant (Figure 59-12). A more complicated system involves pumping both the coolant and the perfusate into a single device such as a shell and tube heat exchanger (Figure 59-15). Many other configurations are also widely used, but these two roughly illustrate the range of possibilities.

59.3.3 Filters. Filters may be used for removal of solid particles such as microscopic clots from the perfusate during recirculation. When a fine grade of porosity is used, a filter can be used for literally "sterilizing" water to be used in preparing perfusate, or for sterilizing perfusate after it is mixed. In sterilizing applications, a filter with precisely controlled pore size is necessary. Such filters are manufactured by General Electric (Nucleopore filters) and Millipore Corporation (see Appendix A). Both filter holders and the filters themselves must be checked for chemical compatibility if they are to be exposed to cryophylactic agents such as DMSO. A typical fine porosity filter holder is shown in Figure 59-14.

59.3.4 Flow Meter. A flow meter gives a direct and continuous indication of the volumetric fluid flow past a given point. Many kinds of mechanizations are employed for this purpose, including deflected paddles in the flow, magnetically suspended balls that are pushed out of position, rotating vanes, positive displacement pumps that are back-rotated by flow, etc. One simple method of monitoring flow involves connection of a differential manometer (Figure 59-13) across a flow restriction. The pressure difference across the restriction is proportional to flow and is visible as an unbalance of liquid levels in the manometer.

59.3.5 Remote Thermometer. A remote thermometer (Figure 59-5) is an instrument which detects electrical voltage or resistance as a measure of temperature. Sensitive probes at the end of long wires produce the proper conditions of voltage or resistance so that the temperature indication is accurate. If probes are matched, the instrument may be switched rapidly among a virtually unlimited number of probes, displaying within a few seconds (so that the meter needle stops moving) an accurate temperature reading at each point. The probes may be inserted into body cavities to monitor physiological temperatures. Probes are available in many forms including hypodermic needles and flat plates which can be taped to body surfaces. A full line of remote (and conventional) thermometers are offered by such suppliers as Cole Parmer and Van Waters and Rogers Scientific (see Appendix A).

59.4 Operational Set-up and Perfusion Procedures. This portion of the section deals with the check-out and use of perfusion equipment. Rather limited preparations are required for simple systems, while the procedures for readying equipment such as system B-1 are more complicated. The chart below (Figure 59-14) shows which steps are applicable in the case of each system previously described (59.2). A column is provided for each system, beneath which the pertinent paragraphs are shown. The user can follow through the sequence of steps applicable to any system by referring to the paragraphs cited.

Step No.	Step Description	System				
		A-1	A-2	A-3	A-4	B-1
1	Assemble Equipment	59.4.1	59.4.1	59.4.1	59.4.1	59.4.1
2	Sterilize Equipment	59.4.2	59.4.2	59.4.2	59.4.2	59.4.2
3	Close All Valves	59.4.3.1	59.4.3.1	59.4.3.1	59.4.3.2	59.4.3.2
4	Fill with Perfusate	59.4.4	59.4.4	59.4.4	59.4.4	59.4.4
5	Test for or Develop Pressure	59.4.5.1	59.4.5.1	59.4.5.2	59.4.5.3	---
6	Fill Heat Exchanger	---	---	---	---	59.4.6
7	Set Inner Loop Pressure	---	---	---	---	59.4.7
8	Connect Equipment to Donor	59.4.8	59.4.8	59.4.8	59.4.8	59.4.8
9	Begin Perfusion	59.4.9.1	59.4.9.1	59.4.9.2	59.4.9.3	59.4.9.4
10	Reservoir Level Maintenance	59.4.10	59.4.10	59.4.10	59.4.10	59.4.10
11	Monitor Parameters	59.4.11	59.4.11	59.4.11	59.4.11	59.4.11

Figure 59-14

59.4.1 Assemble the Equipment. All interconnections between components must be effected, using flexible tubing and clamps as required. In all cases, the perfusate output (injection) tube should be sufficiently long to reach the Donor's input cannula(s).

59.4.2 Sterilize the Equipment. Circulate formaldehyde (37%, U.S.P., available through Renalab Division, Ries Biologicals, Inc., see Appendix A) for no less than ten minutes and (preferably) for up to two hours. Purge the system with sterile saline and test for contamination by the use of Clinitest tablets. Dissolve a Clinitest (available at drug stores) tablet in 10 cc of sterile saline. The color will be a light blue. Now dissolve another clinitest tablet in 10 cc of fluid from the perfusion equipment, supposedly sterile saline. If the second sample is discernably green, to the slightest extent as compared with the first sample, an unacceptably high level of formaldehyde remains. In this case, the equipment must be purged again with sterile saline and then retested.

59.4.3 Close all Valves.

59.4.3.1 Systems A-1, A-2, and A-3. These systems will probably have only tubing clamps on the injection tube. They will still be filled with sterile saline (the reservoir will be very low).

59.4.3.2 System A-4. System A-4 will have a tubing clamp on the injection tube and (1) a pressure and (2) a flow valve. Both valves should be closed. The system will still be filled with sterile saline (the reservoir will be very low).

59.4.3.3 System B-1. System B-1 will have a tubing clamp on the injection tube and eight valves. All valves should be closed excepting the "flow meter" valve (a calibrated, pre-set flow restriction). The system will still be filled with sterile saline (the reservoir will be very low).

59.4.4 Fill with Perfusate. Fill the reservoir with perfusate (depending on the equipment, up to three gallons may be accommodated).

59.4.5 Test For or Develop Pressure ("A" Systems only).

59.4.5.1 Systems A-1 and A-2. Depress the hand pump plunger or squeeze the bulb syringe. Observe the rise in pressure. Assure



that the hand technique used is such that inadvertant overpressures are avoided.

59.4.5.2 System A-3. Elevate the reservoir to a height of from 4 to 6½ feet above the height of injection into the Donor's circulatory system.

59.4.5.3 System A-4. Turn on the embalming machine and open the pressure setting until limiting physiological pressures are reached. Assure that these are not exceeded.

59.4.6 Fill Heat Exchanger (System B-1 Only). Fill the heat exchanger reservoir (Figure 59-12) with crushed ice and water.

59.4.7 Set Inner Loop Pressure (System B-1 Only). Open valves #8, #3 and #5. Turn on the pump. Close valve #5 until pressure  $P_1$  exceeds maximum physiological limits by 50 mm Hg. Open valve #6 until pressure  $P_1$  falls to maximum physiological limits.

59.4.8 Connect Equipment to Donor. This step is not taken until perfusion is about to begin. Full instructions are given in section 60.0.

59.4.9 Begin Perfusion. The following paragraphs describe the operation of equipment during perfusion.

59.4.9.1 Systems A-1 and A-2. Depress and withdraw the hand pump or squeeze and release the bulb syringe repeatedly. Monitor the pressure gauge continuously and never exceed physiological pressure limits (200 mm Hg).

59.4.9.2 System A-3. Perfusion is automatic. Once the injection tube is unclamped, fluid is injected by gravitational force.

59.4.9.3 System A-4. Adjust perfusion pressure to maximum physiological pressure (200 mm Hg), per manufacturer's instructions. Monitor pressure continuously and reduce it if transient flow reduction produces pressures exceeding physiological limits.

59.4.9.4 System B-1. Open valves #4 and #7, observe perfusion pressure  $P_2$ . This pressure should fall no more than 25 mm Hg below maximum physiological pressures (200 mm Hg). Do not change control settings; regulation of pressure is automatic.

59.4.10 Reservoir Level Maintenance. In closed circuit (recirculative) perfusion, reservoir levels will be somewhat constant and continuous addition of new perfusate will not be required. In open circuit perfusion, or in perfusion in which spent perfusate is manually transferred to the reservoir, it will be necessary to assure that reservoir levels do not fall to the point where air is drawn into the system.

59.4.11 Monitor Reservoir Levels and Other Parameters. Reservoir levels, perfusate temperature, and other parameters must be measured as described in section 60.0.

## 60.0

PROCEDURES FOR  
PHASE I PERFUSION

The procedures shown in Figure 60-1 for Phase I perfusion should follow the preparatory steps (57.0) with unbroken continuity.

Action	Subparagraph
Connection of Perfusion Equipment	60.1
Discontinuance of HLR	60.2
Elimination of Air Bubbles During Connection	60.3
Injection of Heparin	60.4
Injection of Perfusate	60.5
Refilling Injection Reservoir	60.6
Dispersion of Blood Coagulation	60.7
Reversal of Flow	60.8
Measurements and Records	60.9

Figure 60-1

60.1 Connection of Injecting Equipment to Cannulas (General). The manner in which the injecting equipment is connected to the cannulas determines the direction of the injection. Improper connection could jeopardize proper perfusion. Figure 60-2 will be helpful in determining how connections are to be made. It is equally important that connections are made in such a manner that no air bubbles are allowed into the system (60.2)

Type of Cannula Used	Region Cannulated	Vessel Cannulated	Flow Direction			
			Input		Output	
			Cannula 1	Cannula 2	Cannula 1	Cannula 2
Standard Embalming Cannulas. (Two per vessel -- connected with a "Y-shaped" stopcock assembly).	Ilio-Femoral	femoral artery	toward leg	toward trunk	---	---
		femoral vein	---	---	from leg	from trunk
	Carotid	carotid artery	toward head	toward trunk	---	---
		jugular vein	---	---	from head	from trunk
	Axillary	axillary artery	toward arm	toward trunk	---	---
		axillary vein	---	---	from arm	from trunk
"T-shaped" (one two-directional cannula per vessel).	Ilio-Femoral	femoral artery	toward leg & trunk		-----	
		femoral vein	-----		from leg & trunk	
	Carotid	carotid artery	toward head & trunk		-----	
		jugular vein	-----		from head & trunk	
	Axillary	axillary artery	toward arm & trunk		-----	
		axillary vein	-----		from arm & trunk	

Figure 60-2

60.1.1 When "T-shaped" cannulas are used, only one cannula is inserted into each vessel (i.e., one into the artery and one into the vein). Each cannula is connected directly to the flexible tubing which leads to the injecting apparatus. The input tube must *always* be connected

to the T-shaped cannula in the artery and the output tube must *always* be connected to the cannula in the vein, except in the event reverse flow is utilized (60.6).

60.1.2 When standard embalming arterial cannulas are used (in embalming, "drainage tubes" are used almost exclusively in the veins rather than cannulas), two cannulas are inserted into each the artery and the vein; one cannula in each vessel pointing in each direction. A "Y-shaped" stopcock assembly (56.0) connects the two cannulas of either vessel. This permits flow through a single flexible tube leading from the injecting apparatus to be directed both up and down the vessel. The input tube from the injecting apparatus must *always* be connected to the stopcock assembly in the artery and the tube carrying output back should *always* be connected to the assembly in the vein, except in the event reverse flow is utilized (60.6).

60.2 Elimination of Air Bubbles During Connection. In all cases, certain steps must be taken to assure that no air bubbles are introduced. At the time of cannulation, the vessels were closed off (with hemostats) above the cannula(s) to prevent air from entering the system when the vessels were incised. These hemostats should still be in place. Paragraphs 60.2.1 through 60.2.4 give full instructions for connecting the tubing from the injecting apparatus to the cannulas without introducing air into the system.

60.2.1 Purge Tubing. To purge air from the tubing leading from the injection apparatus, start flow (hold tubing over reservoir, let the perfusate flow back into reservoir). When satisfied that all air has been eliminated at this point, hemostat the tubing 6 inches from the end which will connect to the cannula (or Y-shaped stopcock assembly).

60.2.2 Purge Cannula/Vessel Area. To purge air from the site of cannulation, inject perfusate into the vessels between the cannula and the hemostat which closes off the vessel. This is done with a hypodermic needle. As the perfusate enters the vessel, the air will be forced out the top of the cannula. When the injected perfusate begins to flow out the top of the cannula, all air should be purged from the site.

60.2.3 Connect Tubing to Cannula. The purged tubing, still clamped with a hemostat, is connected to the open end of the cannula. (Surgical rubber tubing, when forced over the end of the cannula, should effect a good, airtight seal. Greater protection against air leakage can be accomplished by securing this connection with a tubing clamp).

60.2.4 Purge Connection. Air must be purged from the area between the cannula and the hemostat on the tubing. Insert a hypodermic needle through the surgical tubing just below, and as close as possible, to the hemostat. The plunger should be completely depressed when the hypodermic is inserted. As the plunger is pulled outward, the air will be removed. If plunger is pulled out two thirds of its distance before perfusate begins to enter the cylinder, remove the hypodermic needle from the tubing, push plunger all the way in again and reinsert the hypodermic needle into the tubing in the same position described above. Never pull the plunger completely out of the cylinder while the needle is inserted into the tubing (this would allow air to re-enter the tubing). Repeat this procedure until perfusate begins to fill the cylinder of the hypodermic, at which time all air should be removed from the system. The hypodermic needle is removed. All hemostats can be removed when injection is desired.

60.2.5 Prevention of Air Entering During Interruption of Perfusion or Disconnection of Tubing. When injection is to be temporarily interrupted, both *input* and *output* tubes should be clamped and closed before injection is terminated. Even if no tubes are disconnected, air may enter the output lines (with progressive drainage) if this line is not clamped. If tubing is disconnected, particularly at the input side, tubing to the cannulas must be clamped so that the air-tight integrity may be preserved. If tubing is disconnected at the input side, the areas on each side of the disconnection (up to such clamps as may have been put in place) must be purged of air after each reconnection, as described above.

60.3 Discontinuance of HLR. If use of the HLR has not been previously discontinued due to the achievement of low body core temperatures (36.3), it is discontinued as Phase I perfusion begins.

60.4 Injection of Heparin. Heparin, in addition to that normally in the perfusate (58.0) should *only* be injected at this stage of the induction of SSH when it *has not previously* been injected as described in 20.0. When this is the case, 20,000 I.U. sodium heparin, without preservatives, should be injected as described below. Then, the injection of perfusate should begin without delay.

15,000 I.U. heparin should be injected on the trunk side and 5000 I.U. on the other side of the arterial cannulation. When flow is begun, the heparin will to some degree be forced through the circulatory system ahead of the perfusate. This method takes the greatest advantage of heparin administered at this point in the procedure.

to the T-shaped cannula in the artery and the output tube must *always* be connected to the cannula in the vein, except in the event reverse flow is utilized (60.6).

60.1.2 When standard embalming arterial cannulas are used (in embalming, "drainage tubes" are used almost exclusively in the veins rather than cannulas), two cannulas are inserted into each the artery and the vein; one cannula in each vessel pointing in each direction. A "Y-shaped" stopcock assembly (56.0) connects the two cannulas of either vessel. This permits flow through a single flexible tube leading from the injecting apparatus to be directed both up and down the vessel. The input tube from the injecting apparatus must *always* be connected to the stopcock assembly in the artery and the tube carrying output back should *always* be connected to the assembly in the vein, except in the event reverse flow is utilized (60.6).

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60.2.1 Purge Tubing. To purge air from the tubing leading from the injection apparatus, start flow (hold tubing over reservoir, let the perfusate flow back into reservoir). When satisfied that all air has been eliminated at this point, hemostat the tubing 6 inches from the end which will connect to the cannula (or Y-shaped stopcock assembly).

60.2.2 Purge Cannula/Vessel Area. To purge air from the site of cannulation, inject perfusate into the vessels between the cannula and the hemostat which closes off the vessel. This is done with a hypodermic needle. As the perfusate enters the vessel, the air will be forced out the top of the cannula. When the injected perfusate begins to flow out the top of the cannula, all air should be purged from the site.

60.2.3 Connect Tubing to Cannula. The purged tubing, still clamped with a hemostat, is connected to the open end of the cannula. (Surgical rubber tubing, when forced over the end of the cannula, should effect a good, airtight seal. Greater protection against air leakage can be accomplished by securing this connection with a tubing clamp).

60.5 Injection of Perfusate. As perfusate is injected, the input reservoir must be refilled and the contents of the collection container must be discarded. After as much blood as possible has been flushed from the body, the contents of the collection container may need to be reserved for recirculation. The recovered perfusate is cooled to near 0°C and reintroduced into the injection reservoir if the supply of perfusate is likely to be depleted before body core temperatures near 0°C are reached. See 58.1.8 for further discussion.

60.6 Refilling Injection Reservoir. Certain measures must be taken to prevent the introduction of air into the system when new perfusate is added to the reservoir during injection.

60.6.1 Add perfusate by pouring it into a large funnel with tubing attached to its outlet, leading to a point which is both beneath the reservoir surface and remote from the pump intake.

60.6.2 If no other means for prevention of air entering the system is available, completely halt perfusion, recirculation, and other fluid transport in the system until the reservoir is refilled and bubbles have cleared completely.

60.7 Dispersion of Blood Coagulation. Although heparin has been administered to the Donor directly as well as being a part of the perfusate, coagulation of blood constituents may be experienced in varying degrees, depending on the individual circumstances. This clotting can cause a local or total stoppage in perfusion flow.

Blockages can often be detected either by a drop in flow or, in some cases, by swelling of the tissues around the blockage. Blockages due to blood coagulation can often be alleviated by manipulation of the points affected or by application of pressure to the adjacent surface areas. This problem will rarely occur after the majority of the blood has been flushed from the body.

Perfusion of a Donor who has been clinically dead a period of hours with no cardiopulmonary assist will be more difficult than perfusion which is begun under more favorable circumstances. In these latter cases, alternative apparatus will be used while the blood is being removed.

60.7.1 Instead of cannulas, drainage tubes (56.0) will be inserted into the vein, first in one direction, then the other, until all blood and clots are removed.



It may be necessary from time to time to remove the drainage tubes and "spread" the vein with forceps to allow the coagulated blood to escape the vessel opening. This is done by "squeezing" the forceps together, inserting them into the vein, and allowing them to "spring" open. They are then squeezed again before removal.

60.8 Reversal of Flow. The cannula connections (60.1) may be reversed to perfuse the vessels of the pulmonary system (the pathway by means of which blood travels to the lungs for release of carbon dioxide and absorption of oxygen). Expert opinion differs concerning the necessity for flow reversal to clear these vessels, since the tissues concerned are nourished by parts of the systemic system which parallel the pulmonary system and which are perfused without the necessity of flow reversal. In the event that flow reversal is performed, it should be performed only after blood constituents are completely cleared in the normal flow direction, and after body core temperatures have fallen to or below the temperatures at which use of cardiopulmonary resuscitation is discontinued (36.3).

60.9 Measurements and Records. During the time that perfusate is being injected and body core temperature is being reduced, a number of parameters must be monitored and recorded. These include: (a) body core temperature, (b) perfusate temperature, (c) perfusate flow, and (d) pH balance.

Record charts may be used (45.0) for the systematic recording of these measurements. Care should be exercised to show the exact time at which each measurement is taken.

The amount of fluid accumulation or loss in the Donor's body can be determined by the difference between the amount of perfusate which leaves the injection reservoir and the amount of effluent which enters the collection container during a given period of time. Reservoir measurements may be taken (if other means are not available) by means of a sterile scale (ruler) which is inserted into the container and clamped to its side. If the amount of perfusate being injected significantly exceeds the amount of effluent being collected (taking into consideration that a small amount will diffuse into the tissues) a blockage may have occurred.

pH readings should be taken periodically by the use of either a pH meter or pH sensitive tape. If these are not within the desired range, correction should be made with an appropriate buffer (58.3).

## 61.0 CONCURRENT PHASE I PROCEDURES

To this point, the procedures recommended have been concerned solely with the functions of the circulatory and respiratory systems. Cardiopulmonary resuscitation and perfusion, however, should be supplemented by purging and/or conditioning of the digestive tract and the urinary system.

61.1 Digestive Tract; The Stomach. First, the stomach should be cleared of all substances (solid and liquid) using a stomach pump and catheter (or other aspirator). Then a sodium bicarbonate solution (pending further study, 10 grams of sodium bicarbonate in one liter of water) should be introduced and removed ten minutes later, neutralizing acids that may have accumulated. Finally, a flush with one liter of the base perfusate (58.0) should be carried out to provide a stomach content neutral with respect to the perfusate. Ten minutes after introduction of the base perfusate, as much of it as possible should be aspirated (sucked out) using the same equipment as before.

61.2 Digestive Tract; The Intestines. Repeated enemas should be given with the base perfusate (58.0). Each flush may consume one to two liters, depending on the size of the individual.

61.3 The Urinary System. A bladder catheter should be inserted (observe that male and female catheters are different) and the bladder aspirated. Following this, the bladder should be flushed with base perfusate (58.0).

61.4 Implementation. For all of these concurrent procedures, it will be important that other operations in progress not be disturbed (such as cardiopulmonary resuscitation or perfusion). The Donor will have to remain horizontal throughout, and the implementation of concurrent procedures must proceed without reduction of ice contact (in the groin) or compromise of artificial respiration (if in use). The following discussion is conjectural and speculative, and does not represent a description of proven approaches. Taken strictly in that context, the ideas presented may be useful.

61.4.1 Access to the Stomach. A stomach tube or catheter will be necessary. If artificial respiration is underway, tracheal intubation (insertion of a respiratory tube through the mouth, throat, and then into the tracheal tube leading to the lungs) must first have been accomplished. Following this, with artificial respiration in progress, the stomach tube may be inserted. If possible, a remote thermometer probe should be attached to the outside of the stomach tube. This will permit the best possible monitoring of body core temperature.

61.4.2 Access to the Intestines. A relatively large tube must be inserted into the rectum, and must be fixed in position, so it may be used both for injection of the enema and for aspiration of the fluid plus fecal matter (it will be impractical to place the Donor in a vertical position, remove the tube, and induce gravity drainage by abdominal manipulation. The fluid and solid matter will have to be aspirated with a suction pump of some kind). If possible, a remote thermometer probe should be attached to the outside of the large tube. This will permit a second advantageous position for monitoring of body core temperature.

A number of possibilities exist for effecting a seal at the entrance to the rectum. A rubber laboratory cork could be drilled for four holes (one large, three small) through which are threaded the large tube, a remote thermometer probe, and two ends of a nylon retrieval loop. All port clearances in the cork could subsequently be sealed with epoxy cement to prevent leakage. The cork could be sealed within the rectum just beyond the sphincter (donut shaped muscle at the entrance to the rectum). By selection of a suitable size cork and use of a biological lubricant (such as vaseline) a satisfactory seal might be easily established.

61.4.3 Access to the Bladder. A standard urinary catheter can be inserted, and can be used both for aspiration and injection of fluids. As previously observed, an appropriate type (male-female) and size of bladder catheter must be employed.

61.4.4 The Question of Removal of Devices Used in Concurrent Procedures. Elsewhere in this manual, it is suggested that cannulas be left in place following perfusion. This suggestion follows partly from the fact that cannulas will be used in Phase II perfusion, and the tissue in which cannulas are embedded may be inflexible or practically solid when Phase II perfusion is terminated. Another consideration is that future attempts at reanimation may be facilitated by the availability of access to the circulatory system. It is debatable as to the value of leaving the tubes leading to the

digestive system in place. Certainly the remote thermometer probes will be of value in Phase II and afterwards, since monitoring of body core temperature will be necessary until the Donor's condition is stabilized at a final temperature level. It could be argued that access to the digestive tract might be of value to scientists of some future time, in the use of some hypothetical perfusate which might be liquid at liquid nitrogen temperatures, and which might (initially) be more easily introduced into the body via the digestive tract than through the circulatory system. For the present, it is suggested that these tubes may be left in place to some possible future advantage. By use of appropriate connectors, the tubes could be disconnected a few inches outside the body openings through which they were originally introduced.

## 62.0

POST PHASE I  
PERFUSION PROCEDURES

Phase I and Phase II may frequently be executed in the same location, using essentially the same equipment. In these cases, the transition to Phase II will be essentially uninterrupted. In other cases, Phase II procedures will have to be executed at a different facility from that in which Phase I was carried out. In the latter case, a number of intermediate steps must be taken. These are shown in Figure 62-1 and discussed in subparagraphs below.

Action	Subparagraph
Discontinuation of Injection	62.1
Disconnection of Inj. Equip.	62.2
Arrangement of Transportation	62.3
Maintenance of External Cooling	62.4

Figure 62-1

62.1 Discontinuation of Injection. When body core temperature reaches 0°C, the Phase I perfusion is continued, in a recirculating mode, until immediately before Donor must be moved. Then perfusion is terminated.

62.2 Disconnection of Injecting Equipment. The injecting equipment can be disconnected from the cannulas as soon as injection is discontinued. The cannulas should NOT be removed from the vessels, and air should not be allowed to enter the system (60.0). Cannulas should remain in the vessels for use in Phase II. Short lengths of tubing may be attached to the cannulas and clamped off to prevent drainage.

The disconnection should be carried out in such a way that the tubes beyond the clamps contain no air bubbles (60.2.4).

62.3 Arrange for Transportation. Arrangements for transportation of the Donor can be made while the injection apparatus is being disconnected. Trans-phase transportation of the legally dead Donor is discussed in sections 63.0 and 64.0.

62.4 Maintain External Cooling. Every effort must be made to maintain body core temperature at 0°C during the lapse between Phase I and Phase II procedures. Contact with crushed ice on all body surfaces should be carefully maintained. Body core temperature should be measured and recorded periodically.

## 63.0 TRANS-PHASE DONOR MOVEMENT; OPERATIONS

The primary operational concern with trans-phase Donor movement is speed. Therefore it is recommended that where significant distances are involved (several hundred miles or greater) air transportation be secured. If surface travel is to be used, the information in section 39.0 will apply.

63.1 Air Travel; Common Carrier. Operationally, air transport by common carrier may pose problems in maintaining external cooling. Ideally, a society representative would travel with the Donor to effect replenishment of ice as this melts. Even assuming that is permitted, difficulties may arise in providing for the disposal of excess water as ice continues to melt. A special purpose container may be necessary to satisfy airline regulations. These problems may arise whether the craft involved is a passenger or a cargo plane.

63.2 Air Travel; Charter Carrier. It may be possible to effect air transportation by chartered flight, in a relatively small plane. Regardless of how this is accomplished, the penalties will probably involve (1) higher cost and (2) increased travel time. Less problems may be expected in terms of the representative's disposal of excess water, but this will depend on the individual circumstances.

63.3 Special Purpose Containers. The Donor will probably be transported in a water-tight box with a hinged cover, providing for drainage of water to a separate container. A hand pump (see 59.0) might be used to transfer water from the Donor container to the water collection container.

63.4 Comment. This section will be revised and expanded to provide more detail, particularly under 63.3, at a later time.

## 64.0

TRANS - PHASE  
DONOR MOVEMENT; ADMINISTRATION

64.1 Documentation. In line with the discussions of section 39.0, documentation (removal permits, etc.) must be provided as necessary. Variation in state and local regulations makes advance inquiry as to these requirements essential if trans-phase Donor movement is anticipated.

64.2 Use of Common Carriers. Use of common carriers may be advantageous, but laws may prohibit movement of a deceased person unless embalmed by a licensed embalmer (39.0). In some instances in the past, perfusate has been accepted as a form of embalming fluid, and the perfusion was accomplished by a licensed embalmer, so the necessary criteria were satisfied.

In the future, with human organs being transported from city to city by air, the regulations in this regard may be broadened to provide for transportation of whole-body human anatomical gifts. In any case, should trans-phase Donor movement by common carrier be anticipated, thorough advance inquiry and preparations would be appropriate.



## 65.0 STANDARD PROCEDURES: PHASE II

In Phase II of the induction of solid state hypothermia, a cryoprotective agent is added to the Phase I base perfusate. The temperature is then gradually lowered and the percentage of cryoprotective agent is increased, so as to always maintain a liquid state in the Donor's tissues. Finally, a point is reached where lower temperatures produce crystallization regardless of what mixture of cryoprotective agent is used. Important factors are:

- 65.1 The temperature must not be lower than the freezing point of the tissues. The freezing point is dependent on the percentage of cryoprotective agent in the tissues.
- 65.2 Since it takes time for the cryoprotective agent to diffuse outwards from the capillaries, the percentage of cryoprotective agent in some tissues may be substantially less than in others. The percentage in any tissue is somewhat less than that in the perfusate.
- 65.3 The cryoprotective agent may be toxic in large percentages. This toxicity is reduced as the temperature is lowered (1). Therefore, the temperature should be as low as possible at each point without violating factor (65.1) above.

The perfusion methods described in the following sections are responsive to a recent article published in the Manrise Technical Review (2). This procedure

is based largely upon theoretical considerations, and as of October 1972 has never actually been used with whole organisms or even large organs. Also, there are a number of practical problems involved with implementation which may arise when the method is first employed.

Sections 65.0 through 70.0 have been reviewed for approval by Mr. Quaife. His collaboration is an essential element in the information and recommendations to follow.

#### References

1. "Cryoprotective Agents", H. T. Meryman, *Cryobiology*, 8:173-183, 1971.
2. "Mathematical Models of Perfusion Processes", A. Quaife, *Manrise Tech. Rev.*, 2:28-75, 1972.

## 66.0 DIVISION OF ACTIVITIES WITHIN THE PHASE II TEAM

Comments under 52.0 apply to Phase II team organization and responsibilities. Further revisions of this section will include a recommended team organization and division of responsibilities.

Phase II will require careful control of temperature and perfusate composition, but these functions will gradually settle into a routine as time passes. On the other hand, completion of Phase II procedures may require prolonged perfusion, perhaps over several days or more. A team of four persons may be more appropriate than a team of two. Members of the team may elect to work in shifts, since Phase II procedures must be applied continuously until they are completed.

## 68.0 PREPARATION OF PERFUSATE FOR PHASE II

Perfusate for Phase II consists of the Phase I perfusate (see 58.0) with cryoprotective agents added. At this time, a single cryoprotective agent, dimethyl sulfoxide (DMSO), is the recommended additive.

The title of this section contains the word "preparation", implying that the Phase II perfusate is simply pre-mixed and then injected. This would be an oversimplification. DMSO is added to the base perfusate on a progressive basis, such that its percentage is gradually increased as the perfusion proceeds. This operation is inextricably involved with the details of section 70.0, "Procedures for Phase II Perfusion", so the details are not included in this section.

### 68.1 Quality of Dimethyl Sulfoxide Recommended; Sources of Supply.

"Reagent" grade DMSO is considered to be appropriate. Curtin Scientific (see Appendix A) quotes \$50.58 per case (of four gallons) of DMSO when five or more cases are purchased.

68.2 Pre-dilution of DMSO. Prior to adding DMSO to the base perfusate, as part of the perfusion procedure, it may be appropriate to "pre-dilute" the DMSO to dissipate a principal part of DMSO's heat of solution and lower the freezing point of the mixture (1, pp 43,58-9). Dilution with distilled water to a concentration of 80 percent is recommended in Mr. Quaife's paper (1). Observe that this represents the percentage by *weight*, not *volume*. One adds one kilogram of water for every four kilograms of DMSO, to achieve the 80 percent concentration. In practice, it will be simpler to mix DMSO and water by measuring volumes, but one must not forget to correct for the specific gravity of DMSO (approximately 1.1).

With corrections for volumetric change on mixing, using the known densities of DMSO and water at 25°C, add 793 ml DMSO to 218 ml water to get 1 liter 80 percent DMSO (w/w) (2).

68.3 Pre-balance of DMSO with Perfusate Constituents. Before DMSO, either pure or prediluted, can be added to the base perfusate, the constituents present in the Phase I or base perfusate must be added. To one liter (or one gallon, or four gallons) of DMSO, one must add the same amounts of constituents as one would add to distilled water for Phase I perfusate (section 58.0). Note that it does not matter whether or not the DMSO has been prediluted. Constituents are added on the basis of the total fluid volume to be balanced. Particularly, note that the balance is per unit *volume*. One adds a specified quantity of constituents to a liter, gallon, etc. of cryoprotective agent, regardless of what it *weighs*.

#### References

1. "Mathematical Models of Perfusion Processes", A. Quaife, Manrise Tech. Rev., 2:28-75, 1972.
2. Personal Communication; A. Quaife, dated 12-5-72.

## 69.0 EQUIPMENT FOR PHASE II PERFUSION

69.1 General Description. The equipment for Phase II consists of a perfusion system with capabilities extending beyond those necessary for Phase I, and a temperature controlled cabinet which contains the donor during perfusion. Both are described in more detail below.

69.2 Temperature Controlled Cabinet. The temperature controlled cabinet is basically an insulated box with a means for regulated cooling down to  $-60^{\circ}\text{C}$  or below. Commercial units can be procured, with costs generally exceeding \$3000. They are cooled by compressor driven refrigerator coils and are suitable for continuous operation, year in and year out. Alternately, a similar unit may be constructed which makes use of cryogenic gases for cooling. Such a system could be more difficult to regulate, and may not be desirable for long term operation, but its cost may be far less than the type of equipment first described. Further, its range of operation may in principle be extended to the temperatures of cryogenic gases to be used for long term storage. The discussion which follows is limited to temperature controlled cabinets based on the use of cryogenic substances.

69.2.1 Precautions. The gases given off by most cryogenic substances of interest are not breathable, and must be vented to the atmosphere after they are used. The continued release of gaseous nitrogen in an unventilated space could progressively result in reduction of oxygen levels in such a way that unconsciousness or death would follow. The release of carbon dioxide would produce this same effect,

plus the suffocating sensation one experiences when holding one's breath. Thus carbon dioxide gas may give more warning of a dangerous condition than nitrogen, but both are potentially hazardous. Phase II spaces should be well ventilated, particularly if cryogenic gas cooling is used.

69.2.2 Design Considerations for Cryogenically Cooled Temperature Controlled Cabinets. The following general discussion may be useful to those who intend to design their own cabinets.

69.2.2.1 Basic Elements of a Cryogenic Gas Temperature Controlled Cabinet. Figure 69-1 is a block diagram which indicates the possible set of elements in a cryogenically cooled temperature controlled cabinet. The cabinet interior is a well insulated volume capable of containing the Donor plus any other equipment that should be cooled. Its temperature is monitored by a temperature controller, which causes a feeder mechanism to transport cryogenic substances into a cold gas generator when the cabinet interior becomes too warm. A blower or fan recirculates gases through the cabinet and generator so as to maintain uniformity of temperatures within the cabinet. Excess gases are exhausted to the outside atmosphere.

69.2.2.2 General Discussion. For maximum efficiency, all elements within the dotted enclosure should be packaged into a common insulated volume. The blower is essential to correct function of the temperature controller and the cold gas generator.

Perfusion System. The perfusion system, as already suggested, must embody a number of functions not required in Phase I. Since the possible complexities for the sake of convenience are many, and since the immediate purpose is providing a least costly means of upgrading Phase I perfusion equipment for use in Phase II, an "idealized" system will not be discussed. Rather, methods for "adding on" to a basic Phase I perfusion apparatus will be presented.

69.3.1 The Basic System. The Phase I perfusion system to be used as a baseline for upgrading to meet Phase II requirements will be similar to "System B-1" as described in Section 59.0 (59.2.5). In review of this type system's characteristics:

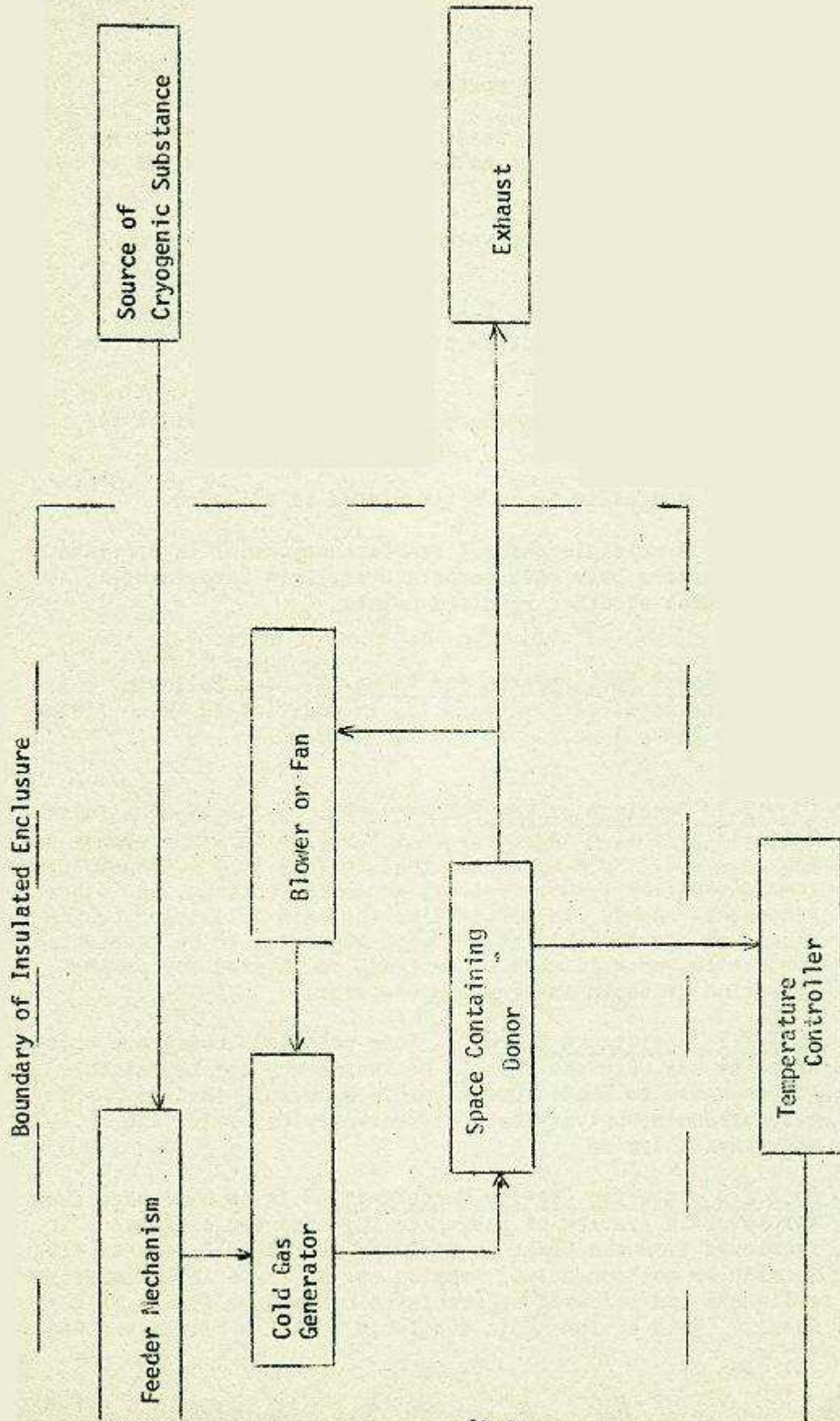


Figure 69-1



- 69.3.1.1 It has a main perfusate reservoir.
- 69.3.1.2 The reservoir feeds one or more pumps. The pumps' outputs flow in parallel to both a heat exchanger and to the Donor.
- 69.3.1.3 Perfusate temperature is monitored at a pump's outlet.
- 69.3.1.4 Injection flow rate to the Donor is measured. Flow (and consequently injection pressure) to the Donor is controlled by valves or other means.
- 69.3.1.5 Pressure is measured at the outlet provided for injection to the Donor.
- 69.3.1.6 Perfusate to be recirculated is filtered.
- 69.3.1.7 A multiple channel remote thermometer is provided which monitors body core temperatures, skin temperatures, and temperatures at other required points.

69.3.2 Additional Requirements for Phase II. The following characteristics are required for Phase II, in addition to those listed for the basic Phase I perfusion system above.

69.3.2.1 Function at Low Temperatures. All equipment in the Phase II perfusion apparatus must function to temperatures as low as  $-70^{\circ}\text{C}$ . Many materials tend to fail at low temperatures; some mechanisms (pumps, valves) cease to function, and other components change characteristics (certain "flexible" tubing is not at all flexible at  $-70^{\circ}\text{C}$ ). Apparatus to be considered for use in Phase II must be designed and tested for proper operation at these reduced temperatures.

69.3.2.2 Resistance to DMSO. Some materials which are suitable for use with the Phase I perfusate may not be suitable for exposure to DMSO. Questionable materials include polycarbonate, polystyrene, styrene-acrylonitrile, and polyvinyl chloride.

69.3.2.3 Specific Gravity Measurement. It is necessary that the specific gravity of perfusate injected and effluent retrieved from the Donor be measured in Phase II, either frequently or continuously. Samples may be taken in hydrometer cylinders and measured by precision hydrometer floats (Figure 69-2). This equipment is available from Van Waters and Rogers<sup>5</sup>

Scientific (VWR Scientific) per Appendix A. A specific gravity meter is available from the Brooks Instrument Division of the Emerson Electric Company (see Appendix A, under "Brooks Instrument"). One of the VWR Scientific hydrometers can be read in .0005 subdivisions in the range of 1.000 to 1.055, and costs less than ten dollars for a hydrometer and cylinder. The Brooks specific gravity meter costs several hundred dollars and is specified for an accuracy of one percent over the range 1.000 to 1.220, implying a useable resolution of .002. In general, a change of one percent DMSO is approximately equivalent to a change of .002 in specific gravity. Since measurements to a fraction of a percent DMSO may be important, the Brooks meter (if used) should be supplemented by manual hydrometers for higher accuracy.

69.3.2.4 Controlled Inflow/Outflow from System. In Phase II, pure (or concentrated solutions of) cryoprotective agents must be continuously added to the main reservoir, and a portion of the effluent must be continuously discarded. The flow rates are small, and must be controlled so that the flow into the main reservoir is the same as the effluent discard outflow. A simple means of mechanizing this would involve the use of a variable speed dual headed roller pump available from Cole Parmer (see Appendix A). Flow meters could be placed in both lines, with needle valves used to balance flow rates. A separate reservoir is required for the input DMSO concentrate. An effluent discard reservoir is required for collection of ejected perfusate.

#### 69.4 Further Discussion of Equipment for Phase II Perfusion.

Phase II perfusion equipment is not yet commercially available in a standard product line of any kind. Figure 69-3 is an early "conceptual" sketch of a Phase II perfusion system. Correspond with Manrise Corporation directly for further information.

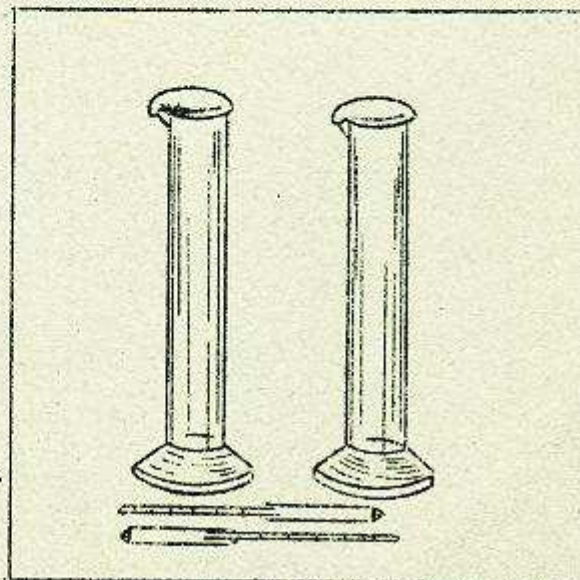


Figure 69-2: Hydrometers & Cylinders

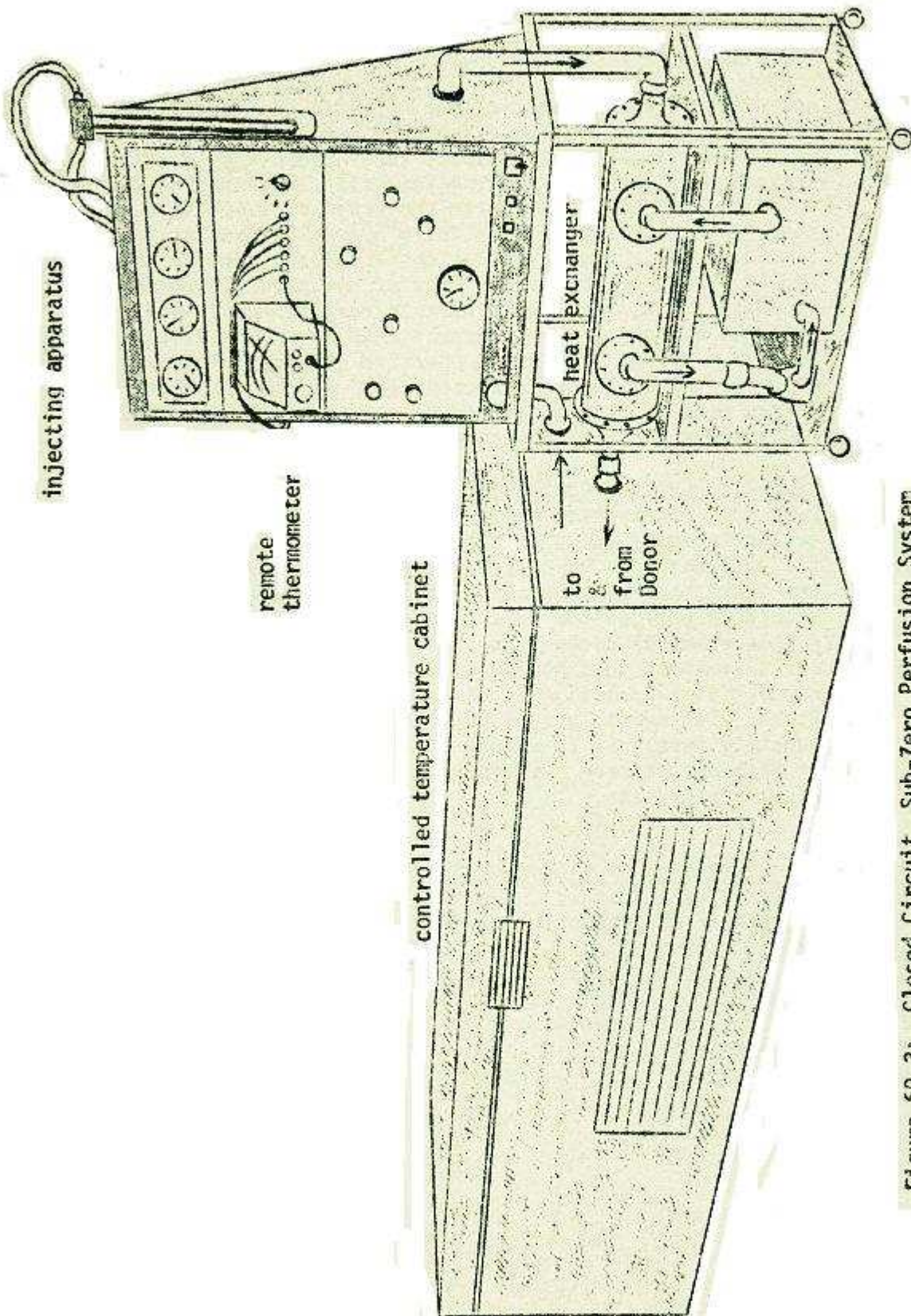


Figure 69-3: Closed Circuit, Sub-Zero Perfusion System

## 70.0 PROCEDURES FOR PHASE II PERFUSION

The procedures which follow are, in all essential respects, the implementation of Part F, "Procedures for Phase II Perfusion" in Art Quaife's paper (1). See the explanation in 70.10.

70.1 Initial Conditions. Using equipment fulfilling the minimum requirements given in section 69.0, the main reservoir is charged with not less than fifteen liters of Phase I perfusate, and Phase I recirculation at 0°C is in progress. The auxiliary reservoir is filled with DMSO concentrate and the flow rate into and out of the system is zero.

70.1.1 Stop Perfusion. Terminate injection to the Donor. The system now contains a fixed amount of Phase I perfusate, continuously being recirculated to the heat exchanger. All effluent is discarded.

70.1.2 Add DMSO. Prebalanced DMSO (see 68.3), either pure or diluted, is added directly to the main reservoir until the concentration reaches five percent (w/w). This is achieved by adding DMSO gradually until the specific gravity of the mixture corresponds to the five percent DMSO level in Figure 70-1.

70.2 Inject Ten Liters. Perfusion is begun, and continued until all except five liters have been injected. All effluent is discarded. At this point the DMSO concentration in the effluent is measured, and the factor K is calculated:

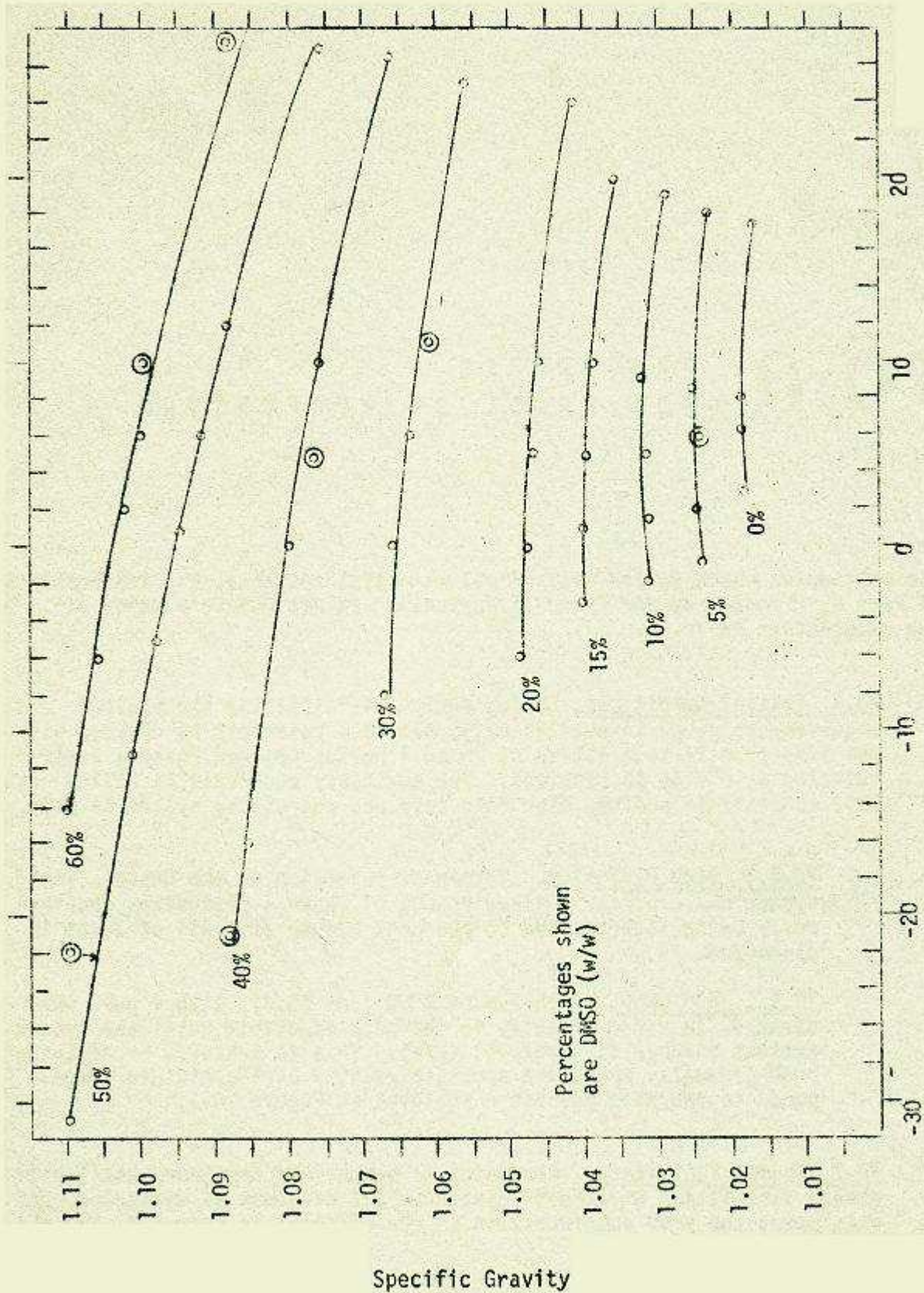


Figure 70-1: Specific Gravity vs Temperature for Quaiife's (Q-3) Solution with DMSO (revised)

$$K = 1 - \frac{C_1}{.05}$$

$C_1$  is the DMSO concentration in the effluent.  $K$  is an indication of how much DMSO is being absorbed. For example, if no DMSO is being absorbed, then  $C_1 = .05$  and  $K$  is zero. If all DMSO is being absorbed,  $C_1 = 0.0$  and  $K$  is 1.0. As before, the concentration of DMSO in the effluent is found by measuring the specific gravity of a sample of the effluent and consulting Figure 70-1.

**70.3 Continue Perfusion with Recirculation.** After the first ten liters have been injected, perfusion is continued, but the effluent is recirculated (with filtration) to the main reservoir for reinjection.

**70.4 Start Adding Concentrated DMSO and Discarding Effluent.** As soon as possible after recirculation is begun, concentrated DMSO is started slowly flowing into the main reservoir and a like flow rate of effluent (see 69.3.2.4) is discarded. First, the proper input-output flow rates must be calculated. A quick estimate may be made to begin with:

" $K$ " has already been calculated (70.2 above). The perfusion flow rate " $J$ " (liters/min) must now be observed from the injection flow meter.  $C_d$  is the fraction of DMSO in the DMSO-concentrate reservoir (examples: for pure DMSO,  $C_d = 1.0$ ; for 80% DMSO w/w,  $C_d = 0.8$ ). The perfusate first injected is assumed to be five percent. Calculate  $J_d$ :

$$J_d = \frac{(J)(K)(.05)(1.5)}{C_d}$$

The factor 1.5 is simply an initial approximation to more complex terms; further information is provided in 70.6.

For example:

If perfusion flow ( $J$ ) = 2.0 liters/min

If  $K = 0.2$

If  $C_d = 0.8$

$$\text{then } J_d = \frac{(2.0)(0.2)(0.05)(1.5)}{0.8} = 0.0375 \text{ liters/min}$$

In the example given, the 80 percent DMSO solution would be sent into the main reservoir at 0.0375 liters per minute. Effluent would be discarded at the same rate. The initial determinations of " $K$ " and " $J_d$ "

are subject to many errors, and redetermination of "K" and modifications to "J<sub>d</sub>" will be needed frequently, in the beginning. Therefore, one must proceed at once to those steps called for in 70.5 and 70.6. Also a thorough understanding of Phase II as outlined in 70.10 is essential.

70.5 Continue Recirculation; Perform Essential Measurements and Calculations. Once the inflow-outflow (concentrated DMSO; effluent discharge) has started, recirculation to the Donor is continued. Measurements and calculations are now required for controlling the operation.

70.5.1 Data Sheet. Figure 70-2 is the data format for use in controlling Phase II perfusion. It is assumed that users of this procedure will have an extensive supply of these sheets.

70.5.2 Filling in Basic Information. Fill in the Donor's weight in kilograms, opposite "M<sub>b</sub>". Calculate W<sub>s</sub> and W<sub>w</sub> as shown below.

$$W_s = .053 M_b \quad \text{NOTE: } 1 \text{ pound equals } 0.4536 \text{ kilograms}$$

$$W_w = .575 M_b$$

Fill this data in at the top of all data sheets used for any particular Donor.

70.5.3 Filling in Lines of Recorded Data on Data Sheets. The time of measurement of effluent specific gravity and DMSO concentration (see 70.2) is entered under "t". The flow rate of perfusion observed at the time is entered under "J".  $\rho_p$  is the specific gravity of perfusate in the main reservoir, and the concentration  $C_p$  is the corresponding value from Figure 70-1. Enter these values on the data sheet under the appropriate columns.  $\rho_1$  is the effluent specific gravity, and  $C_1$  is its corresponding value of DMSO concentration from Figure 70-1. Fill these values in also, on each line, as measurements are made.

70.5.4 Calculation of Quantities on the First Line. Only one quantity (A) is calculated, on the first line:

$$A = J(\rho_p C_p - \rho_1 C_1)$$

All other quantities are entered as zero.

70.5.5 Calculation of Quantities on Second and Later Lines. On each line, "A" is calculated the same as on the first line: from data to the left, on the same line, by the same formula used above.





The other quantities are calculated from data including quantities from *previous* lines. When a quantity is taken from a previous line, it will be shown in the equation as having a circle around it.

For B:

$$B = \frac{A + \textcircled{A}}{2}$$

The triangle ( $\Delta$ ) is called delta; it represents the time elapsed (in minutes) between the last measurement and the present measurement. Thus:

$$\Delta = t - \textcircled{t} \quad \text{and} \quad U = \textcircled{U} + B\Delta$$

Finally, the concentration of DMSO in weakly circulated tissues ( $C_w$ ) is calculated:

$$C_w = \frac{U - W_s C_p}{W_w}$$

In briefest explanation: "A" is the DMSO uptake rate in each measurement. "B" is the average DMSO uptake rate over the time between the last measurement and the current measurement. " $\Delta$ " is the time between measurements, and "B $\Delta$ " is the amount of DMSO absorbed into the body during  $\Delta$ . "U" is the total DMSO absorbed by the body up to the corresponding time "t". In the calculation  $C_w$ , it is assumed that the strongly circulated parts of the body are fully equilibrated with the perfusate. The rest of the DMSO absorbed is assumed to be uniformly distributed throughout the weakly circulated tissues.

#### 70.6 Continue to Modify Inflow-Outflow Rates as Appropriate.

At any time, from the latest line of data, one may recalculate "K" and then "J<sub>d</sub>" (inflow-outflow rate):

$$K = 1 - \frac{C_1}{C_p}$$

$$W_b = W_s + W_w \quad \left[ W_b \text{ is calculated only once. This value is then used throughout Phase II.} \right]$$

$$J_d = \frac{J K C_{po} \left( \frac{W_p}{W_b} + 1 \right)}{C_d - C_p + K C_{po}}$$

where: (see definition of symbols on next page)

- $J_d$  = Inflow-outflow rate (liters/min)  
 $J$  = Perfusion flow rate (liters/min)  
 $K$  = Value calculated from DMSO concentrations  
 $C_{po}$  = Concentration of DMSO in initial injection  
 $W_p$  = Weight of perfusate external to Donor (Kg)  
 $W_b$  = Weight of Donor's liquid-state body constituents (Kg)  
 $C_d$  = Concentration of DMSO in DMSO concentrate reservoir  
 $C_p$  = Concentration of DMSO in reservoir (at the very beginning,  
 $C_p = C_{po}$ )  
 $C_1$  = Concentration of DMSO in effluent

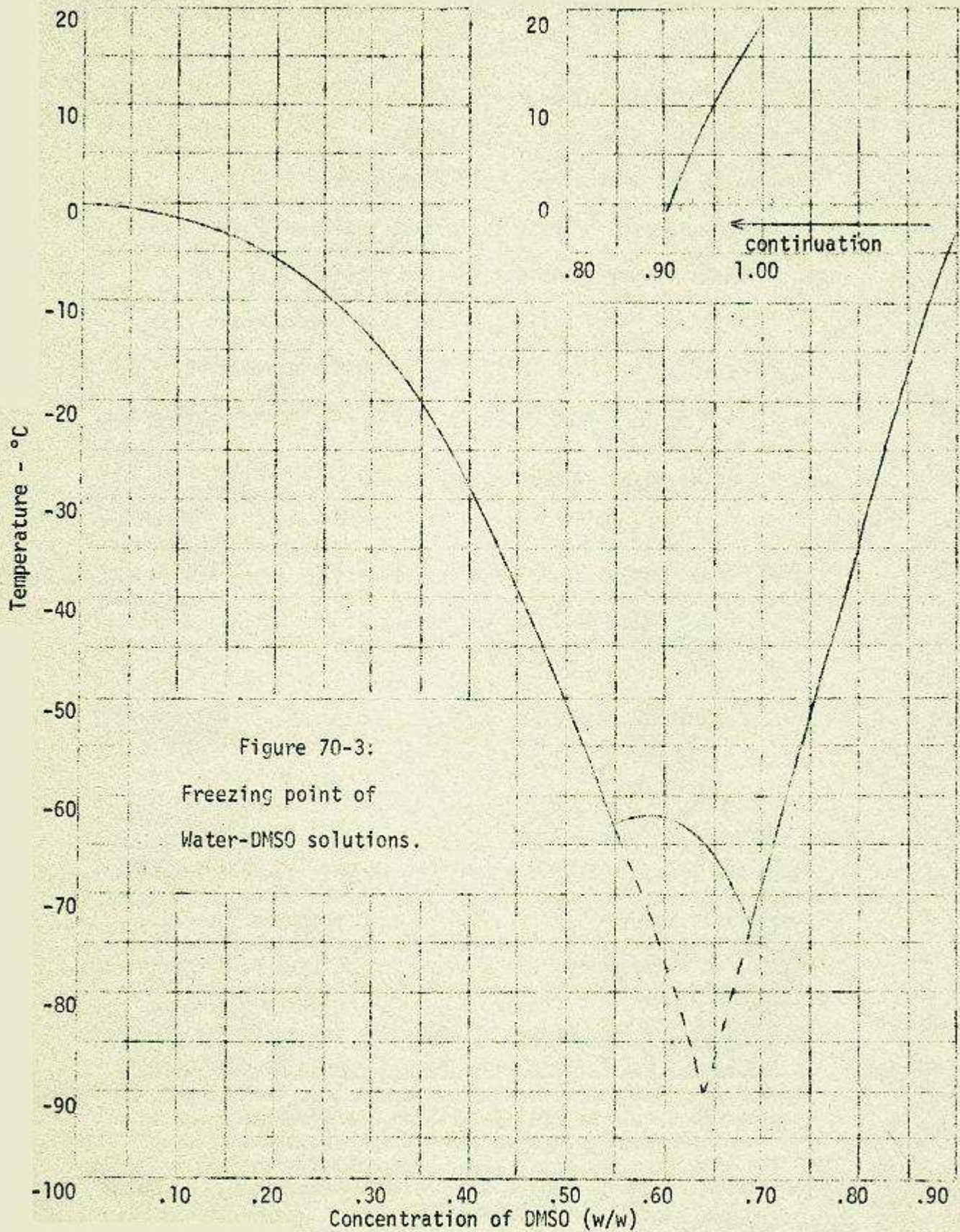
After recalculation of  $J_d$ , the inflow-outflow rates should be modified accordingly. A chart or log should be maintained to indicate the value of  $J_d$  in use at any time. An explanation of the above choice of  $J_d$  is given in Section 70.10, and particularly in 70.10.5.

70.7 Frequently calculate "Time to Completion". The remaining time " $t_c$ " to complete Phase II perfusion may be calculated, at any point, using the equation:

$$t_c = \frac{(.64 - C_p) \Delta}{C_p - C_p}$$

Since any single value of  $t_c$  will be subject to measurement errors, a string of five or more calculations should be averaged together for greater accuracy. Any value of  $t_c$ , of course, is valid only for the temperature and time at which it is calculated. For this reason, as temperatures are changed and as time advances, frequently recalculate  $t_c$ .

70.8 Lower the Temperature of Perfusate and the Donor's Exterior to the Lowest Permissible Level at any Point; DO NOT Increase  $C_p$  Above 0.64. The Donor's exterior temperature and the perfusate temperature should be held just above the freezing point of the DMSO concentration predicted for weakly circulated tissues. Thus, at any time, the value of  $C_w$  may be compared with Figure 70-3 and the lowest allowable control temperature determined. As noted in



70.7, lowering of temperature will extend the "time of completion". Therefore, should a limiting "time of completion" be reached (practical circumstances dictate against further extension of Phase II) then the temperature is held constant until the value of  $C_w$  reaches 0.64. A careful record should be maintained of the temperature control points used and the times at which changes are made. When  $C_p$  reaches 0.64, it is held constant at that value until the completion of perfusion.  $J_d$  is reduced as necessary to prevent  $C_p$  from exceeding 0.64. It may be necessary to discontinue inflow-outflow to and from the system ( $J_d = 0.0$ ) for some periods of time. Finally,  $C_1$ ,  $C_p$ , and  $C_w$  should all converge toward 0.64 (with  $C_w$ , this is somewhat related to the overall accuracy of measurements and calculations). If feasible, perfusion should be continued for several hours after all three values are essentially 0.64, and then discontinued. Phase II perfusion is completed.

70.9 Suppose No Inflow-Outflow Subsystem is Available. It is possible that one may have Phase I perfusion equipment fulfilling all requirements for Phase II except the provisions for inflow-outflow control (per 69.3.2.4). In this event, a minor modification to the procedure will still permit Phase II to be accomplished.

70.9.1 After determining the proper inflow-outflow rates per 70.4, concentrated DMSO is added to the main reservoir and effluent is discarded by manual methods. For example, if the inflow-outflow is determined to be 0.0375 liters per minute (as in 70.4), one would simply inject 37.5 milliliters of concentrate per minute into the main reservoir and extract 37.5 milliliters of effluent per minute from the effluent collection reservoir, perhaps by means of large, calibrated hypodermic syringes, etc.

70.9.2 It is relatively unimportant whether the effluent is extracted continuously or periodically. One could simply remove 37.5 milliliters in a few seconds, once per minute, for satisfactory operation. Far more important, however, would be constant flow into the system. The injection of concentrated DMSO into the main reservoir would (in the example given) be 37.5 milliliters per minute. A gravity feed device, for example, could be arranged to deliver about one milliliter every 1.60 seconds. Obviously, performing Phase II with less sophisticated equipment is more demanding of the perfusion team, since more attention to DMSO injection and minute by minute withdrawal of effluent continues hour after hour, perhaps for days, without interruption.

70.10 Further Information. At this point, a brief explanation is given as to the rationale for Phase II procedures. This is, at best, an overview of the contents of Mr. Quaife's Paper (1) and no substitute for a detailed reading of it. Questions of interpretation may arise and should be referred to Manrise Corporation or Mr. Quaife directly.

70.10.1 Ideas in Other Parts of the Manual. The introductory section to Phase II procedures (65.0) describes the basic Phase II processes and factors of primary importance. Other preceding sections, particularly 68.0 and 69.0, will contribute to the reader's readiness to comprehend the rationale behind Phase II procedures.

70.10.2 The "Gradient" and Estimation of Total Body DMSO Concentration. Even as the first DMSO perfusate is injected (70.2), the body begins absorbing some of the DMSO. Thus, the perfusate leaving the body has less DMSO than the perfusate entering it. This difference of percentages is termed the "gradient" and can be used, along with the flow rate, to calculate the rate of DMSO absorption in the body. Further calculations, based on frequent measurements of gradient and flow rates, permit estimation of DMSO concentration in the body. This in turn permits figuring (from a graph) which temperatures are safe, at any point, in that they will not cause freezing to tissue. Finally, when the body is estimated to have absorbed as much DMSO as is useful, Phase II perfusion is finished.

70.10.3 Stabilizing the Gradient. The procedures specified for Phase II are gauged to make the body concentration of DMSO build up at the same rate as the perfusate concentration. If this is done correctly, the gradient will be stable, and this will signify that the body is absorbing DMSO at a relatively constant rate. The supporting line of reasoning, while rather complex, is briefly explained as follows:

The flow rate of DMSO entering the perfusion system from the outside (and the flow rate of effluent discarded) is intended to produce a condition where the gradient ( $C_p - C_1$ ) stabilizes. Thus if everything proceeds according to plan, perhaps the initial percentages are 5% DMSO in, and 2% out (a difference or gradient of  $5\% - 2\% = 3\%$ ). Later, assuming an unchanging gradient, the percentage would be 12% in, 9% out; then 39% in, 36% out; and so on. Also, the estimated body concentration  $C_w$  should lag behind the other two by about the same margin. Thus at first  $C_w$  would be 0% (5% less than the perfusate and 2% less than the effluent). Later, corresponding to the foregoing examples,  $C_w$  should be about 7% (for 12% in, 9% out) and 34% (for 39% in, 36% out). If the gradient changes, and keeps on changing, or if the "in" and "out" percentages start to get well ahead of the estimated body percentages, then adjustments

are required. This will almost certainly be the case, since the foregoing explanation is based on a mathematical approximation of the processes which will occur. Any lack of exactness will be reflected in the degree of readjustments that are necessary.

#### 70.10.4 What is Actually Happening When the Gradient Starts

Changing? If the gradient starts getting smaller, this means the body is absorbing DMSO less rapidly than predicted. If the gradient gets larger, DMSO is being absorbed more rapidly than previously estimated.

#### 70.10.5 Changes of Flow Rate ( $J_d$ ) Into and Out of the System

to Compensate for a Changing Gradient. If the gradient gets smaller, "K" will decrease, so flow rates ( $J_d$ ) into and out of the system must be reduced. The body is soaking up DMSO slower than expected, and the method of correction is to slow up the addition of concentrated DMSO to the system. The gradient, "K", and the required inflow-outflow " $J_d$ " will stabilize if repeated corrections are made.

Conversely, if the gradient rises, the body's absorption rate has increased, therefore, "K" will increase and so must " $J_d$ ". The body is absorbing DMSO more rapidly, and the addition of DMSO is speeded up accordingly. One thing, very important, is to keep comparing estimated body DMSO concentration  $C_w$  with the concentrations in the perfusate ( $C_p$ ) and the effluent ( $C_1$ ). It is possible that by following the given procedure, ( $C_p - C_w$ ) may become greater than 10%. This is undesirable, so in this case, inflow-outflow should be stopped ( $J_d = 0.0$ ) and restarted only when  $C_p - C_w$  is less than 10 percent.

70.10.6 "Built-in" Compensation Factors. Suppose perfusion flow rate "J" (different from  $J_d$ ) slows up, perhaps due to higher viscosity at lower temperatures. Perhaps, however, due to lower diffusivity at the lower temperatures the gradient does not rise (as it usually would with decreased J) but remains constant, along with the calculated value of K. Obviously, the body's rate of absorption of DMSO will decrease (due to lower flow rate) even though the gradient and "K" are the same. The calculation for " $J_d$ ", however, involves multiplying by "J". Thus, in this case,  $J_d$  would be decreased as "J" decreased and the rate of adding concentrated DMSO to the system would be slowed properly. In other ways also, the procedures tend to compensate for changes which could perturb the system. One last reminder: *Don't ever let  $C_p - C_w$  exceed 10% (0.10), and don't let  $C_p$  exceed 64% (0.64).*

# 71.0

## POST PHASE II PROCEDURES

Following Phase II perfusion, temperatures are lowered to those used for permanent storage. This section provides guidelines that may help accomplish this, based on a number of assumptions concerning the equipment available.

71.1 Reasons for Lower Temperatures. At the completion of Phase II all cellular constituents are still in liquid state. These temperatures are too high for permanent storage. Over the course of many years, continuing chemical changes within cells may reduce potential viability. Alternatively, lowering the temperature after Phase II will cause solidification of all liquid state matter, possibly with some degree of crystallization. This, too, may be harmful. Current recommendations are to continue lowering temperature until the boiling point of liquid nitrogen is reached. (The temperature of carbon dioxide evaporation is too high for long term storage. Recrystallization within cells occurs at this temperature, with associated reduction of cell viability.)

71.2 Means for Lowering Temperature. If a low temperature cabinet as described in 69.2 is available, with provisions for switchover from liquid carbon dioxide to liquid nitrogen, the temperature may be reduced in a highly controlled way to liquid nitrogen's boiling point.

71.3 Rate for Lowering Temperature. Theoretically, the rate of decreasing temperature through those regions where solidification occurs would be *extremely* rapid (snap freezing). This is infeasible without yet to be developed hyperbaric techniques.

71.3.1 Without snap freezing, the fastest controlled cooling rates would require total submergence in a fluid cooled far below the Donor's core temperature, having a boiling point higher than core temperature. This also, however, presupposes the availability of equipment far more sophisticated than that required for Phase II. Even assuming the existence of this type apparatus, the achievable cooling rates would be far less than those now held to be optimum. Fast cooling, therefore, cannot be used effectively at this time.

71.3.2 Temperature may be decreased, at a far slower rate than that projected for snap freezing or submergence in cold-boiling liquids, by circulating a cooled gas about the Donor. Nitrogen is the preferred gas, as intimated in 71.2.

#### 71.4 Procedure for Lowering Temperature.

71.4.1 Gas Purging Procedures. The low temperature cabinet should be switched over to the use of liquid nitrogen well prior to reaching the temperature of carbon dioxide solidification. Provision for an hour's operation on liquid nitrogen, above  $-78.5^{\circ}\text{C}$ , should adequately purge all carbon dioxide gas and prevent the later formation of  $\text{CO}_2$  "frost" on the Donor.

71.4.2 Lowering the Temperature. Cold nitrogen gas should be circulated about the Donor at a temperature  $40^{\circ}\text{C}$  lower than the Donor's body core temperature. As core temperature falls, gas temperature is also lowered to maintain the  $40^{\circ}\text{C}$  differential, until the gas temperature reaches that of boiling nitrogen. Biologists later concerned with demonstrations of viability will, as a result, have a known temperature reduction profile as a reference for such comparative testing as may be necessary. The figure of  $40^{\circ}\text{C}$  is arbitrary, but should result in a sufficient thermal gradient for reduction of temperatures to those of liquid nitrogen in a reasonable period of time. The recorded cooling *rate* (body core temperature as a function of time) as measured over the entire period is the parameter which should be known for future reference.

71.5 Procedures for Maintaining Storage Temperatures. Submergence in liquid nitrogen is the preferred technique for maintaining the desired storage temperature ( $-196^{\circ}\text{C}$ ). *Total* submergence ensures uniformity of temperature. *Partial* submergence may not. This point should be considered in the design of new containers. Ideally, liquid nitrogen levels in the storage capsule would be maintained so as to ensure total submergence at all times (perhaps by using an auxiliary liquid nitrogen storage reservoir and a level controller).



Another approach to assuring uniformity of temperature involves providing an inner capsule or container of high thermal conductivity which is in contact with liquid nitrogen at all times. Any heat picked up from gas phase nitrogen external to the inner container is quickly conducted away to the liquid nitrogen heat sink. One manufacturer recommends aluminum as a suitable construction material; the inner container may contain perforations to prevent bouyancy problems.

## 72.0 SECTIONS OF THE MANUAL YET TO BE COMPLETED

In a sense, all of the sections of the manual are "yet to be completed". We will revise the manual on a continuous basis, improving its contents and keeping it technically current.

72.1 Improvements Possible Now. Many areas of the manual can be beneficially expanded, now or in the immediate future. For example, Section 8.0 will develop into a comprehensive guide on organizing cryonics societies. Section 10.0 will be expanded to contain a "check-list" version of the procedures for inducing SSH with reference to the appropriate sections containing detailed information. Section 71.0 will soon contain information on low temperature capsules and where to obtain them. In this respect, then, the manual will be in a state of growth for some time.

72.2 Updating. In some areas, the manual's treatment is nearly as complete as current knowledge permits. With the inclusion of sections through 72.0 the manual, in its first printing, is considered to be complete. Improvements from this point forward will be considered a part of the revision service. As better equipment becomes available, descriptions will be included in sections such as 38.0, 59.0, and 69.0. The sections on "Medical Tests" (40.0 through 44.0) will become a detailed set of guidelines on research measurements of value, as these are formulated.

72.3 Sections 72.0 and Those to Follow. Gradually, as procedures used in successful reanimation of whole organisms are perfected, more sections will be added, based on experimental protocols proposed for use in reviving humans.

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