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INFORMATION FOR AUTHORS

Manrise Technical Review. At this time, the most widely recognized means of increasing the probabilities of surviving clinical death involve the induction of solid state hypothermia, a low temperature state in which chemical and biological processes are essentially arrested. Most information published in MTR will be directly relevant to this subject.

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MANRISE
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EDITORIAL

It has been proposed that all dying persons be frozen, pending the development of means for their reanimation.[1] It has also been suggested that this should not be done at all until the capability for reanimation has been demonstrated.[2] Perhaps there is a third position which can bridge that gap.

First, a glance at current capabilities. Cells of most types can be frozen and thawed with some retention of viability, in an isolated state. With tissue samples, there is a lesser degree of success. Attempts to freeze whole organs or organisms and revive them have largely failed. "Freeze now" advocates assert that the difficulties encountered with larger specimens generally stem from thawing damage and from a lack of understanding of how to correct and repair freezing damage. This contention may be correct, but it has not yet been satisfactorily proven. On the positive side, were this premise verified, then fundamental feasibility of the "freeze now" approach could be validated.

More simply, if a whole organism is frozen now, and then small samples of all important types of cells are rewarmed by various methods and shown to be viable, then the possibility of later reanimation is fundamentally demonstrated. It may not be practical to rewarm an entire organism and revive it at this time, since the development of a very advanced technology is required. Yet confidence *can* be established that when such technology is developed, those persons frozen earlier by *proven* techniques will be potentially revivable. This is the most immediate objective to be sought.

Several considerations are involved in this demonstration of feasibility. First, highly controlled methods of freezing whole organisms must be developed, along with similarly sophisticated means for extracting small samples and rewarming them. Experimentation must then be pursued until a whole organism freezing protocol exists which can be verified by rewarming samples of all important types of cells with essentially complete retention of viability. At this point, it will have been established that a low temperature state can be induced in whole organisms where cells are potentially viable. This level of confidence is expected to be sufficient to induce the necessary long term research for development of the means of reanimation.

It is expected to be sufficient, since many persons will understand that the rest of the task is primarily a matter of technological development. Each cell of an organism frozen by the verified method will be able to be restored to a normal living state, individually. No cell of such an organism can then be regarded as fundamentally "dead". The research that follows will result from the fact that many will want it done, and will support it. (continued on page 77)

MATHEMATICAL MODELS OF PERFUSION PROCESSES

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Very little empirical data is available concerning the actual efficacy of alternate procedures for freezing and thawing whole mammals to the temperature of liquid nitrogen. In order to currently formulate recommended procedures for the induction of solid state hypothermia in humans, it is thus necessary to rely partly upon extrapolation from the results of experiments treating the freezing of cells and organs, and to an even greater extent upon theoretical analysis of the effects of alternate procedures. In this paper, we formulate mathematical models of many of the processes that take place during the induction of SSH in humans. Primary focus of the treatment is to analyze the rate of removal of heat from the body, and the rate of buildup of cryoprotectant in the body cells during perfusion. Specific formulas are also developed giving the quantity of cryoprotectant and the length of time required to accomplish perfusion, the cooling profile to be followed, and many others. The conclusions of the theory are used to formulate a specific recommendation as to a best current perfusion procedure. In many cases, the unknown values of the body parameters appearing in the equations require that approximating assumptions be made in order to achieve numerical results. It is expected that these results will be refined considerably when further information becomes available concerning the values of these parameters.

Introduction

As currently conceived, the induction of solid state hypothermia (SSH) in the human body takes place in three phases. During Phase I, the blood is replaced by a suitable balanced salt solution and the body temperature is lowered to 0°C as rapidly as possible. In Phase II, a concentration of cryoprotectant [assumed in this paper to be dimethyl sulfoxide (DMSO)] is built up in the body water, while continuing to lower the body temperature in such a way as to remain in liquid state. Phase III begins when the desired terminal concentration is achieved; the body is then lowered to liquid nitrogen temperature where it is stored in solid state. During the first two phases, systems will be required to recirculate chilled perfusate through the body. This paper treats the mathematics of such systems.

Section I. Limitations of the Analysis.

One is faced with grave difficulties in attempting to formulate equations valid during all of Phase I and Phase II perfusion. All of the physical constants involved, such as diffusion and thermal conductivity, heat capacities, densities, viscosities, and thus achievable flow rates, are temperature dependent. In most cases, little data is available concerning the values of these constants at temperatures well below 0°C. Even when there is theory available to predict their temperature dependence, its inclusion would usually make the rest of the analysis intractable. Thus, the equations developed herein can only be considered valid over limited temperature ranges; the values chosen for the constants appearing therein should be those appropriate to that range.

A note on the choice of variables: In most equations developed herein, it would be conceptually simpler if such variables as DMSO concentration and flow distribution were expressed on a per unit *volume* of perfusate (or tissue) basis. But in view of the fact that the two accurate graphs available giving freezing point of DMSO solutions versus temperature both use concentration per unit *weight*, and similarly for the data presented in Table 1 below, we will instead use weight concentrations. Appendix 1 gives the relation between these two possible choices of variables. We have also written the equations in such a way as to maximize the formal analogy between heat conduction and diffusion.

Section II. Macrocirculation of the Blood.

In normal circulation, there exists a wide variation in the percentage of the cardiac output delivered to different tissues. For example, on a per gram of tissue basis, the kidneys receive over 150 times as much blood as do the skeletal muscles. We can approximate the normal blood flow by dividing the body into two parts: (a) Strongly circulated tissue, consisting of the brain, heart muscle, kidneys, and organs of the hepatic-portal circulation (mainly the liver), and (b) Weakly circulated tissue, consisting of the remainder of the body. The breakdown of these two parts is given in Table 1.

Tissue	Mass	Blood Flow
Strongly circulated	4.6 kg.	3.76 liters/min.
Weakly circulated	58.4 kg.	1.64 liters/min.

Table 1

Distribution of cardiac output in a 63 kg. man at rest. [From (1), page 240]

This division has important implications in the induction of SSH. During Phase I, the vital internal organs, including the brain, will be cooled much more rapidly than the rest of the body. In Phase II, it greatly limits the rate of buildup of cryoprotectant in the greater bulk of the body, since it receives only about 30% of the total flow.

Presumably the above figures for distribution of flow are altered during perfusion subsequent to clinical death, due to cessation of the normal vasomotor regulatory mechanisms, and since the perfusate is being introduced and exhausted abnormally through the femoral arteries and veins. The author does not possess any information concerning the extent of this alteration.

Section III. Microcirculation of the Blood; Diffusion and Heat Conduction.

Let us consider a uniform medium whose temperature distribution is $T(\vec{r},t)$, where $\vec{r}$ and t represent position and time respectively. Then Fourier's Law determines the change of temperature with time:

[1] $$\frac{\partial T}{\partial t} = D\vec{\nabla}^2 T$$

where $\vec{\nabla}^2$ is the Laplacian operator; in rectangular coordinates,

$$\vec{\nabla}^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$$

The constant D is called the *thermal diffusivity* constant for the medium. This equation, together with initial and boundary conditions, completely determines $T(\vec{r},t)$.

If we let $C(\vec{r},t)$ be the concentration of a substance diffusing through a medium, then precisely the same equation, with C replacing T, describes this diffusion (Fick's Second Law). In this case, D is the *diffusion constant* for a pair of substances. An excellent general reference to this subject is (2).

As chilled perfusate is circulated through the vascular system, heat transfer will be effected, particularly in the capillaries. A typical capillary has radius .004 mm., length .5 mm., and blood flows through at a rate of .5 mm./sec. [From (1), pg. 43; see also (3), pg. 559 and (4), pg. 503].

Each capillary supplies the tissue immediately surrounding it, which we may envision to be a cylinder of tissue coaxial with an inner cylinder representing the capillary, as in Figure 1.

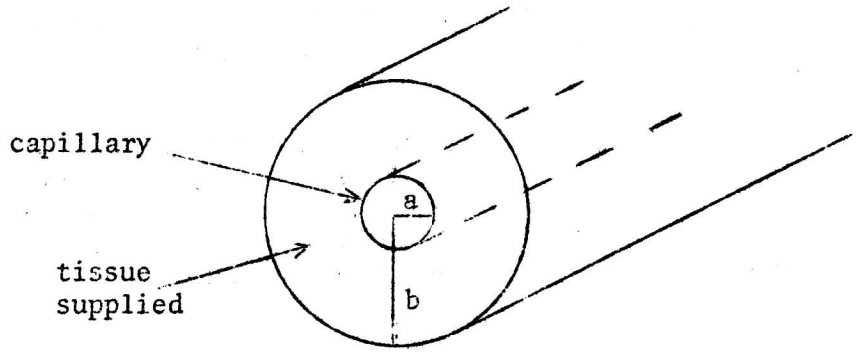

Figure 1

Roughton (5) calculates that for average tissue, b/a ≈ 17.7. In strongly circulated tissue such as heart muscle we have b/a ≈ 2.5, while for poorly circulated tissue, b/a ≈ 125.

Suppose that we maintain the surface r = a of the capillary at a temperature T_0, while the rest of the tissue in the outer cylinder is initially at a temperature T_1. We let $\overline{T}(t)$ be the *average* temperature within the outer cylinder. Roughton gives the solution to [1] in this case:

$$[2] \qquad \frac{T(r,t) - T_0}{T_1 - T_0} = \pi \sum_{n=1}^{\infty} \frac{J_0(x_n)Y_0(rx_n/a) - J_0(rx_n/a)Y_0(x_n)}{\left[\frac{J_0(x_n)}{J_1(bx_n/a)}\right]^2 - 1} \exp\left[\frac{-Dx_n^2 t}{a^2}\right]$$

$$[3] \qquad \frac{\overline{T}(t) - T_0}{T_1 - T_0} = \frac{4}{(b/a)^2 - 1} \sum_{n=1}^{\infty} \frac{\exp\left[\frac{-Dx_n^2 t}{a^2}\right]}{x_n^2 \left\{\left[\frac{J_0(x_n)}{J_1(bx_n/a)}\right]^2 - 1\right\}}$$

Here J_0 and J_1 are the Bessel functions of the first kind, of order 0 and 1; Y_0 and Y_1 are the Bessel functions of the second kind, and $x_1, x_2, x_3 \ldots$ are the positive roots of the equation:

$$[4] \qquad J_0(x)Y_1(bx/a) - Y_0(x)J_1(bx/a) = 0$$

Now the thermal diffusivity of a medium is obtained from its thermal conductivity K by the relation $D = K/\rho h$, where ρ and h are the density and heat capacity of the medium. References (6) and (7) give values of K and ρh for average biological tissue; in both cases, $D = 1.1 \times 10^{-3}$ cm^2/sec., which is the value we will use.

The author has written a computer program to calculate T and $\overline{T}$ from equations [2] and [3]. Thermal equilibrium is reached very rapidly. In average tissue, both T(b,t) and $\overline{T}(t)$ reach 99% of their final value in about .32 sec. Even in poorly circulated tissue, 99% equilibration is reached in 15 seconds. These times are very short when compared to the rate at which the temperature of the perfusate (which determines T_0) will change. As a consequence, during the circulation of chilled perfusate, no significant thermal gradients will exist within the tissue serviced by a capillary. Thus, the temperature at the walls of the capillary will be almost identical to the average temperature of the tissue.

As an element of perfusate passes through the capillary, it will absorb heat from the surrounding tissue. To determine the final temperature of the perfusate upon emerging from the capillary, one must solve exactly the same problem for the inner cylinder $r \leq a$ as was just treated for the outer region $a \leq r \leq b$. The solution may be found in (2), which also gives graphs of T(r,t) and $\overline{T}(t)$ as functions of Dt/a^2 (pg. 67 & pg. 72). When $Dt/a^2 = 1.0$, $\overline{T}$ has already achieved over 99% of its equilibrium value. In our case, this occurs when t = .00014 sec. Since the time of transit through the capillary is about 1 sec., we see that complete heat transfer will easily have been effected.

Thus, the conclusions of the above discussion are:

A. Each tissue can be represented by a single temperature T.

B. Circulating perfusate will emerge from this tissue at the same temperature T.

We will see later, however, that significant thermal gradients will exist between different organs.

Unfortunately, the same conclusions do not hold for DMSO diffusion through tissues. The author has been unable to find a published value of the diffusion constant for DMSO through biological tissue. We can obtain an *upper bound* on this value by taking the diffusion constant for DMSO in water; how close the true value comes to this upper bound depends largely on the extent to which DMSO is slowed down in crossing cell and capillary membranes. Based upon its molecular weight, and the diffusion constants for other compounds in water [see reference (8)], we should have approximately

$$D_{DMSO,water} = 4.5 \times 10^{-6} \text{ cm}^2/\text{sec}$$

at 0°C. If we use this value in [2] and [3], we find that 99% equilibration is reached in about 78 seconds in normal tissue, and about 3700 seconds (1 hour!) in poorly circulated tissue.

These conclusions are in very rough agreement with data cited by Pegg (9, pg. 173) who mentions equilibration times for vascular perfusion at 0°C of about 30 minutes (type of organ not stated).

Thus we see that conclusion A will not hold for DMSO diffusion; significant gradients will exist within individual tissue. The approximation that will be used to describe this state of affairs is given in the next section.

Section IV. Black Box Description of Heat Exchangers, and of the Body as a Medium for DMSO Diffusion.

We consider a heat exchanger as in Figure 2:

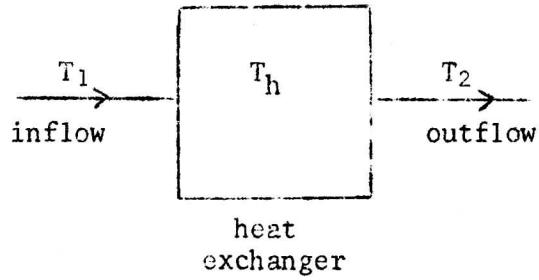

Figure 2

The exact relation between the temperature T_2 of the outflow and that T_1 of the inflow will depend upon the construction of the exchanger, the type of controls available, etc. For our purposes, we will consider a simple type of exchanger whereby the perfusate is brought in close proximity with a coolant, maintained at a constant temperature T_h. For example, in Phase I the coolant would be ice water at 0°C. In Phase II, it might be ethyl alcohol at dry ice temperature.

As an element of perfusate passes through the exchanger, it will undergo cooling approximately according to Newton's law of cooling:

$$[5] \qquad \frac{dT}{dt} = A(T_h - T)$$

where A is a constant characteristic of the exchanger. The solution to this equation is:

[6] $$T(t) = T_h + (T_1 - T_h) \exp(-At)$$

and if t_f is the time of transit through the exchanger, we get:

[7] $$T_2 - T_1 = H(T_h - T_1) \qquad 0 < H < 1$$

where $H = 1 - \exp(-At_f)$. (Note that T_2 and T_1 are measured at times differing by t_f; we ignore this minor difference).

Exactly the same considerations can be applied to the flow of a DMSO solution through the body. In that case, our conclusion would be:

[8] $$C_2 - C_1 = K(C_b - C_1) \qquad 0 < K < 1$$

where C_b represents the average body concentration of DMSO that the perfusate comes in proximity with. This is not as precise as in the case of the heat exchanger, since the perfusate will primarily exchange with fluid near the capillary walls, which is at a higher concentration than the average C_b. We nonetheless assume the existence of a K making this relationship valid. More precisely, in Section VII we will use *two* constants K_s and K_w to describe the body, corresponding to the partition of body tissue given in Section II.

Section V. Cooling the Body.

We will now set up the equations describing the rate of removal of heat from the body. We consider a recirculating system as in Figure 3.

The upper box represents the partition of the body tissue into strongly and weakly circulated parts, of masses M_s and M_w kg., and flows J_s and J_w liters/min., as given in Section II. The total flow $J = J_s + J_w$ is assumed constant. Throughout this paper, we will use lower case letters to represent the fractional parts of upper case quantities; i.e., $j_s = J_s/J$, $m_s = M_s/(M_s + M_w)$, etc.

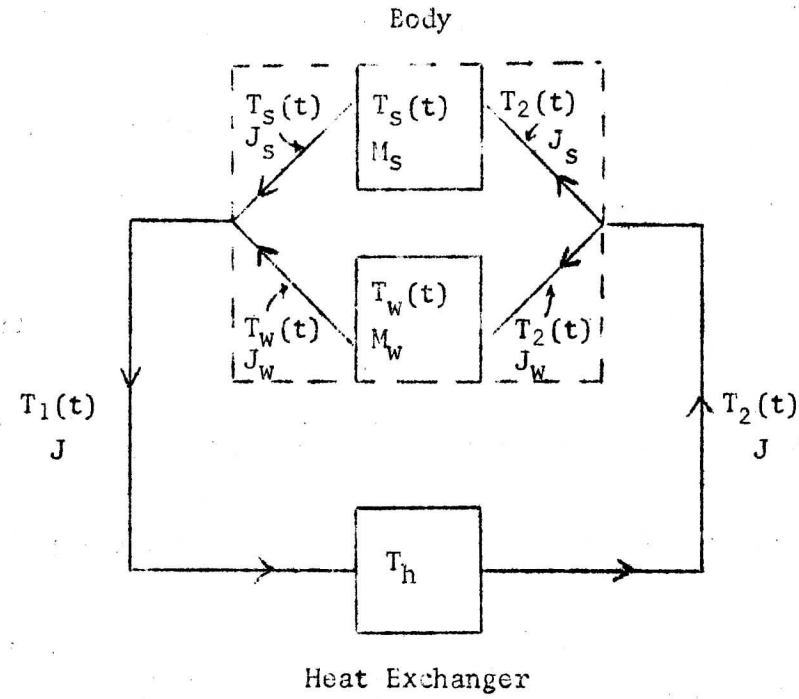

Figure 3

The perfusate passes through a heat exchanger, where its temperature is lowered according to equation [7]. We let

h_b = heat capacity of the body, in kcal/kg°C

h_p = heat capacity of the perfusate

ρ_p = density of the perfusate, in kg/liter

From (10, p. 995), $h_b \approx .83$ at 37°C. During Phase I, h_p will be close to that of water, 1.0. Similarly, $\rho_p \approx 1.0$. These values would all be different in Phase II. We let

[9] $T_{so} = T_s(0)$; $T_{wo} = T_w(0)$

In the case of greatest interest, $T_{so} = T_{wo} = 37°C$.

We assume that the body is insulated, so that there is no gain or loss of heat to the surroundings. We further assume that the production of heat energy via metabolism has been arrested by oxygen deprivation. Then, since total heat energy is conserved, the differential equations governing body temperature are:

$$[10] \qquad h_b M_s \frac{dT_s}{dt} = h_p \rho_p J_s (T_2 - T_s)$$

$$[11] \qquad h_b M_w \frac{dT_w}{dt} = h_p \rho_p J_w (T_2 - T_w)$$

[Note that we have tacitly assumed here that in passing through the body, (h_p) out = (h_p) in, and also that (ρ_p) out = (ρ_p) in. This will be true in Phase I, but involves a slight approximation in Phase II since DMSO concentration is different in the outflow than in the inflow. We note that at 20°C, DMSO has a heat capacity of .49 and a specific gravity of 1.1 (11, pg. 219).]

The temperature T_1 is given by:

$$[12] \qquad T_1 = j_s T_s + j_w T_w$$

Combining [7] with [12], we get:

$$[13] \qquad T_2 = HT_h + (1 - H)(j_s T_s + j_w T_w)$$

Substituting [13] into [10] and [11], we get:

$$[14] \qquad \frac{dT_s}{dt} = \frac{J_s h_p \rho_p}{M_s h_b} \{[(1 - H)j_s - 1]T_s + (1 - H)j_w T_w + HT_h\}$$

$$[15] \qquad \frac{dT_w}{dt} = \frac{J_w h_p \rho_p}{M_w h_b} \{(1 - H)j_s T_s + [(1 - H)j_w - 1]T_w + HT_h\}$$

Note that these equations are of the form:

$$[16] \qquad \frac{dT_s}{dt} = a_1 T_s + b_1 T_w - (a_1 + b_1) T_h$$

$$[17] \qquad \frac{dT_w}{dt} = a_2 T_s + b_2 T_w - (a_2 + b_2) T_h$$

These, and other linear differential equations appearing in this paper, are most easily solved by the method of the Laplace transform [see, e.g., reference (12)], or can be solved directly [reference (13), pg. 468]. We let

$$[18] \qquad \gamma = \sqrt{(a_1 + b_2)^2 + 4(a_2 b_1 - a_1 b_2)}$$

$$[19] \qquad \lambda_1 = \frac{a_1 + b_2 + \gamma}{2}$$

$$[20] \qquad \lambda_2 = \frac{a_1 + b_2 - \gamma}{2}$$

It is easy to verify that the following relations hold:

$$[21] \qquad \lambda_1 + \lambda_2 = a_1 + b_2$$

$$[22] \qquad \lambda_1 - \lambda_2 = \gamma$$

$$[23] \qquad \lambda_1 \lambda_2 = a_1 b_2 - a_2 b_1$$

$$[24] \qquad (\lambda_i - a_1)(\lambda_i - b_2) = a_2 b_1 \qquad \text{for } i = 1,2$$

Next, we let:

$$[25] \qquad A_1 = \frac{1}{\gamma}\left[(\lambda_1 - b_2)(T_{so} - T_h) + b_1 (T_{wo} - T_h)\right]$$

$$[26] \quad A_2 = \frac{1}{\gamma}\left[(b_2 - \lambda_2)(T_{so} - T_h) - b_1(T_{wo} - T_h)\right]$$

$$[27] \quad B_1 = \frac{1}{\gamma}\left[(\lambda_1 - a_1)(T_{wo} - T_h) + a_2(T_{so} - T_h)\right]$$

$$[28] \quad B_2 = \frac{1}{\gamma}\left[(a_1 - \lambda_2)(T_{wo} - T_h) - a_2(T_{so} - T_h)\right]$$

Then, using the properties [21] to [24] of the λ_i's, it is easy to verify directly that the solution to[16] and [17] is:

$$[29] \quad T_s = A_1\exp(\lambda_1 t) + A_2\exp(\lambda_2 t) + T_h$$

$$[30] \quad T_w = B_1\exp(\lambda_1 t) + B_2\exp(\lambda_2 t) + T_h$$

For purposes of comparison, suppose that we had treated the body as a single unit, and ignored the difference in flow distribution ("uniform body" approximation). Then the equation governing the change in body temperature would be:

$$[31] \quad \frac{dT_b}{dt} = \frac{Jh_p\rho_p}{M_b h_b} H(T_h - T_b)$$

whose solution is:

$$[32] \quad T_b = T_h + (T_{bo} - T_h)\exp\left(\frac{-Jh_p\rho_p}{M_b h_b} Ht\right)$$

Section VI. Phase I Perfusion.

We will use the above results to develop cooling profiles for the 63 kg. reference man of Table 1. We assume that it is possible to achieve a perfusion flow of 4 liters/minute, as opposed to the resting cardiac output of 5.4 liters/minute, and reduce J_s and J_w proportionally.

Esmond (14) gives values of H for a number of different heat exchangers. At flow rates of 4 l/min, the best of them have a value H = .4. If we take $T_h = 0$, $T_{so} = T_{wo} = 37°C$ and substitute these values into our model, it becomes:

[33] $T_s = 12.3 \exp(-.017t) + 24.7 \exp(-.428t)$

[34] $T_w = 37.6 \exp(-.017t) - .6 \exp(-.428t)$

Notice that the last two terms vanish very rapidly. Thus, the model predicts that T falls very quickly to about 12°C while T_w remains relatively near 37°C; from this point on, both T_s and T_w fall to 0°C at the same exponential rate.

For comparison, with the same assumed parameters, the "uniform body" approximation becomes:

[35] $T_b = 37 \exp(-.0306t)$

These three functions are plotted in Figure 4.

In most studies of body temperature in man, the body is divided into a *core* zone, consisting of the cranial, thoracic and abdominal cavities, and parts of the muscular mass; and a *peripheral* zone, consisting primarily of the skin and some of the subcutaneous tissue (1, pg. 528). The core temperature, as recorded by a rectal or esophogeal thermometer, is sometimes taken to represent the temperature of 2/3 of the body, while the skin temperature is taken to represent the remaining 1/3 (10, pg. 994). Our model implies that large thermal gradients will exist even within the core zone during vascular cooling. Such severe gradients are apparently not encountered in clinical hypothermia.

There are two possible explanations for this discrepancy: (a) The strongly circulated organs of mass M_s are, quite naturally, those with the highest metabolic rate. Thus, during hypothermia, their greater rate of heat production counteracts the cooling effects of the chilled blood; and (b) Probably less important, there is heat transfer by conduction from adjacent organs. The assumption in our model that oxygen deprivation has effected arrest of metabolism is not strictly true, and no attempt has been made to correct for the heat transfer between adjacent organs. Thus, our model almost surely overestimates the rate at which the vital organs of mass M_s are cooled.

A more serious source of potential error is that mentioned in the last paragraph of Section II. I believe that the partitioned model should predict quite well if the proper values of the flow distribution during perfusion can be ascertained.

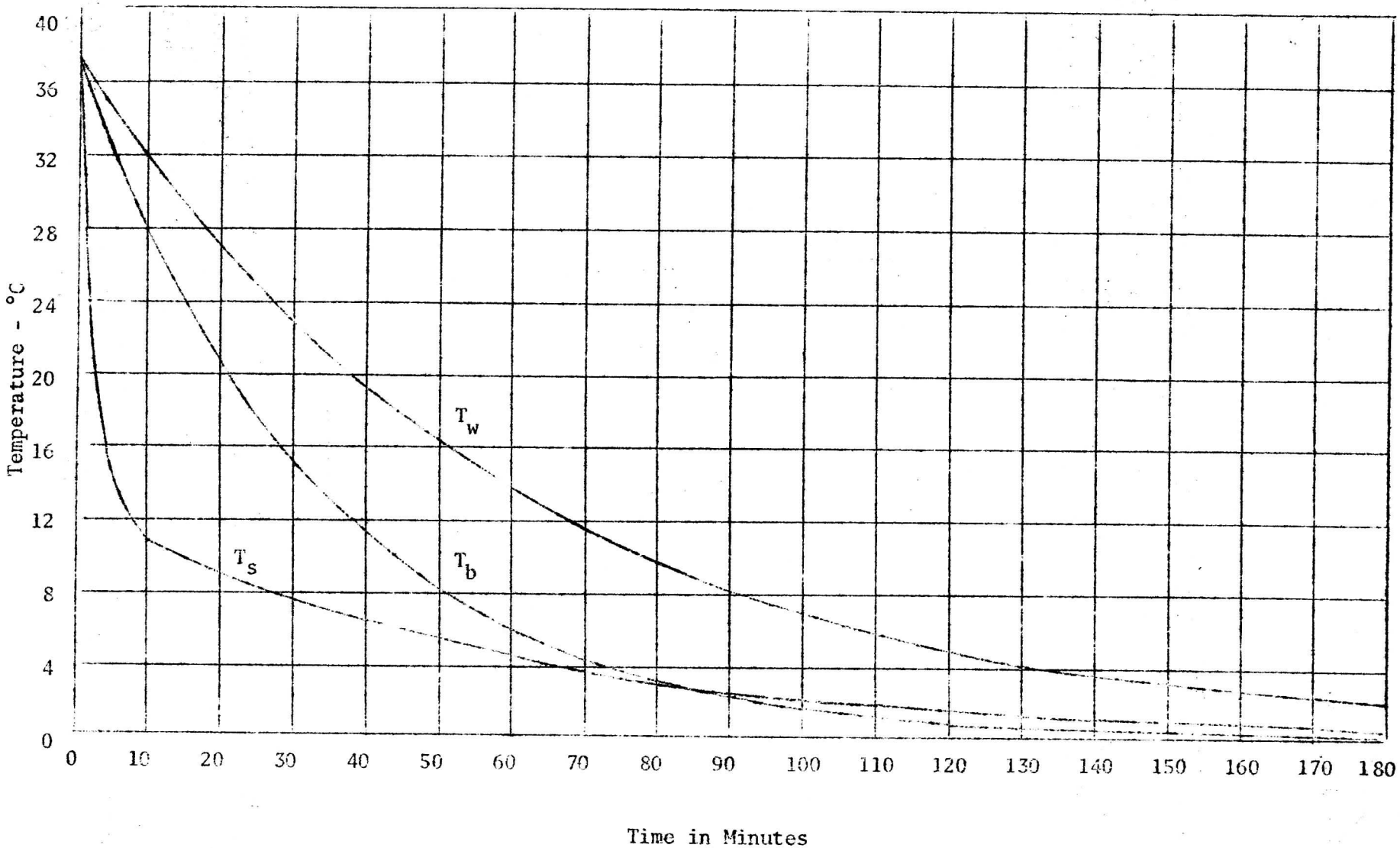

Figure 4

Predicted cooling curves for a 63 kg. man, with perfusion flow of 4 liters/minute and heat exchanger efficiency H = .4. T_s and T_w are from the partitioned body model, while T_b is from the uniform body approximation. Due to the limitations mentioned in the text, the *actual* rates of cooling of T_s and T_w probably lie closer to T_b than do the predicted curves.

In clinical hypothermia, the heat exchange unit is normally placed in series (as we have done), since placing it in parallel would require a larger priming volume for the external circuitry, and it is undesirable to have to dilute the blood to this extent. In Phase I perfusion, however, we are not faced with this limitation. A more desirable placement of the heat exchanger [similar to that employed by Manrise Corporation in its prototype perfusion machine; see reference (15), Section 59.0] is contained in Figure 5:

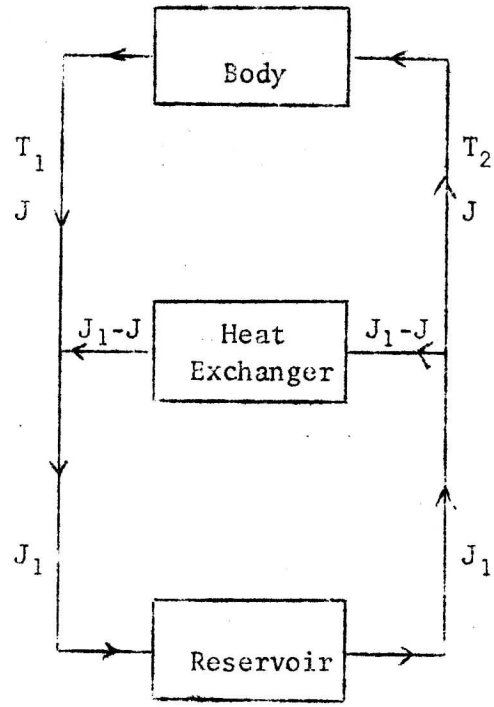

Figure 5

With this circuitry, effluent is mixed with chilled perfusate in the reservoir before returning to the body. While the flow rate J is limited by the physiological pressures tolerable within the vascular system, the flow rate J_1 can be raised to much higher levels. This permits much more rapid removal of heat from the system.

All of the equations developed in Section V apply equally well to this case; we need only choose the appropriate value of H which makes equation [7] valid. With high enough flow rates J_1, we should be able to achieve $T_2 \simeq T_h$, i.e., $H \simeq 1$.

In this section, we have assumed that all Phase I cooling takes place in closed circuit mode. However, in practice it will first be necessary to operate in open circuit, at least until all of the blood has been flushed out. This will require perhaps 20 liters of perfusate, or about 5 minutes of open circuit flow. This will not materially affect the time estimates given above for total Phase I perfusion. But in virtue of the very rapid cooling it is possible to give the vital organs, and most importantly the brain, it is highly desirable that this initial perfusate be pre-chilled.

Section VII. Cryoprotection.

We next treat the problem of building up the concentration of DMSO in the body water to the desired terminal level. We consider a recirculating system as in Figure 6:

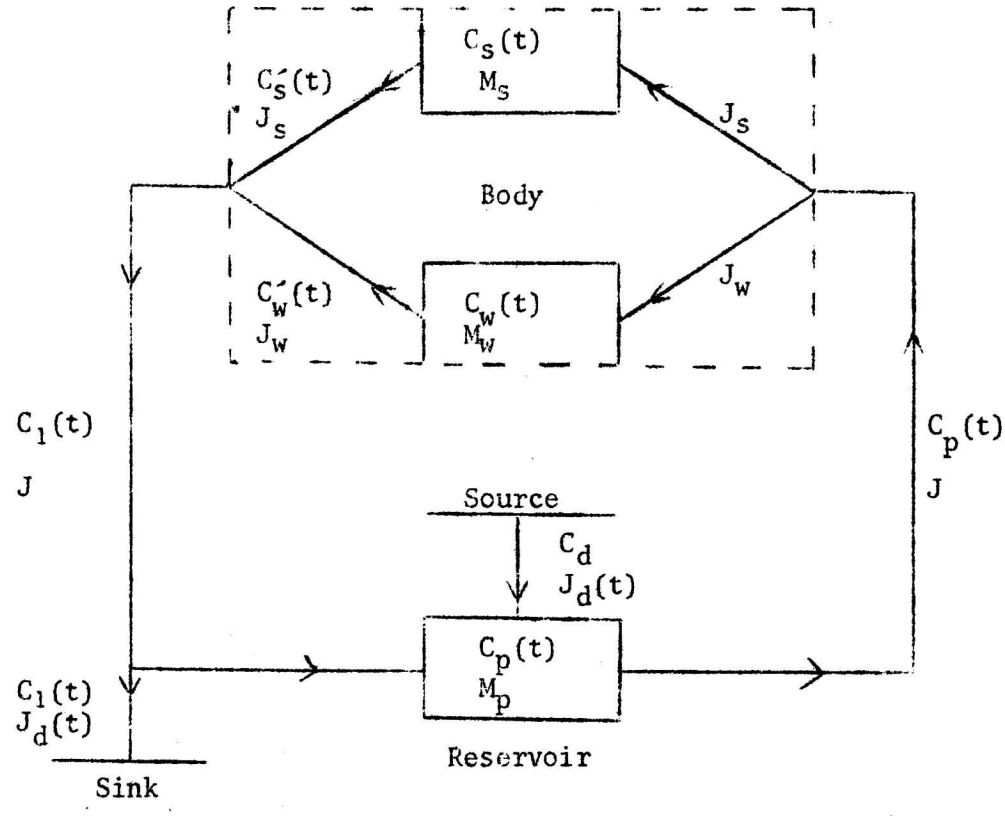

Figure 6

The circuitry is quite similar to that used in Section V for the removal of heat. The functions C_s', C_w', C_1 and C_p represent DMSO concentration in kg/kg perfusate. We have shown in Section III that significant gradients will exist within body tissue; thus the functions C_s and C_w are *average* concentrations in kg/kg liquid phase. The lower box is an external reservoir. In order to continually increase the concentration of DMSO in the perfusate, we have provided for a source of perfusate at a (higher) concentration C_d to flow into the reservoir at a rate $J_d(t) \leq J$, while perfusate of lower concentration C_1 is drained off at the same rate. The source C_d must be prepared so as to contain the same concentration of salts as in the base perfusate.

We could also consider continuously adding perfusate to the (much larger) reservoir without draining off old solution. At first sight this might appear more efficient, since no DMSO is sent down the drain. However, Appendix 2 contains a proof that this second method requires more DMSO than the method we employ above.

We assume that the reservoir is kept well stirred, so that DMSO concentration is uniform throughout. As a consequence, the concentration of the perfusate leaving the reservoir will be exactly the (average) value in the reservoir, namely C_p.

We let

W_s = mass of the liquid phase (water) within M_s

W_w = mass of the liquid phase (water) within M_w

W_p = M_p (for symmetry)

W_b = $W_s + W_w$

W = $W_s + W_w + W_p$ (the total mass of liquid involved).

We again let

[36] $$C_{so} = C_s(0) \quad ; \quad C_{wo} = C_w(0) \quad ; \quad C_{po} = C_p(0)$$

In the case of greatest interest we will have $C_{so} = C_{wo} = 0$, but we also allow for the case where a prior perfusion has built these up to non-zero values.

In accordance with the model developed in Section IV, we assume the existence of constants K_s and K_w such that:

[37] $$C_s' - C_p = K_s(C_s - C_p) \qquad 0 < K_s < 1$$

[38] $C_w' - C_p = K_w(C_w - C_p) \qquad 0 < K_w < 1$

and we define

[39] $K = j_s K_s + j_w K_w$

Now the concentration C_1 is given by:

[40] $C_1 = j_s C_s' + j_w C_w'$

By using the three linear relations [37, 38, 40], the six (time dependent) concentration functions appearing in Figure 6 can be reduced to the three independent functions C_s, C_w, and C_p.

Since the total mass of DMSO is conserved, it is easy to see that the differential equations governing these functions are:

[41] $$W_s \frac{dC_s}{dt} = J_s \rho_p (C_p - C_s')$$

[42] $$W_w \frac{dC_w}{dt} = J_w \rho_p (C_p - C_w')$$

[43] $$W_p \frac{dC_p}{dt} = \rho_p [J_d C_d + (J - J_d) C_1 - J C_p]$$

(In this Section, as in Section V, we ignore changes in the density of the perfusate as it passes through the body).

Now from equations [37, 38, 40], we have:

[44] $C_1 = C_p + j_s K_s (C_s - C_p) + j_w K_w (C_w - C_p)$

Substituting [37, 38, 44] into [41, 42, 43], we get:

[45] $$\frac{dC_s}{dt} = \frac{J_s K_s \rho_p}{W_s} (C_p - C_s)$$

[46] $$\frac{dC_w}{dt} = \frac{J_w K_w \rho_p}{W_w} (C_p - C_w)$$

[47] $$\frac{dC_p}{dt} = \frac{\rho_p}{W_p} \{J_d(C_d - C_p) + (J - J_d)[j_s K_s (C_s - C_p) + j_w K_w (C_w - C_p)]\}$$

Together with the initial conditions [36] and the known function $J_d(t)$ (depending upon the perfusion method; see below), these three equations completely determine the three concentration functions $C_s(t)$, $C_w(t)$, and $C_p(t)$.

At this point, we will consider three different methods of introducing cryoprotectant into the body, which correspond to three different choices of $J_d(t)$.

Method A. No Additional DMSO Added to the Reservoir.

We assume that $J_d \equiv 0$; i.e., that the perfusate originally placed in the reservoir is allowed to recirculate until some desired level of equilibration is achieved. It has previously been suggested that the whole perfusion process be carried out by iterating this method in discrete steps, each step requiring the replacement of the (equilibrated) perfusate in the reservoir with a new perfusate of higher concentration.

We first let

$$C_f = \frac{1}{W} (W_s C_{so} + W_w C_{wo} + W_p C_{po})$$

It should be clear that C_s, C_w, and C_p will all converge to the final value C_f.

Now from [45, 46, 47] with $J_d = 0$, we get:

[48] $$W_s \frac{dC_s}{dt} + W_w \frac{dC_w}{dt} + W_p \frac{dC_p}{dt} = 0$$

[49] $$W_s C_s(t) + W_w C_w(t) + W_p C_p(t) = \text{const.}$$

By setting $t = 0$, we find that const. $= WC_f$. Thus,

[50] $$C_p(t) = \frac{1}{W_p}\left[WC_f - W_sC_s(t) - W_wC_w(t)\right]$$

The above equation simply expresses the fact that the total mass of DMSO present is conserved. Substituting [50] into [45] and [46] yields:

[51] $$\frac{dC_s}{dt} = \frac{J_sK_s\rho_p}{W_sW_p}\left[-(W_s + W_p)C_s - W_wC_w + WC_f\right]$$

[52] $$\frac{dC_w}{dt} = \frac{J_wK_w\rho_p}{W_wW_p}\left[-W_sC_s - (W_w + W_p)C_w + WC_f\right]$$

which are of the form:

[53] $$\frac{dC_s}{dt} = d_1C_s + e_1C_w - (d_1 + e_1)C_f$$

[54] $$\frac{dC_w}{dt} = d_2C_s + e_2C_w - (d_2 + e_2)C_f$$

These equations are of exactly the same form as [16] and [17], which were previously solved. The solutions are thus:

[55] $$C_s(t) = D_1\exp(\lambda_1 t) + D_2\exp(\lambda_2 t) + C_f$$

[56] $$C_w(t) = E_1\exp(\lambda_1 t) + E_2\exp(\lambda_2 t) + C_f$$

Where the λ's, D's, and E's are determined from the d's and e's in exactly the same manner as in solving [16] and [17].

Since the author has no estimate as to the values of K_s and K_w, and since I will later recommend a better perfusion method, no graphs or numerical estimates of approach to equilibrium are provided.

Method B. Constant Perfusate Concentration.

We assume that J_d is regulated in such a way that C_p is maintained at the constant value C_{po}. Then [45] and [46] become:

$$[57] \qquad \frac{dC_s}{dt} = \frac{J_s K_s \rho_p}{W_s} (C_{po} - C_s)$$

$$[58] \qquad \frac{dC_w}{dt} = \frac{J_w K_w \rho_p}{W_w} (C_{po} - C_w)$$

These equations are easily solved:

$$[59] \qquad C_s(t) = C_{po} + (C_{so} - C_{po}) \exp\left(\frac{-J_s K_s \rho_p}{W_s} t\right)$$

$$[60] \qquad C_w(t) + C_{po} + (C_{wo} - C_{po}) \exp\left(\frac{-J_w K_w \rho_p}{W_w} t\right)$$

Equation [43] becomes:

$$[61] \qquad 0 = \rho_p [J_d C_d + (J - J_d) C_1 - J C_{po}]$$

so that

$$[62] \qquad J_d = J \frac{C_{po} - C_1}{C_d - C_1}$$

Using [44, 59, and 60] one obtains an explicit expression for $J_d(t)$. However, unless very sophisticated control mechanisms were available, it is unlikely that the resulting equation would ever be used to regulate $J_d(t)$. In practice, it would be much simpler to continuously monitor the concentration C_p in the reservoir, and add and drain in such a way as to keep it (nearly) constant.

Method C. Constant Gradient Across the Body.

We increase J_d in such a way as to maintain $C_p - C_l$ at a constant value. Before solving the equations, it will be helpful to first introduce some notation. We let

[63] $w_s = W_s/W_b \quad ; \quad w_w = W_w/W_b$

[64] $k_s = j_s K_s/K \quad ; \quad k_w = j_w K_w/K$

[65] $C = k_s(C_{po} - C_{so}) + k_w(C_{po} - C_{wo})$

[66] $C_{bo} = w_s C_{so} + w_w C_{wo}$

[67] $L = \dfrac{JK\rho_p}{W_b}$

Now our assumption is that $C_p - C_l$ = const. From [44], we have:

[68] $C_p - C_l = KC$

and again using [44] , we get:

[69] $C_p = K_s C_s + k_w C_w + C$

Substituting [69] into [45] and [46] , we have:

[70] $$\frac{dC_s}{dt} = \frac{k_s}{w_s} L\,[(k_s - 1)C_s + k_w C_w + C]$$

$$= \frac{k_s k_w}{w_s} L\,(-C_s + C_w + C/k_w)$$

$$[71] \qquad \frac{dC_w}{dt} = \frac{k_s k_w}{w_w} L (C_s - C_w + C/k_s)$$

We again solve these equations by the method of the Laplace transform. If we let

$$[72] \qquad \lambda = \frac{k_s k_w}{w_s w_w} L$$

$$[73] \qquad A_0 = C_{bo} + \frac{w_w(k_s - w_s)}{k_s k_w} C$$

$$[74] \qquad A_1 = LC$$

$$[75] \qquad A_2 = C_{so} - A_0$$

$$[76] \qquad B_0 = C_{bo} + \frac{w_s(k_w - w_w)}{k_s k_w} C$$

$$[77] \qquad B_1 = LC$$

$$[78] \qquad B_2 = C_{wo} - B_0$$

Then the solutions to [70, 71] are:

$$[79] \qquad C_s(t) = A_0 + A_1 t + A_2 \exp(-\lambda t)$$

$$[80] \qquad C_w(t) = B_0 + B_1 t + B_2 \exp(-\lambda t)$$

Let us consider the case of greatest interest, where $C_{so} = C_{wo} = 0$. Then we have $C_{bo} = 0$, and $C = C_{po}$. Substituting the A's and B's into [79] and [80] yields:

$$[81] \qquad C_s(t) = C_{po}\left\{\frac{JK\rho_p}{W_b}t + \frac{w_w(k_s - w_s)}{k_s k_w}\left[1 - \exp(-\lambda t)\right]\right\}$$

$$[82] \qquad C_w(t) = C_{po}\left\{\frac{JK\rho_p}{W_b}t + \frac{w_s(k_w - w_w)}{k_s k_w}\left[1 - \exp(-\lambda t)\right]\right\}$$

If we substitute [81, 82] into [69] , we get:

$$[83] \qquad C_p(t) = C_{po}\left\{1 + \frac{JK\rho_p}{W_b}t + \frac{(k_s - w_s)^2}{k_s k_w}\left[1 - \exp(-\lambda t)\right]\right\}$$

Note that [81, 82, 83] all contain the transient term in $\exp(-\lambda t)$. Once this term has vanished, C_s, C_w, and C_p all increase linearly with t, and at the same rate.

For the sake of reference, we will again solve the equations in the uniform body approximation. In this case we have $w_s = j_s$, $K_s = K_w = K$, so that $k_s = w_s$. Thus, the solution is:

$$[84] \qquad C_b(t) = C_{po}\frac{JK\rho_p}{W_b}t$$

$$[85] \qquad C_p(t) = C_{po}\left(1 + \frac{JK\rho_p}{W_b}t\right)$$

Returning to the partitioned body case, the flow J_d necessary to maintain the constant gradient across the body is obtained from [47]:

$$[86] \qquad \frac{dC_p}{dt} = \frac{\rho_p}{W_p}\left[J_d(C_d - C_p) + (J - J_d)(-KC_{po})\right]$$

$$[87] \qquad J_d = \frac{\frac{W_p}{\rho_p}\frac{dC_p}{dt} + JKC_{po}}{C_d - C_p + KC_{po}}$$

An explicit expression for $J_d(t)$ is obtained by substituting for C_p from equation [83].

This perfusion method cannot be continued indefinitely, since J_d cannot exceed J. Note that by the time this limit is approached, the transient term in [83] will have essentially vanished, and so we will have $dC_p/dt \approx JK\rho_p C_{po}/W_b$. Thus, from [87], this constraint is:

$$[88] \qquad J \geq \frac{(\frac{W_p}{W_b} + 1)\, JKC_{po}}{C_d - C_p + KC_{po}}$$

$$[89] \qquad C_p \leq C_d - \frac{W_p}{W_b} KC_{po}$$

Of the three perfusion methods treated in this section, Method C seems clearly the best. It builds up the concentration of DMSO in the body water as rapidly as possible, subject to the constraint that we never exceed a specified gradient across the body. This constraint is approximately the same as specifying a maximum allowable DMSO gradient *within* the body. I recommend carrying out the entire perfusion this way, at least until C_p reaches the desired terminal concentration C_f, at which time Method B should be adopted.

Further results concerning this method will be developed in Section IX.

Section VIII. Freezing Point of DMSO-Water Solutions

References (16) and (17) both give graphs of the freezing point of DMSO-water solutions as a function of weight concentration of DMSO. These graphs are not entirely in agreement; in the range C = .4 to C = .5, the graph in (17) lies about 4°C higher than that in (16). It is not clear how this difference should be solved, since:

A. The graph in (16) is readable to an accuracy of about 1/2 degree, but the accuracy of the data is not stated; and

B. Although the text of (17) indicates that their measurements are accurate to within 1°C, the graph itself is only readable to within about 3°C.

In Figure 7 we have given a composite of these two graphs, which is based primarily upon the data given in (16).

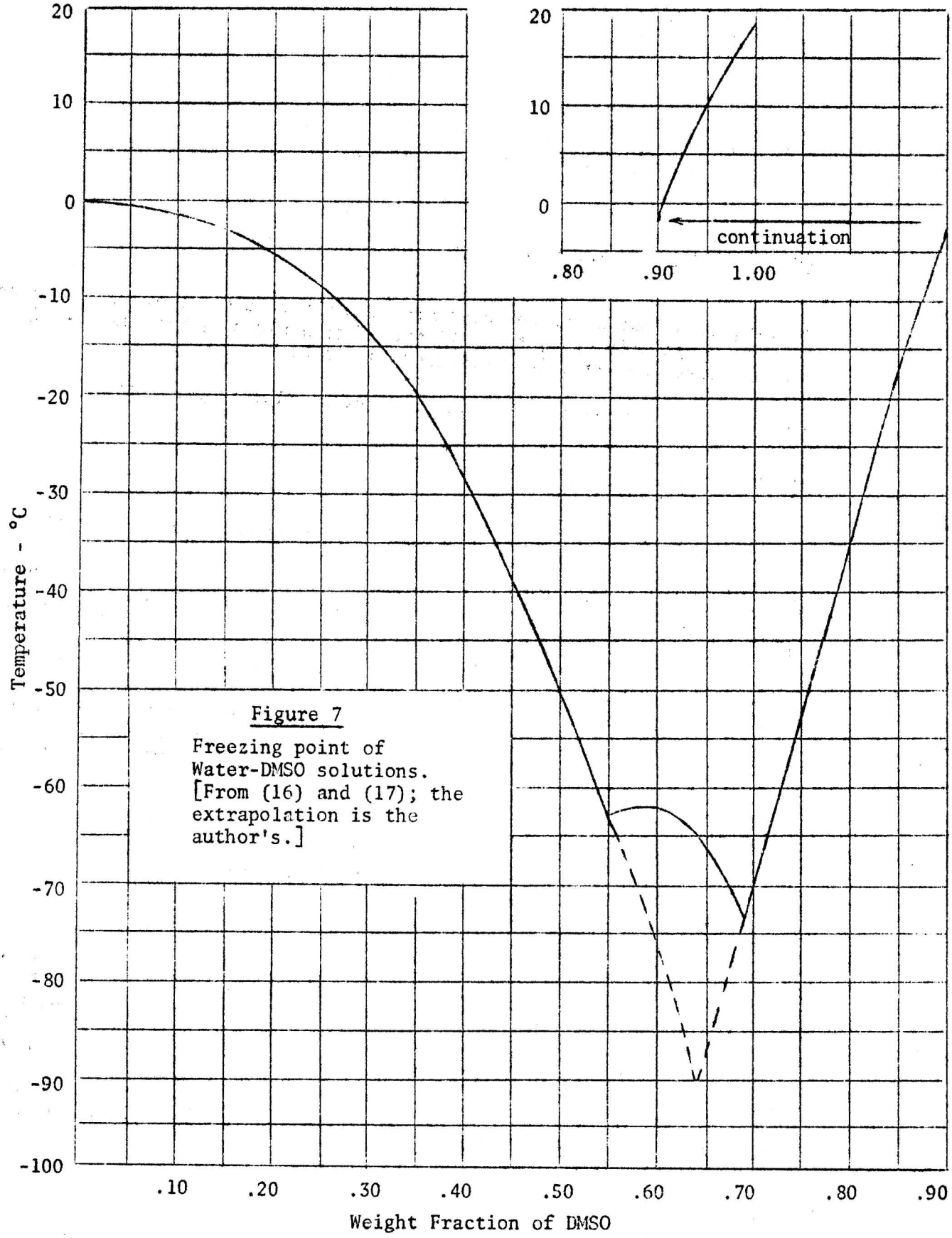

Figure 7

Freezing point of Water-DMSO solutions. [From (16) and (17); the extrapolation is the author's.]

The author has carried out a multiple regression on the computer to determine the best polynomial fit to Figure 7 between C = 0 and C = .55. The best fit is given by:

[90] $T_{fr} \simeq -.4 + 12.8\ C - 162.3\ C^2 - 123.0\ C^3$

Passing to a fourth degree polynomial did not reduce the error sum of squares. With this approximation, the estimate of standard deviation is $\hat{\sigma} = .81$, and the maximum observed error in prediction is 1.1°C.

Almost as good a fit is obtained with the simpler polynomial:

[91] $T_{fr} \simeq 26.1\ C - 252.1\ C^2$

In this case, $\hat{\sigma} = .97$, and the maximum observed error in prediction is 1.6°C.

Reference (17) states that the formation of a DMSO trihydrate, which occurs at C = .59, prevents reaching temperatures lower than -63°C in liquid phase (approaching this concentration from the side C = 0). But the authors mention the possibility that the presence of salts in the solution (as we will have) may prevent the formation of this trihydrate, eliminating the trihydrate eutectic crystallization that otherwise occurs at C = .55. If this is true, then the lowest temperature achievable in liquid phase is obtained by extrapolating the outer curves to their intersection. (17) gives this temperature at between -105°C and -115°C, but this author's extrapolation (shown in Figure 7) gives this temperature at about -90°C, obtained when C ≈ .64.

Section IX. Phase II Perfusion.

The approach to Phase II perfusion that we will develop is based upon recommendations originally set forth by Gouras (18), which in turn were based primarily upon the work of Farrant [(19); see also (9)]. In this approach, we attempt to progressively increase the concentration of DMSO in the body cells as temperature is lowered, in such a way as to prevent any freezing of cell water until the lowest temperature possible. This method completely avoids one of the major sources of damage that normally occurs in the freezing of tissue, the toxic effects of the high concentrations of salts that result when cell water freezes out.

A major problem in attempting to utilize this approach is to estimate the actual concentration of DMSO within the body cells as perfusion is carried out; for this purpose, we will utilize the theory developed in Section VII. The temperature dependence of the constants involved, and the unknown values of K_s and K_w, will require us to make a number of compromises in order to obtain numerical results. These approximations will be detailed as they arise.

In order to achieve the simultaneous removal of heat from the body and buildup of cryoprotectant, we unite the circuitry of Figure 5 with that of Figure 6, resulting in the circuitry of Figure 8:

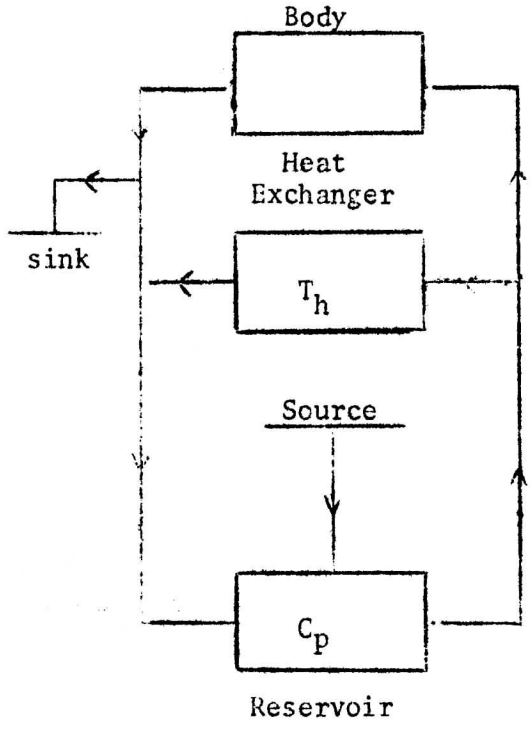

Figure 8

In light of the possibility mentioned at the end of Section VIII, we will recommend achieving the final concentration $C_f = .64$ of DMSO in the body water. Thus, we assume that the recommended Method C of Section VII is employed until the perfusate concentration C_p reaches this value C_f. At this time, Method B is adopted until the body concentration of DMSO reaches a value sufficiently close to C_f. In order to carry out this approach, it is necessary to select values for the two parameters C_{po} and C_d. It will be useful to first develop further consequences from the theory of Section VII before considering the factors that govern the choice of values for these parameters.

Part A. Relation Between C_p, C_s, and C_w.

We can obtain numerical estimates of the rate of buildup of C_s and C_w as a function of C_p if we make the simplifying assumption that $K_s = K_w$, so that $k_s = j_s$ and $k_w = j_w$. We use [81, 82, 83] and consider times large enough that transient terms in $\exp(-\lambda t)$ becomes negligible. Thus we have:

$$[92] \qquad C_s(t) \simeq C_{po} \left[\frac{JK\rho_p}{W_b} t + \frac{w_w(j_s - w_s)}{j_s j_w}\right]$$

$$[93] \qquad C_w(t) \simeq C_{po} \left[\frac{JK\rho_p}{W_b} t + \frac{w_s(j_w - w_w)}{j_s j_w}\right]$$

$$[94] \qquad C_p(t) \simeq C_{po} \left[1 + \frac{JK\rho_p}{W_b} t + \frac{(j_s - w_s)^2}{j_s j_w}\right]$$

from which we see that:

$$[95] \qquad C_s(t) \simeq C_p(t) - \frac{w_s}{j_s} C_{po} \qquad \text{for large } t$$

$$[96] \qquad C_w(t) \simeq C_p(t) - \frac{w_w}{j_w} C_{po} \qquad \text{for large } t$$

From data given in (1, pg. 302), we have that $W_s \simeq .73\ M_s$, $W_w \simeq .62\ M_w$, and $W_b \simeq .63\ M_b$. Thus, using our 63 kg. reference man of Table 1, we have:

$$[97] \qquad w_s \simeq .08 \ ; \qquad w_w \simeq .92$$

$$[98] \qquad j_s \simeq .70 \ ; \qquad j_w \quad .30$$

and so we have:

$$[99] \qquad C_s \simeq C_p - .1\ C_{po} \qquad \text{for large } t$$

$$[100] \qquad C_w \simeq C_p - 3.0\ C_{po} \qquad \text{for large } t$$

Thus, while the concentration C_s keeps right up with C_p, the concentration C_w eventually falls 3 C_{po} behind.

It should be pointed out that the eventual lag (C_p - C_w) which develops gives a measure of the maximum DMSO gradient that is encountered in the body during perfusion. Compare equation [100] with the uniform body model of equations [84, 85] in which (C_p - C_b) maintains the constant value C_{po}. We see that, with the same choice of C_{po}, three times larger gradients are encountered in the partitioned body model as in the uniform body model.

Part B. Length of Time and Quantity of DMSO Required.

An estimate of the length of time required to reach $C_p = C_f$ can be obtained from equation [94]:

$$[101] \qquad t_f = \frac{W_b}{JK\rho_p}\left[\frac{C_f}{C_{po}} - 1 - \frac{(j_s - w_s)^2}{j_s j_w}\right]$$

Note that this formula is based upon the unrealistic assumption that the product JK remains constant throughout Phase II perfusion. For this reason, no numerical estimate for the value of t_f will be provided. An estimate can be obtained if the average value of JK over this temperature range can be ascertained. The formula does serve the purpose of showing that the total perfusion time required is essentially inversely proportional to the value chosen for C_{po}.

The total quantity of DMSO required to carry out the Method C part of Phase II perfusion depends upon the choice of C_{po} and C_d, and is given by:

$$[102] \qquad Q(C_{po}, C_d) = C_{po}W_p + \int_0^{t_f} C_d\rho_p J_d(t)\,dt$$

where $J_d(t)$ is obtained from [87] and [86]. The integral that results from this substitution cannot be evaluated in closed form. We can, however, evaluate this integral if we use the uniform body approximation [85] to determine $C_p(t)$ in [87]. In this case,

$$[103] \qquad Q(C_{po},C_d) = C_{po}W_p + C_d \int_0^{t_f} \frac{\rho_p \left(\frac{W_p}{W_b} JK + JK\right) dt}{C_d - C_{po}\left(1 + \frac{JK\rho_p}{W_b} t\right) + KC_{po}}$$

$$Q = C_{po}W_p - C_d(W_p + W_b) \log\left[C_d - C_{po}\left(1 + \frac{JK\rho_p}{W_b} t\right) + KC_{po}\right]\Bigg|_0^{t_f}$$

$$[104] \qquad Q(C_{po},C_d) = C_{po}W_p + C_d(W_p + W_b) \log\left(\frac{C_d - C_{po} + KC_{po}}{C_d - C_f + KC_{po}}\right)$$

where above, 'log' is the *natural* logarithm.

Note that this expression is independent of J, and so is not affected by the fact that J decreases at low temperature.

As a function of C_{po}, this expression assumes its minimum value when $C_{po} \longrightarrow 0$, as can be verified by the usual method of solving the equation $\partial Q/\partial C_{po} = 0$. In this case, we have:

$$[105] \qquad Q(0+,C_d) = C_d(W_p + W_b) \log\left(\frac{C_d}{C_d - C_f}\right)$$

Here, the "0+" is to indicate that the quantity Q is the limiting value as C_{po} approaches zero from the plus side. (Of course, no perfusion of DMSO at all takes place when we actually have $C_{po} = 0$).

It can be seen from equations [95] and [96] that the uniform body approximation becomes exact when $C_{po} \longrightarrow 0$. Also, in this case, the whole of Phase II perfusion can be carried out by Method C; since $C_b \approx C_p$, there is no need for (and no further DMSO required from) Method B.

Part C. Choice of C_{po}

We now have enough information to weigh the factors involved in choosing a value for C_{po}. There are at least two advantages to choosing a *small* value for this parameter:

i. As shown in Part B, the total quantity of DMSO required is minimized.

ii. DMSO gradients within the body are minimized, reducing osmotic stresses. In this connection, see the remarks at the end of Part A of this section. If the partitioned body model proved to be the case, one would choose a value of C_{po} only 1/3 as large as one would select in the uniform body model.

The only disadvantage to such a choice is the length of time required to complete the perfusion [equation 101], which exposes the body to toxic concentrations of DMSO for undesirable periods of time.

On the basis of these considerations, it appears to the author that a reasonable compromise choice is $C_{po} = .05$. This value will be assumed throughout the rest of the paper.

Part D. Choice of C_d

A major consideration in choosing C_d is to minimize the total quantity of DMSO required, as expressed in equation [105]. By again setting $dQ(0+,C_d)/dC_d = 0$, we find that $Q(0+,C_d)$ assumes its minimum value when $C_d = 1$; i.e., when the source is pure DMSO. In this case, again using the 63 kg. reference man, we can obtain a numerical estimate of the quantity of DMSO required. From [105], we have:

$$[106] \qquad Q(0+,1) = 1\ (5 + 40)\ \log\left(\frac{1}{1 - .64}\right)$$

$$= 46 \text{ kg. DMSO}$$

On the other hand, there are at least two reasons for choosing a value for C_d less than 1:

i. DMSO has considerable heat of solution; Reference (15) gives this value as 60 cal/gm. Prediluting the DMSO with the balanced salt solution employed would allow much of this heat to be evolved prior to use, rather than placing the burden for its removal on the heat exchanger.

ii. Pure DMSO freezes at 18°C, and thus cannot be prechilled significantly. But if, for example, we chose $C_d = .8$, the solution can be chilled to as low as -34°C without freezing.

With this choice of C_d, we have:

[107] $Q(0+,.8) = 58$ kg. DMSO

which entails roughly a 25% increase in the quantity of DMSO required. This does not seem prohibitive, and so we will recommend $C_d = .8$ as the source concentration.

It must be pointed out that the end value obtained in [107] is based upon assumed values for a number of parameters (in particular, body weight), and upon several approximating assumptions which tended to underestimate the actual amount of DMSO required. The author recommends that in preparing for an anticipated perfusion, at least 100 kg. of DMSO be kept on hand -- especially if the prospective patient is of above average body size.

The quantity of DMSO required can be greatly reduced if an efficient method for extracting DMSO from the effluent can be developed. In this case, no DMSO need be sent down the drain -- after extraction, the DMSO is simply added to the source C_d.

F. R. Chamberlain reports to me that a relatively simple method to accomplish this extraction can be developed. His proposed scheme will be the subject of a later MTR article. If such a method were perfectly efficient, then the theoretical minimum value of the quantity of DMSO required could be approached:

[108] $Q_{min} = (W_p + W_w)\, C_f = 29$ kg. DMSO

where we have used the same values for the parameters as were employed above.

Part E. Removal of Body Heat Versus Buildup of Cryoprotectant.

The choice of Method C for introducing cryoprotectant into the body still leaves open the question of the best temperature profile for removing heat from the body. In order to profitably discuss this question, we will employ the uniform body approximations appearing in equations [32] and [84], since this will allow

us to cancel a number of temperature dependent quantities against each other. The results obtained below would be essentially the same if we treated the two parts M_s and M_w of the body separately. For further simplification, we use [91] rather than [90] to describe the DMSO-water freezing curve. We also assume that the heat exchanger is cooled by a liquid at dry ice temperature, so that $T_h = -79°C$.

To complete the description of the Phase II perfusion procedure to be employed requires specifying a function $T_b(C_b)$, which gives the desired body temperature to be obtained as a function of the body concentration of cryoprotectant. Ideally, this dependence is chosen in such a way as to minimize the total damage done to the body due to exposure to toxic concentrations of DMSO, decay and other harmful reactions that continue to take place in the body, and other deleterious effects. The determination of the optimal function is a problem to be solved by the methods of the Calculus of Variations. Since the complexity of assessing the damage done to the body, and the temperature dependence of the constants involved, both make this a rather difficult problem, the author intends to defer an attempted solution as the subject for a later article. For the present, we will assume that the optimal approach is to make T_b hug the freezing curve T_{fr} as closely as possible. This solution would surely be optimal if the product JK, which determines the rate of buildup of cryoprotectant in the body, remained constant all the way down to -63°C. [See also (9), which proposes using this presumed solution in experimental organ preservation.]

Thus, the focus of this Part will be solely to demonstrate that $T_b(C_b)$ *can* hug the freezing curve. I.e., using Method C to build up cryoprotectant in the body, the heat exchanger will still be able to remove body heat rapidly enough as to maintain $T_b \approx T_{fr}$.

We first note that from [91],

$$[109] \qquad \frac{dT_{fr}}{dC_b} \approx 26.1 - 504.2\, C_b$$

But on the other hand, the maximum rate at which we can reduce body temperature is obtained from [31] and [84]:

$$\frac{dT_b}{dC_b} = \frac{dT_b}{dt} \Big/ \frac{dC_b}{dt}$$

$$= \frac{h_p W_b H}{h_b M_b K C_{po}} (T_h - T_b)$$

So we see that as C_b increases and T_b decreases, dT_{fr}/dC_b becomes more and more negative, while dT_b/dC_b becomes less and less so. Thus, it will be most difficult for dT_b/dC_b to keep up with dT_{fr}/dC_b as the terminal concentration C_f is approached. Now the lowest temperature we will attempt to achieve is $T_b = -62°C$, when $C_b = .55$. [Reference (17) gives $T_{fr}(.55) = -63° \pm 1°C$]. At this point we have:

$$[111] \qquad \frac{dT_{fr}}{dC_b} = -252$$

At the very low flow rates that will prevail at this temperature, the heat exchanger will become nearly perfectly efficient, and we will have $H \approx 1$. The worst case assumption we can make (for the thesis we are trying to establish) is that $K = 1$; its value will certainly be much less. Using these values in [110], we get:

$$[112] \qquad \frac{dT_b}{dC_b} < \frac{1}{.83} .63 \frac{1}{1} \frac{1}{.05} [-79 - (-62)] = -258$$

This value exceeds (negatively) the value in [111], which establishes our contention.

Part F. Procedure for Phase II Perfusion.

In this part, we describe in greater detail the step by step procedure to be used in carrying out the recommendations of Part E. We will address some of the practical questions of measurement and control, and provide methods for handling the expected deviations from our theoretical predictions resulting from the approximations and idealizations of the models we have employed.

In order to give a numerical discussion of Phase II perfusion, we will now postulate values for several more parameters. We assume that at 0°C it is possible to maintain a flow rate of $J = 2$ liters/minute, and that the volume of the external reservoir is $W_p = 5$ liters. Note also that for our 63 kg. reference man, the volume of the blood in the circulatory system is about $V_c = 5$ liters (1, pg. 280). We will also assume in this part that DMSO concentration is monitored by measurement of the specific gravity of the perfusate and the effluent.

Initial Stages of the Procedure.

We begin by preparing a quantity of perfusate at concentration $C_{po} = .05$ sufficient to fill the external reservoir, and to displace all of the Phase I perfusate currently in the vascular system, amounting to a total of over 10 liters of perfusate. Five or more liters of perfusate are now allowed to flow through the body, while the flushed perfusate is sent to the drain, and then the reservoir is filled with the remaining five liters. Note that the length of time required to flush the circulatory system is approximately V_c/J, which under our assumptions is 2.5 minutes.

At this point we measure the concentration of the effluent, and thus make an initial determination of the value of K. From equation [68], we have:

$$[113] \qquad K = 1 - \frac{C_1}{.05}$$

This value of K is used to select the initial source flow rate J_d, according to equation [87]. Note that this equation contains the term dC_p/dt, for which we could substitute either the partitioned body model of [83], or the uniform body model of [85]. As emphasized repeatedly in Section VI, the actual situation at this point surely lies somewhere between these two extremes. Note that:

$$[114] \qquad \left.\frac{dC_p}{dt}\right|_{t=o} = \frac{C_{pc} J K \rho_p}{W_b}\left[\,1 + \frac{(k_s - w_s)^2}{w_s w_w}\,\right] \qquad \text{(partitioned body model)}$$

$$= .015K \qquad \text{(with assumed parameter values)}$$

$$[115] \qquad \left.\frac{dC_p}{dt}\right|_{t=o} = \frac{C_{po} J K \rho_p}{W_b} \qquad \text{(uniform body model)}$$

$$= .0025K \qquad \text{(with assumed parameter values)}$$

Thus we see that *initially* there is a large difference (a factor of 6) between this value as determined by the two different models. (This difference disappears as soon as the transient terms in the partitioned body model vanish).

Substituting into [87] and using the assumed parameter values, we have:

[116] $J_d(0) = .23K$ liters/minute (partitioned body model)

[117] $J_d(0) = .15K$ liters/minute (uniform body model)

In practice, an initial value of J_d lying somewhere between these two values should be chosen. Of course, effluent is sent down the drain at the same rate.

Further Regulation of Perfusate Concentration.

Ideally, from here on we would regulate C_p in such a way as to maintain $C_p - C_1 = .05K$. However, there are two practical considerations which mitigate against this procedure:

i. The initial determination of K made above is subject to a considerable degree of error; and

ii. K is not truly a constant. As perfusate temperature is lowered, K will *increase* as a consequence of the reduced flow rate J, but will *decrease* as a consequence of the reduced rate of DMSO diffusion within the body. The net effect is very likely to be a reduced value of K.

We emphasize again that the goal of this method is to maintain $C_p - C_1$ at a constant value, since this will result in the body concentration increasing at the same rate as C_p (or C_1). In light of the expected deviations from constancy that will result from either *i.* or *ii.* above, we need to specify a method for restoring this constancy. The method is the same in either case.

Suppose that after an interval of time, C_1 has crept closer to C_p, so that $C_p - C_1$ is smaller than at the previous measurement. One might expect that the remedy is to *increase* J_d (or equivalently, dC_p/dt) in order to restore the constant difference. However, if C_1 is approaching C_p, this indicates a *reduced* value of K, i.e., that the body is not taking up DMSO as rapidly as was previously assumed. Thus in fact the remedy is just the opposite, we *decrease* dC_p/dt, and continue to do so until $C_p - C_1$ remains constant. By the same argument, if $C_p - C_1$ becomes larger, we must *increase* dC_p/dt (i.e. J_d) until this difference remains constant.

In order to supply sample curves for all of these functions during the initial stages of perfusion, we make the additional supposition that K = .2. Figure 9 gives the concentration curves that result from the partitioned body model. Note that the effects of the transient terms in this model become negligible within about two hours. Figure 10 gives similar curves in the uniform body model. In Figure 11 we give the initial source flow rates J_d corresponding to each of these two models.

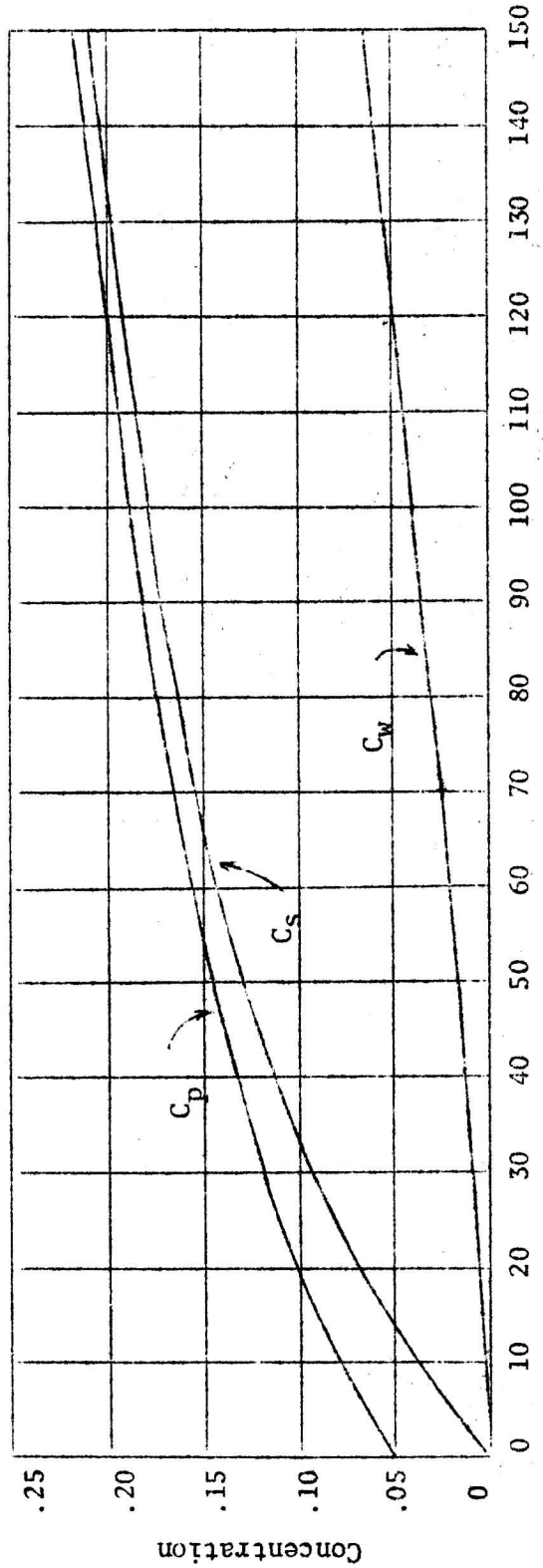

Figure 9

Sample concentration curves for the initial stages of perfusion, using the *partitioned* body model with K = .2. Other assumed parameter values are those for the 63 kg. reference man, with C_{po} = .05, C_d = .8, and flow rate J = 2 liters/minute.

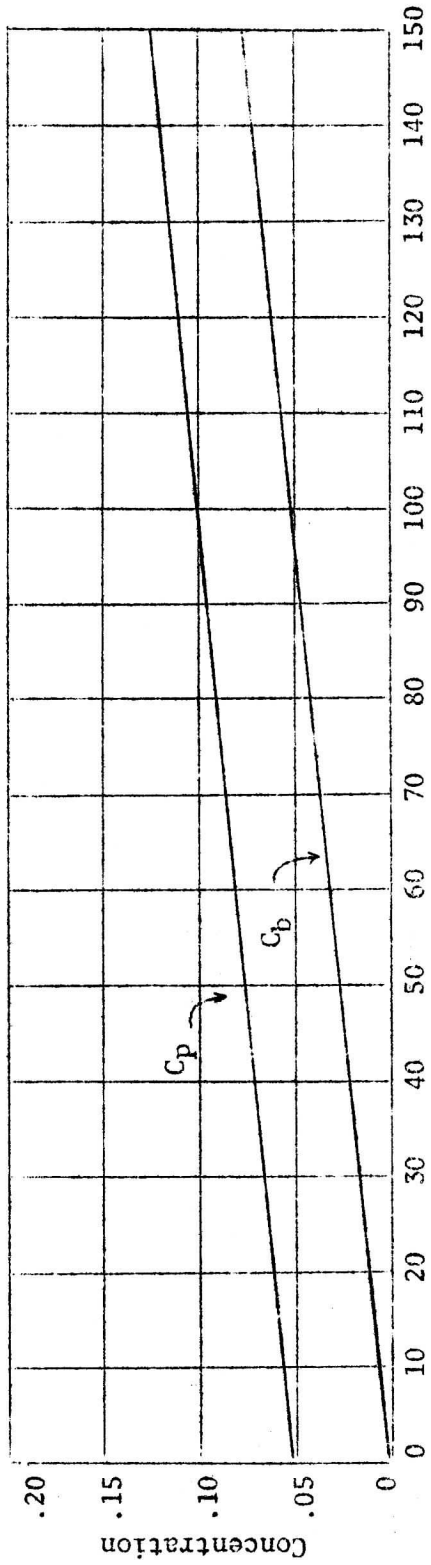

Figure 10

Sample concentration curves for the initial stages of perfusion, using the *uniform* body model with K = .2. Other assumed parameters are the same as in Figure 9.

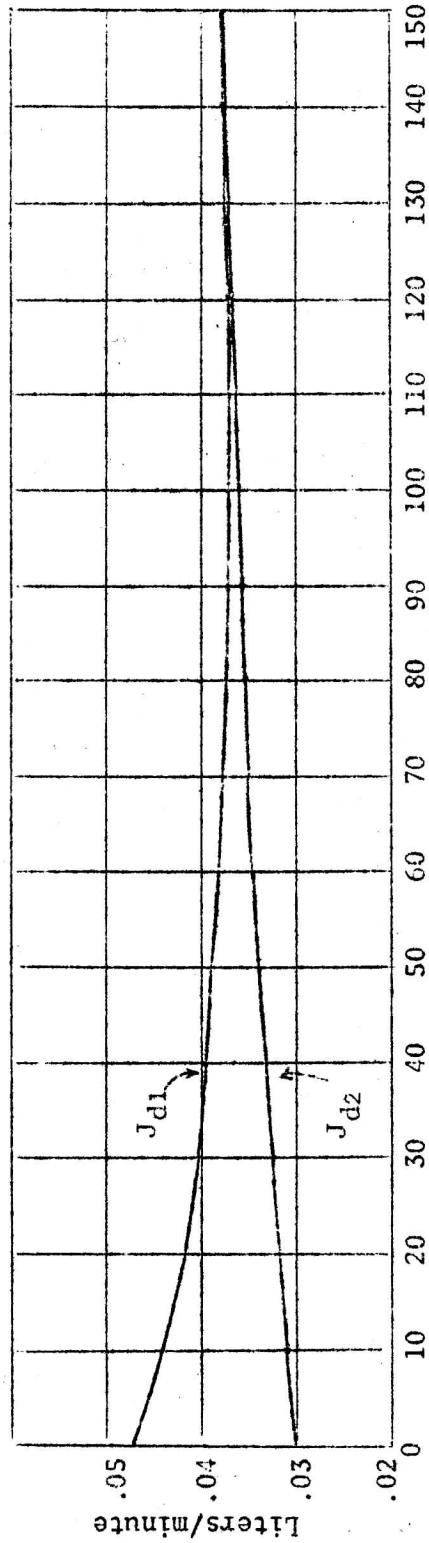

Figure 11

Sample source flow rates. J_{d1} is for the partitioned body model, while J_{d2} is for the uniform body model. Parameter values are the same as in Figures 9 and 10, with the additional assumption W_p = 5 liters for the external reservoir size.

Control of Perfusate Temperature

We will assume that it is possible to maintain adequate perfusion flow all the way down to -62°C. This assumption may well prove unrealistic, or may require perfusion to be carried on for an inordinate length of time. In this case, perfusion will have to be accomplished at higher temperatures. In any event, the procedure detailed below (as outlined in Part E) gives perfusate temperatures that one should not fall below.

If any methods for *directly* measuring the minimum body concentration of DMSO are available, then one would simply lower the body temperature in such a way as to never fall below the freezing curve given in Figure 7. Lacking such means for direct measurement, as we almost certainly will, we must rely upon the theory developed above.

Using the partitioned body model, we can use equation [100] to obtain an estimate of the minimum body concentration of DMSO as a function of the perfusate concentration. This equation, valid for large t, is very conservative in the early stages of perfusion (compare with Figure 9, where it becomes valid in about two hours). This, however, makes little difference to the recommended cooling profile, since one cannot begin to lower body temperature below 0°C until a substantial concentration of DMSO has built up in the body (e.g., one cannot achieve even -5°C until the minimum body concentration is at least .20).

But due to the adjustments in perfusate concentration required to compensate for the errors *i.* and *ii.* mentioned above, equation [100] may not continue to give a close estimate of the actual minimum DMSO concentration in the body. Fortunately, it is relatively easy to make a *direct* calculation of the body concentration of DMSO from the data that will be recorded during perfusion. For if we let U(t) be the total amount of DMSO taken up by the body up until time t, we have:

$$[118] \qquad U(t) = \int_0^t J(\rho_p C_p - \rho_1 C_1)\,dt$$

This equation, which simply expresses the conservation of the total amount of DMSO present (net body uptake is the difference between the inflow and the outflow), is valid in *any* model, regardless of changes in J, K, or any other parameter. (Compare this with equations [41,42]: The only difference is that here we have explicitly allowed for the change in perfusate concentration as it passes through the body. Since we are now assuming that specific gravity is used to monitor DMSO concentration, values of both ρ_p and ρ_1 will be readily available, and so we are making use of the slight additional precision that their small difference permits).

Since all of the quantities appearing in the integrand of [118] will be continuously monitored during perfusion, the integral can be continuously evaluated by any one of the standard numerical methods. In Figure 12, we give a sample worksheet for calculating this integral, using the simplest of numerical methods, the trapezoidal rule. The format of the calculation assumes that measurements are made at irregular time intervals. The formulas would be somewhat simpler if all observations were made at *constant* time intervals, but in actual practice they will be made much more frequently during the early stages of perfusion (when we are still fixing the true value of K) than in the later stages. Slightly greater precision in calculating this integral would be obtained by the use of Simpson's rule, with a minor increase in the complexity of the running calculation. (See any calculus textbook for a discussion of these alternate methods for evaluating definite integrals).

Possessing this information as to the total DMSO uptake by the body, we can further keep a running estimate of the minimum body concentration of DMSO, which parallels equation [100]. Regardless of whether or not the partitioned body model, as manifested in [100], continues to hold exactly, we can use it as a *worst case* assumption for the purpose of determining the minimum body concentration of DMSO. Now in this model, we see from equation [99] that C_s eventually (almost) catches up with C_p. When this occurs (after the transient terms vanish), we can easily see that the minimum concentration in the rest of the body is given by:

$$[119] \qquad C_w(t) = \frac{U(t) - W_s C_p(t)}{W_w}$$

We will use this estimate of the minimum body concentration to control the temperature T_p of the perfusate. T_p is chosen to lie just above the freezing curve given in Figure 7, with C_w used for the x-coordinate. We have previously demonstrated in Part E that the heat exchanger should have no difficulty in removing heat from the perfusate rapidly enough to permit following this profile. But, as has previously been emphasized, the reduced rates of flow and of diffusion at low temperatures are very likely to prevent following this cooling profile all the way down to -62°C. Another limiting factor may simply be the time (and energy) available to the perfusion team. Note that it is very easy to keep a running estimate of the further time required to complete Method C perfusion, if perfusion is to be continued at the *current* temperature. One simply evaluates the current slope of the $C_p(t)$ curve from the data sheet, and uses the equation

$$[120] \qquad \text{time until completion} = \frac{.64 - C_p}{\frac{dC_p}{dt}}$$

Recorded Data

Patient's Weight M_b = _____

Calculated Data

Estimated W_s = _____

Estimated W_w = _____

t	J	ρ_p	C_p	ρ_1	C_1	A	B	Δ	U	C_w
t_0	J_0	ρ_{p0}	C_{p0}	ρ_{10}	C_{10}	A_0	0	0	0	0
t_1	J_1	ρ_{p1}	C_{p1}	ρ_{11}	C_{11}	A_1	B_1	Δ_1	U_1	C_{w1}
t_n	J_n	ρ_{pn}	C_{pn}	ρ_{1n}	C_{1n}	A_n	B_n	Δ_n	U_n	C_{wn}

Formulas for Calculated Data

$$W_s = .053M_b$$

$$W_w = .575M_b$$

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

$$B_n = (A_n + A_{n-1})/2$$

$$\Delta_n = t_n - t_{n-1}$$

$$U_n = U_{n-1} + B_n\Delta_n$$

$$C_{wn} = (U_n - W_s C_{pn})/W_w$$

Figure 12

Sample worksheet for calculating the total body uptake U of DMSO, using the trapezoidal rule for integration. From the running values of U we can at any time calculate C_w, the estimated minimum body concentration of DMSO.

Whenever this time becomes prohibitive, further efforts to lower perfusate temperature should be discontinued, and perfusion carried out at the current temperature.

When the terminal concentration $C_f = .64$ is reached, we switch over to Method B, and maintain this constant perfusate concentration. We continue to estimate the minimum body concentration of DMSO by the method of calculation given in Figure 12. (Of course, the product $\rho_p C_p$ remains constant from here on, which leads to a simplification in the calculations). Perfusate temperature is regulated in exactly the same way as in Method C perfusion.

In determining when to cease Method B perfusion, we have two possible criteria to use:

i. The calculated minimum body concentration (Figure 12) yields $C_w \approx .64$. But accumulated measurement and computational errors over the full course of perfusion will render this number not completely accurate.

ii. The concentration of the effluent is $C_1 = .64$. But note that due to the expected decrease in K as temperature is lowered, C_1 could be fairly close to .64 while C_w remains some distance away.

So we see that it is important to continue perfusion until C_1 is verified to remain stable at .64 for some period of time. This final approach to equilibrium will be quite slow, especially at very low temperatures.

Phase II perfusion terminates when the desired equilibrium is achieved. At this point Phase III begins, whereby the body temperature is lowered to -196°C for storage in solid state.

(references on page 74)

or inversely,

$$[126] \qquad F_d = \frac{1}{\frac{1}{\rho_w C_d} + \frac{1}{\rho_d} - \frac{1}{\rho_w}}$$

These relations are valid at any temperature, when we substitute values of ρ_d and ρ_w correct for that temperature. In particular, at 20°C we have:

$$[127] \qquad C_d = \frac{1}{1 + \left(\frac{1}{F_d} - \frac{1}{1.1}\right)}$$

$$= \frac{11\ F_d}{11 + F_d}$$

and inversely,

$$[128] \qquad F_d = \frac{11\ C_d}{11 - C_d}$$

Appendix 2. Proof that the Recommended Method of Adding and Draining DMSO is Optimal

Consider the alternate procedure, where DMSO is continuously added to the (much larger) reservoir without draining any off. We have:

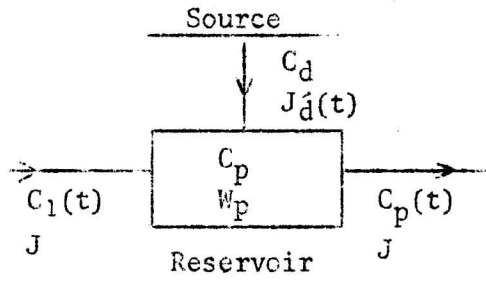

Figure 13

The differential equation governing this process is:

$$[129] \qquad \frac{d(C_p W_p)}{dt} = \rho_p (JC_1 + J_d' C_d - JC_p)$$

In this case the mass W_p is not constant, but rather we have $dW_p/dt = \rho_p J_d'$. Thus:

$$[130] \qquad W_p \frac{dC_p}{dt} + C_p \rho_p J_d' = \rho_p (JC_1 + J_d' C_d - JC_p)$$

and so

$$[131] \qquad J_d' = \left[\frac{W_p}{\rho_p} \frac{dC_p}{dt} + J (C_p - C_1) \right] \Big/ (C_d - C_p)$$

whereas from [43], we have:

$$[132] \qquad J_d = \left[\frac{W_p}{\rho_p}\frac{dC_p}{dt} + J\,(C_p - C_l)\right] \Big/ (C_d - C_l)$$

Now since $C_l < C_p$, we have that $J_d < J_d'$, as claimed.

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This material will be finished and mailed to MTR subscribers who own manuals as quickly as possible. We realize the urgency associated with the availability of this information and will do our best to avoid unnecessary delays.

whereas from [43], we have:

$$[132] \qquad J_d = \left[\frac{W_p}{\rho_p} \frac{dC_p}{dt} + J\,(C_p - C_l) \right] \Big/ (C_d - C_l)$$

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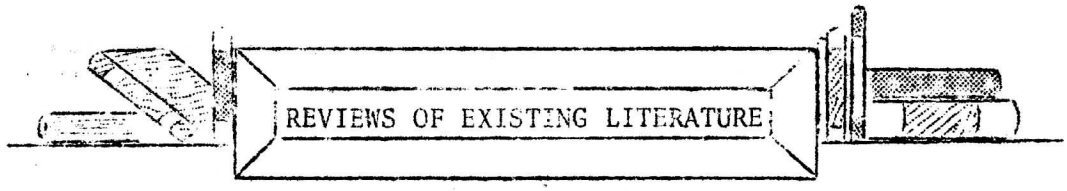


ice formation vs. cell survival

This study points out that the formation of intracellular ice crystals may not be totally imcompatible with viability in certain cells. The degree and cause of cryoinjury vary from cell to cell.

["The Ultrastructure of Small Bowel Epithelium During Freezing", by T. Makita, A. Khalessi, F. M. Guttman, and E. B. Sandborn, *Cryobiology*, 8:25-45, 1971.]

The well established concept that intracellular ice is a principal factor in cryoinjury has been supported, according to the authors, by little concrete evidence. This study of the damage caused by the formation of intracellular ice approached the problem in two ways. First, a replica was made while the cell was frozen (temperatures not cited) and the ultrastructure examined. Second, cells which were treated with various cryoprotective agents, frozen, and subsequently thawed, were studied to draw a correlation between the resultant viability of tissues and the changes in the ultrastructure of the cells.

This study was performed on segments of the small bowel of dogs. Various concentrations of DMSO, glycerol, and a mixture of these two were utilized by intravascular perfusion. A freeze-etching device was employed for production of the replicas. In order to assess the damage caused by the freezing and thawing, the frozen tissues were compared with control and perfused tissues that were not frozen. Each specimen was biopsied (a) before freezing, (b) while frozen, (c) immediately after thawing, and (d) in specimens which survived from 4 days to 2 months after the experiment. The text is well supported with 33 different photographs of the tissue replicas.

Glycerol and DMSO (and a combination of the two) were compared as to their relative effectiveness as cryoprotective agents in the preservation of ultrastructure detail as well as the prevention of the formation of ice crystals. It was found that a combination of 10% DMSO and 10% glycerol resulted in the *least* amount of change to the ultrastructure of the cell. Surprisingly, though, the

specimens with a lower percentage of ice crystals did not show an increased incidence of survival. The greatest survival rate was experienced in segments perfused with DMSO even though the amount of ice formation was greater.

The most significant effect of the freezing and thawing was shown to be membrane permeability. The capillary endothelial, intestinal epithelial, and interstitial cells varied in their ability to withstand the effects of freezing, the first being more vulnerable than the others.

"From these studies", the authors conclude, "it becomes evident that cells and intact organs may survive freezing and thawing under conditions which allow considerable intracellular ice crystal formation and even quite severe damage to the plasma membrane of endothelial and other cells". They also suggest that studies which include further observation of cellular ultrastructure and function, membrane permeability, and survival of tissues may contribute valuable information in this field leading to an improved understanding of the mechanism of cryoinjury and methods for adequate protection during freezing and thawing procedures.

LLC

Φ Φ Φ

Editorial (continued from page 27)

The next hundred years or so, barring a catastrophic end to civilization, should include advances in medicine and gerontology opening up the eyes of everyone to a proper view of human life, as potentially endless, full of activity, enjoyment, and growth. Accidental deaths and residual fatal illnesses will be handled in a way that seeks to return life to those who die. Whether or not the means of reanimation have been developed, those who are dying will be frozen.

But few persons will be frozen without a demonstration of feasibility such as that described above. Most will demand proof that the procedures used will, at the very least, lead to a state in which all cells are potentially viable. This much we must do alone, now, without the general support of society. If we do not take this first step, we may not have a chance to take many others. We must offer proof that what we are doing produces results.

Have you noticed how fast the years keep going by? There is no time to waste!

References

1. *Prospect of Immortality*, R.C.W. Ettinger, MacFadden.
2. *Suspended Animation*, Robert Prehoda, Chilton.

F. R. Chamberlain

total blood washout

This procedure for treating a hepatic coma involves total body perfusion with chilled perfusate similar to that employed in Phase I of the induction of solid state hypothermia. Data on cooling rates achievable were acquired, as well as further conclusions that the use of blood proteins may be of value in preventing fluid buildup in tissues.

["Asanguineous Hypothermic Total Body Perfusion (TBW) In The Treatment of Stage IV Hepatic Coma", by G. Klebanoff, D. Hollander, A. B. Cosimi, W. Stanford, and W. T., Kemmerer (all of the USAF MC), in the Journal of Surgical Research, 12:1-7, January, 1972.

All the blood of a dying adult male was temporarily replaced with chilled ringer's lactate, following which fresh blood was administered with rewarming. Perfusate (10°C) was injected at a rate of up to 2.4 liters per minute into a femoral artery and removed from both a femoral vein and the jugular. During perfusion, body core temperatures dropped from 37°C to their lowest values (21.5°C esophageal and 24.5°C rectal) in ten minutes. pH fell from 7.34 to 6.92 in this same period, and hemocrit dropped to a low of one percent. Blood replacement was complete 15 minutes after the beginning of perfusion, and body temperature was up to near normal 25 minutes later.

The patient first made marked improvement and was consious of his surroundings in contrast to a former quasi-comatose condition. Still, a massive infection developed (apparently unrelated to the perfusion procedure) and pneumonia contributed to the patient's death from respiratory failure about five days following the blood wash out.

Despite diuretic treatments following perfusion, accumulations of fluids in the tissues were excessive and these contributed to the pulmonary congestion that deepened into pneumonia. Colloids were not used in the perfusate and the authors' evaluation was that albumin might have had a beneficial effect, since the patient's normal blood protein stores were probably largely depleted.

The authors conclude that the treatment had a generally beneficial effect (notwithstanding the outcome) and that the technique would be developed as an important adjunct to the treatment of disorders where only total blood replacement can provide the necessary conditions to start a patient on the road to eventual recovery.

FRC

Φ Φ Φ

manual of survival

The British Cryogenic Society has published a "Manual of Survival" which contains a very broad collection of concise statements concerning safety, rescue, and the avoidance of dangerous or harmful habits. Major sections include "Indoors", "In the Open", "Traveling by Car", "Traveling by Rail", "Traveling on Water", "Eating and Drinking", "Smoking", "Illness", "General", "If the Worst Comes to the Worst", and "Saving Other People". In British currency, the price (for non-members) is 80p. The British Cryogenic Society's address is 339 Eastwood Road, Rayleigh, Essex, SS6-7LG, Great Britain.

FRC

Φ Φ Φ

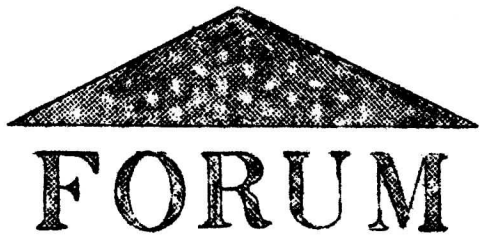
expert design guidance in biotechnology now available free

The below note was published in *Product Engineering*, December 21, 1970.

"Engineers can now get free expert design guidance on products proposed for use by doctors and hospitals. The service is available from the American Institute of Biological Sciences, 3900 Wisconsin Avenue, N.W., Washington D. C., through its Bioinstrumentation Advisory Council headed by Dr. John H. Busser, executive secretary. The service is intended to help engineers dealing in mechanical and electronic problems avoid design mistakes found too frequently in systems promoted for dealing with living organisms. The AIBS service won't substitute totally for having trained biological consultants but may steer engineers past some hazards in early developmental stages."

Φ Φ Φ

Correction to Volume 2 - Number 1. The last sentence on page 4 should read as follows: "In a second group, after removal, the *livers* were flushed and cooled with C_4, then stored for 6-7 hours at 4-6°C before being transplanted."



FORUM

Question: Regarding Phase I in the instruction manual (Instructions for the Induction of Solid State Hypothermia in Humans): In section 58.0 (subsection 58.1.8) you state that open circuit perfusion is to be run until the Donor's body core temperatures are near 0°C. Wouldn't it be just as effective to replace open circuit perfusion with recirculation once the blood constituents have been removed? Blood wash-out would, in most cases, occur prior to the accomplishment of temperature regimes near 0°C and result in a savings in the cost and required amount of perfusate.

Answer: When recirculation is first begun, as indicated in 58.1.8.1, sodium chloride (salt) will begin to build up in the perfusate, as the intracellular constituents in the perfusate go into the tissues and replace the salt that is present at normal body temperatures. Thus, the perfusate becomes "contaminated" with salt and periodic flushing of the entire system (as suggested in 58.1.8.2) should be beneficial in continuing the replacement of intercellular constituents with those similar to intracellular constituents. This point is also made in 58.3.2.4, where salt buildup and the necessity for periodic replacement with fresh perfusate is stressed. If it is practical to run open circuit from start to finish in Phase I, this would be the most desirable mode of operation.

Question: In section 59.0 (subsection 59.4.2) of the SSH instruction manual, it is recommended that formaldahyde (37% U.S.P) be used to sterilize the injection apparatus. Should the formaldahyde be used full strength, or should this be diluted?

Answer: In artificial kidneys a 2 percent solution (50:1 dilution) of the 37 percent formaldahyde is used for sterilization. Thus, the actual fraction of formaldahyde used is (.37)(.02) = .0074 or 3/4 of one percent. This diluted mixture is left in contact with the equipment for no less than 2 hours in effecting satisfactory sterilization. The fractions and percentages given above for mixing this sterilizing agent are volumetric ratios.

Question: The elimination of air bubbles from the system during connection of the tubing from the injecting apparatus to the cannulas in the Donor is discussed in section 60.2 of the manual. It is stated that hemostats were used to close off the vessels (to be cannulated) before the incisions were made. However, section 55.0 "Cannulation Techniques and Procedures", makes no mention of this. Was this just omitted from the earlier section as an oversight, or is this a contradiction of recommendations?

Answer: In the appropriate paragraphs of 55.0 (55.2.2.4, 55.3.3.4, and 55.4.2.3) mention is made of applying homostats to both sides of the vessel, but only for those cases where the circulatory system is under pressure (heart lung resuscitator still in use). In fact, hemostats should be applied in *all* cases, as a preventative measure to ensure air pockets do not develop in the vessels. Future revisions of 55.0 will be changed to show this measure as a practice to be used in preventing air bubbles from being formed.

Question: Section 62.0 refers to cases where Phase I and Phase II procedures are carried out in separate facilities. Under what circumstances would this be done?

Answer: Phase II requires relatively sophisticated and expensive equipment, so centrally located Phase II centers will most often serve a number of outlying Phase I facilities where the immediate and time critical cooling and initial perfusion must take place. After these initial steps are taken, time will be available for transportation of the Donor to the nearest Phase II center.

Are there questions regarding the induction of SSH which have been puzzling you? Are there parts of MTR articles or the Manrise instruction manual which are unclear? No matter how elementary, or how complicated, your questions might seem to you, chances are they have been bothersome to others as well. Perhaps, instead of questions, you have observations and recommendations which would interest other readers of MTR. In many cases, these ideas will be new to both MTR readers and its writers.

So that readers can benefit from all questions, comments and suggestions received, Forum will print these followed by answers or appropriate comments. You are urged to submit your questions and other contributions to FORUM, Manrise Technical Review, Box 731, La Canada, California, 91011. Please mark: "For Publication" at the top of your correspondence. If you wish to have your name withheld, please write "withhold" after your signature.

There is no need for reticence. We are all engaged in a struggle to develop an entirely new area of knowledge in the most rapid and effective way. MTR's fundamental purpose is the sharing of ideas, and in FORUM, all are welcome to participate.

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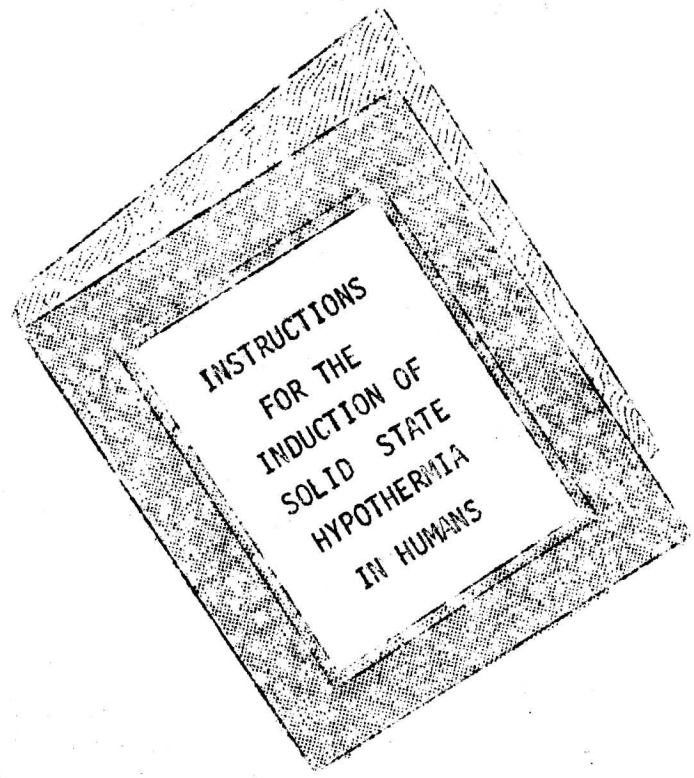

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