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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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References. All references should be cited in the text, indicated by arabic numbers, in parentheses, on the line. The reference list must appear on a separate sheet in order of citation, not alphabetical order. The following minimum data will be given: names of authors, complete title of article cited, name of journal (standard abbreviations acceptable), volume number, first and last page numbers, year of publication.

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*effective March 1, 1972

C O N T E N T S

ORIGINAL ARTICLES

REVIEWS OF EXISTING LITERATURE

MANRISE
TECHNICAL
REVIEW

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RESEARCH PROPOSALS

Technical Journals usually publish, or rather report, work that has been accomplished. In general, more manuscripts are submitted than can be accommodated, there being more work in progress than can reasonably be documented in periodicals. This is not yet the case within the cryonics area. All known work directly oriented toward solid state hypothermia in humans is essentially self-sponsored by the authors themselves. There are by far more problems to be explored than there are dollars to support the work. Can this condition be changed? We think so.

MTR intends to solicit and publish research proposal outlines from persons qualified to perform work relevant to solid state hypothermia. No commitment exists as to publication of any or all materials submitted, but all reasonably complete and logically organized program descriptions will be printed. We think this will accomplish the following ends:

(a) The proposal author will be credited with the conception of a needed investigation. Even if this work is later performed and reported upon by a different investigator, the credit for identification of the problem and establishing its importance will be shared by the originator.

(b) The proposal may be seen by societies or individuals who will have both the means and the inclination to support the research. The address of the proposal's author will be shown, and sponsors may choose to contact the proposer directly and finance the research activity planned.

(c) Review and comment may be received by MTR from other researchers concerning improvements to the proposal, which would be passed on to the author directly

(d) As time passes, and as a larger and larger number of proposals are printed, it will become possible for non-profit societies to develop research programs from the pool of talents represented by published proposals. By contacting the most able individuals, and by proper organization and administration, these societies may develop a solid basis for soliciting large donations in support of their programs.

Instructions for formulating research proposals will be provided to any readers who inquire. These instructions will include outlines and advice as to how proposals may be effectively formulated and presented to prospective sponsors. Whether or not you intend to submit proposals to MTR for publication, this material will be useful to you.

FRC

RECOMMENDED MODIFICATIONS TO COLLINS' SOLUTION FOR USE AS THE BASE PERFUSATE IN INDUCING SSH

by Art Quaife

In 1969, Collins et al. (1) reported the successful hypothermic preservation of dog kidneys for periods of 24 hours, using a perfusate (C_4) with ionic composition similar to intracellular fluid. Since then, many other investigators have attempted to duplicate these results, and to test the efficacy of this solution in preserving other organs, such as the liver and the heart. This article will summarize the results of these further experiments, and consider their implications as to composition of the perfusate to be used in inducing SSH.

Several early attempts to confirm Collins' results were less than successful. Smellie et al. (2) duplicated Collins' experiment exactly, but found that after reimplantation of the kidneys and contralateral nephrectomy (removal of the other kidney), all of their dogs died within 12 days. Frost et al. (3) were able to substantiate Collins' results when kidneys were cooled *immediately* upon removal and then stored for 12 hours. But when kidneys were given an initial 15 minutes warm ischemia time before cooling, they were not able to sustain life after reimplantation.

Most researchers, however, were able to successfully use C_4 solution, finding it superior to previously tested perfusates. Løkkegaard et al. (4) used a perfusate like C_4, with papaverine replacing both procaine HCl and phenoxybenzamine (which is of interest in connection with Watkins' results below). They perfused and then stored pig kidneys for 24 hours, with 100% survival of pigs after reimplantation and contralateral nephrectomy. Other researchers have succeeded in extending the storage time to 48 hours (5,6,7,8). Rudolf et al. (9) removed kidneys from dogs and perfused them *continuously* for 24 hours at 4°C. Both Collins' solution and Ringer's lactate with albumin added successfully preserved the organs, with Collins' yielding lower BUN (blood urea nitrogen) levels after reimplantation. Albumin was essential to the Ringer's lactate; using solution from which it was removed, none of the kidneys thus treated survived.

Dempster (10) raised many issues concerning the use of C_4, including the suggestion that phenoxybenzamine is not necessary. In this vein, Watkins et al (11) suggested that the early failures described above may have been due to the complexity of the perfusate and its

preparation, which they attempted to simplify. In their experiment, dog kidneys were removed, perfused, stored for 24 hours at 4°C, then reimplanted with contralateral nephrectomy. They used three different perfusates:

1. Collins' C_5, which is identical to C_4 except for the addition of albumin and the method of mixing. It requires the preparation of two separate solutions, which are mixed at the time of use.

2. WP-1: C_5 with heparin and phenoxybenzamine omitted.

3. WP-3: WP-1 with magnesium and procaine HCl omitted. Only one solution need be mixed to prepare WP-3.

Except for three technical failures, all dogs survived after reimplantation. Furthermore, the BUN levels returned to normal most rapidly in group 3 dogs, almost as rapidly in group 2 dogs, and somewhat more slowly in group 1 dogs. The authors explain these results by offering theoretical reasons why the four ingredients omitted in WP-3 may be unnecessary or even harmful. They believe that further simplification of the perfusate may be possible.

The authors point out that their transplants (autografts) were carried out under optimal conditions. The rigorous test of any preservation method is in clinical use, where in removing a kidney from a cadaver for transplant, other adverse factors are normally encountered. Clinical test of WP-3 has been initiated, but no report of results is given in their article.

In their original paper, Collins et al. (1) mentioned a problem precipitate caused by phenoxybenzamine. In later work, Collins, Hartley and Clunie reverted to the use of C_3 solution, from which this drug is omitted. They found no significant difference between their results and those obtained using C_4 (12,13,14). They suggest (14) that procaine HCl in C_3 may produce an equivalent effect. Both act as vasodilators and help suppress vasospasm (12,14,15).

They compared C_3, which is free of colloids, with other solutions containing the colloids dextran or albumin (12). Swelling upon perfusion was most marked using C_3, but this did not seem to impair survival.

In perfusing the whole human organism while inducing solid state hypothermia (SSH), we are faced with the problem that different organs may have different metabolic requirements, making it impossible to devise one perfusate that is best for all. For example, Abouna et al. (16) reported their attempts to preserve canine livers with C_4. In a control group, six canine liver homografts were removed and transplanted into unrelated hosts. In a second group, after removal, the kidneys were flushed and cooled with C_4, then stored for 6-7 hours

at 4-6°C before being transplanted. No immunosuppression was used in either group. The control dogs all survived for 5-10 days until suffering death from rejection. However, all the dogs in the second group died quite soon, with a mean life of 13 hours. The authors conclude that C_4 solution is neither sufficient nor desirable in the preservation of livers for transplantation. Of course, the distinctive feature of C_4 is the high concentration of potassium employed, viz. 115 mM/l (millimoles per liter). In earlier work, Abouna (17) had already shown that concentrations of K above 30 mM/l in the perfusate did not improve liver viability after cooling and storage.

In another study, Abouna et al. (18) describe a solution that *does* permit the successful transplantation of livers after 6 hours cold storage. Among other differences from C_4, this solution contains 30 mM/l of K. Also of interest is the magnesium concentration, 8 mM/l, as opposed to 30 mM/l in C_4.

In other research, Calman and Bell (19) attempted to store isolated rat hearts at 25°C for periods of 1½ hours after suitable perfusion. The control perfusate was saline with heparin, buffered to pH 7.0 with tris-HCl. Three parameters were varied separately in the test perfusates: potassium added, phenoxybenzamine added, and pH varied. The results are summarized in the following table:

	Number of rats	Number Survived	Significance of Difference from Control
Control	20	7	
50 mM/l KCl added	20	15	$p < .02$
100 mM/l KCl added	20	1	
25 mg/l phenoxybenzamine added	20	18	$p < .001$
(those below stored 1 hour)			
pH = 6.5	20	5	
7.0	20	19	$p < .02$
7.5	20	13	
8.0	20	5	

The authors draw the following conclusions:

A) Addition of K in amounts of 50 mM/l significantly increased viability, while 100 mM/l permitted almost no survival. (Note that the former concentration is closer to that used by Abouna in successfully storing livers, while the latter is still less than that of C_4).

B) Addition of phenoxybenzamine significantly increased viability. The authors suggest that this drug acts by stabilizing lysosomes, since perfusion with this substance is known to prevent the release of lysosomal enzymes.

C) pH 7.0 at 25°C, rather than pH 7.4, was found to be optimal. The authors note that this same lower pH was successfully used by Collins.

This last conclusion is further substantiated by a study of Sinha et al. (20). When using a (modified) C_4 solution with pH 7.3, no kidneys survived 24 hours cold storage. By simply reducing the pH to 7.0, all kidneys thus treated survived.

It has been proposed that C_4 solution be used as the base perfusate in inducing solid state hypothermia in humans. The studies summarized above all bear on the question of possible modifications of this perfusate. To this writer, the implications are as follows:

A) It has been noted that a precipitate occurs when C_4 is mixed with DMSO; it is suspected that Mg is responsible (21). From Watkins' study, it may be possible to completely eliminate Mg from the perfusate, or at least greatly reduce its concentration. The formula recommended below gives a concentration equal to that found in extracellular fluid (22, p. 622).

B) It is probably possible to eliminate procaine HCl from C_4.

C) In this writer's opinion, Watkins' work does *not* support the omission of heparin from the perfusate. He points out that heparin is relatively useless in kidney perfusion, since the blood is being flushed out as soon as the heparin arrives, making anticoagulation pointless. However, considerably greater periods of time, with much recirculation of blood, are required to flush all blood from the whole body in SSH.

D) Similarly, the positive results of Calman and Bell mitigate against eliminating phenoxybenzamine. Also, since its effects

overlap those of procaine HCl, it would seem unwise to eliminate both.

E) pH of the solution should be 7.0 at 25°C.

F) As a compromise between the requirements of different organs, it is probably necessary to reduce the concentration of K, say to 45 mM/l. Note that this reduced level is still about 8 times the concentration that appears in Ringer's solution.

G) C_4 solution is hypertonic, having an osmolarity of 405 mM/l (11) as opposed to about 300 mM/l in intracellular fluid. Ringer's type solutions are normally isotonic. In virtue of the changes suggested above, it is not clear what the desired osmolarity of the resultant solution should be. The solution used by Abouna et al. (18) has osmolarity 335 mM/l, while that used by Calman and Bell containing 50 mM/l of K has osmolarity of 350 mM/l. The formula suggested below also yields an osmolarity of 350 mM/l. If the amount of NaCl utilized is raised to 2.54 gms., the osmolarity would be raised to that of C_4. If all of the NaCl is omitted, the osmolarity would be reduced to 318 mM/l, nearly isotonic. The amount of H_2PO_4, HPO_4, and HCO_3 has been left unchanged, so the pH is the same as C_4.

These observations lead to the following recommendation for the composition of the perfusate Q_2 to be used in inducing SSH [a preliminary version (Q_1) was published in (23)]:

Components	Gms.
KH_2PO_4	.92
$K_2HPO_4 \cdot 3H_2O$	4.37
$NaH_2PO_4 \cdot H_2O$	1.15
$Na_2HPO_4 \cdot 7H_2O$	6.12
NaCl	.93
$NaHCO_3$	.84
Glucose	25.0
Distilled, deionized water to one liter	

This solution is sterilized by autoclaving, stored on shelf, and refrigerated before using.

Immediately before use, add:

Components	Gms.
Dextran 40	50.0
Heparin	[5000 units]
Phenoxybenzamine	.025
$MgSO_4 \cdot 7H_2O$	.37

To remove any possible (undetected) precipitate, the solution is now filtered through a 5-10 micron pore size sintered glass filter (14).

The above order of mixing is similar to that employed by Dr. Peter Gouras (24).

The composition of this solution in mM/l is: Na 80, K 45, HPO_4 42, Cl 16, H_2PO_4 15, HCO_3 10, Mg 1.5, SO_4 1.5, glucose 139. Total osmolarity is 350, and pH is 7.0 at 25°C.

There are indications that DMSO is incompatible with dextran (25). If this is true, then the above solution would only be suitable for Phase I of the induction of SSH; i.e., lowering the body temperature to 0°C. At that point, the above solution with dextran omitted would have to be employed.

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FORUM

Are there questions regarding the induction of solid state hypothermia which have been puzzling you? Are there parts of MTR articles or the Manrise instruction manual (Instructions for the Induction of Solid State Hypothermia in Humans) which are unclear? No matter how elementary, or how complicated, your questions might seem to you, chances are they have been bothersome to others. 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 most fundamental purpose is the sharing of ideas, and in FORUM, all are welcomed to participate.

EVALUATION OF CANNULA FLOW DYNAMICS, WITH EMPHASIS ON THE "T-SHAPED" CONFIGURATION

by F. R. Chamberlain

Cannulas are the tubes, inserted into arteries and veins, through which fluids are introduced into and expelled from, the body during perfusion. It is desireable that fluid injected into a vessel be injected both up and down the vessel (in both directions) so that no portion of the body will be bypassed. Similarly, in the case of a vessel from which fluids are removed, it is desireable that fluid be extracted from both directions.

In the past, it has been suggested that "T-shaped" cannulas be used, instead of a pair of individual curved tubes, since the arrangement achieved is less complex externally. If a pair of conventional cannulas are used, the input must be divided externally by a "Y" or "T" shaped connector, and then two additional tubes are used to join the connector to the two cannulas. Each of the two cannulas must be very securely tied, since back pressure tends to force them out of the vessels into which they are inserted.

By comparison, the T-shaped cannula is far simpler to use. A single incision, somewhat longer and differently oriented (than that used with a conventional cannula) is all that is required. The T-shaped cannula is still tied securely, but the forces tending to push the cannula out are self cancelling. Externally, there is only a single tube to connect. By all appearances, the T-shaped cannula would be more desireable. However, T-shaped cannulas are not without problems.

The T-shaped cannula is not so easily obtainable as the conventional cannula. No medical applications are known to exist. Experimenters some times make use of plastic (polyethylene or polypropylene) "T"s intended for coupling surgical tubing. These materials may have limitations. In an even more fundamental way, the "T" configuration may be comparatively inefficient if very high rates of flow are desired. These two considerations will be discussed individually.

Materials

Polypropylene, one of the materials of which tubing connector "T"s are made, has many desireable characteristics. It is machineable, so that openings can be widened, and it is strong enough to be serviceable with comparatively thin walls. It also has excellent chemical resistance to DMSO. Unfortunately, polypropylene has a "brittleness temperature" of 0°C. It may be unsuitable for Phase II perfusion because of a predisposition towards failure at low temperatures.

Polyethylene, a DMSO resistant material with excellent properties at low temperatures, lacks the structural advantages of polypropylene. In tubing, its wall thickness is not offered in values less than 1/16 inch (.062"), 2-3 times greater than polypropylene. "T" connectors of polyethylene, for the same outside sizes, will probably be significantly smaller inside.

Stainless steel could be formed into excellent "T" cannulas, but the manufacturing processes would be more expensive than for plastic items.

Flow Efficiency; Wall Resistance

In long lengths of circular tubing, approximately horizontal and of constant inside diameter, friction with the wall is a predominant source of pressure loss. A simplified version of the formula for this pressure loss is:

$$P_L = \frac{(Q)(\mu)(\ell)}{(14,078)(d^4)}$$

where:

P_L = Pressure loss (psi)

Q = Flow (liters/min.)

μ = Viscosity (centipoises); (water at 4°C $\cong$ 1.5)

ℓ = Tubing length (feet)

d = Tubing inside diameter (inches)

As an example of this, figure 1 shows the length of tubing as a function of flow in gallons (rather than liters) per minute for a family of pressure-loss curves. Two families are superimposed on the graph. The solid lines are for tubing .500" inside diameter (a possible dimention for surgical rubber or tygon tubing to be used in heat exchanger hook-up lines, etc.). The dotted lines are for tubing of .430" inside diameter (corresponds to stainless steel of .500" outside diameter with .035" wall thickness). By reference to figure 1, for example, one can see that a 1.0 psi drop across 22 feet of 0.430" I.D. tubing represents a flow rate of 3.9 gallons per minute. One can convert these numbers to other sizes of tubing as follows:

(a) Determine the inside diameter of the new size tubing (example; ¼ inch).

(b) Take the ratio of the ½" diameter tubing to the new size tubing, and take the fourth power of it (multiply the ratio times itself four times). Per example, ½ ÷ ¼ = 2; 2x2x2x2=16.

(c) In extracting information from the chart, divide lengths of tubing or gallons per minute by the fourth power of the ratio (e.g., 16), or multiply the pressure drop by this figure.

(d) Example: ¼ inch (I.D.) tubing, 10 feet long, with a pressure drop of 5 psi: How many gallons per minute? Going into the chart, assuming ½ inch tubing, read off the lower scale, 76 gallons per minute. Dividing by 16, obtain flow rate of 4.75 gallons per minute.

Flow Efficiency; Form Factors

In "T" shaped connectors, pressure losses occur because of the change of direction of travel, as well as from wall resistance. These losses are higher than wall resistance losses in all regimes of interest.

The expression:

$$h_L = K \frac{V^2}{2g}$$

where:

h_L = head loss, feet

V = velocity, ft/sec

g = gravity, ft/sec^2

relates pressure drop to stream velocity. "K" is dimensionless, and is

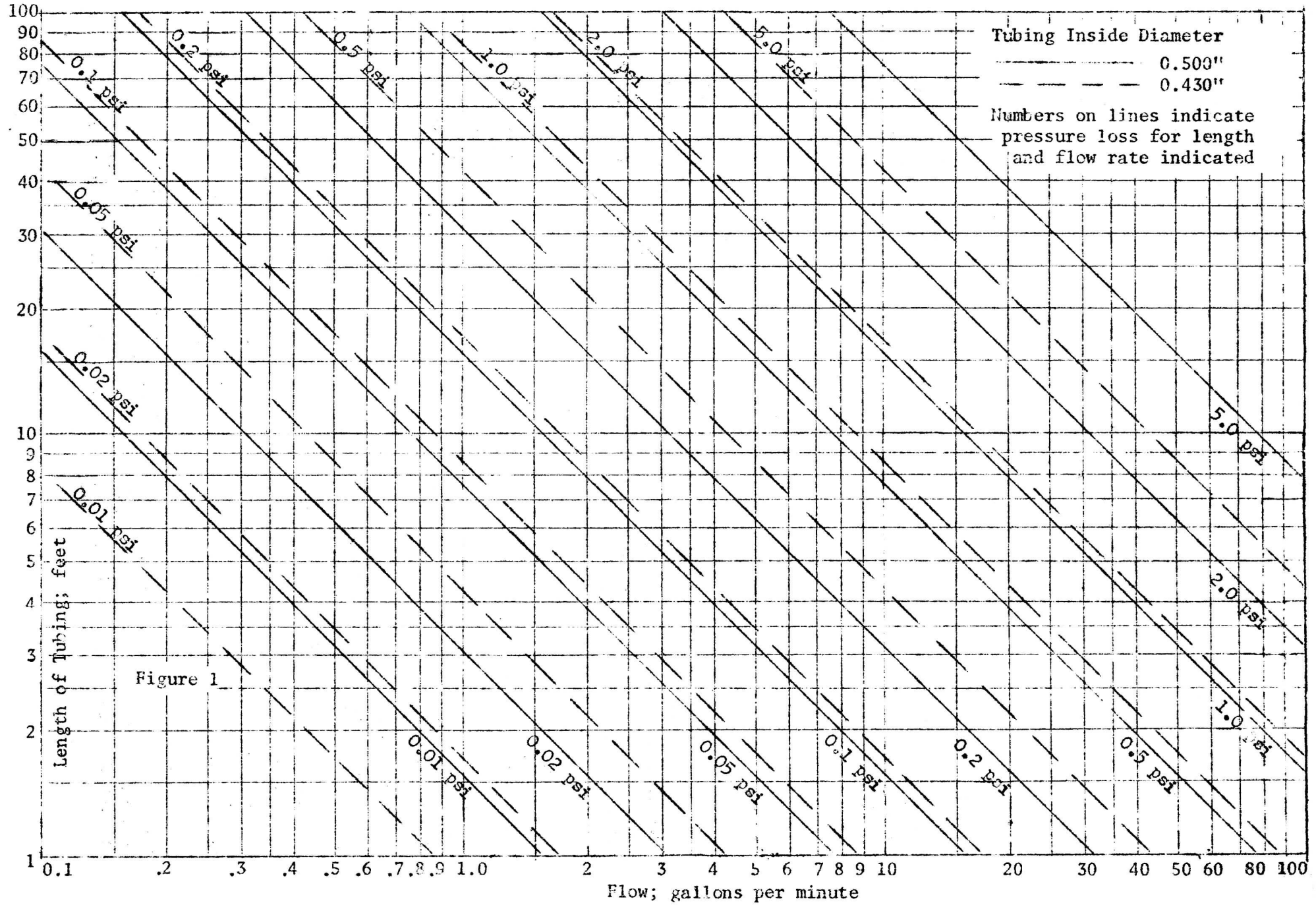
Tubing Inside Diameter
0.500"
0.430"
Numbers on lines indicate pressure loss for length and flow rate indicated
0.1 psi
0.2 psi
0.5 psi
1.0 psi
2.0 psi
5.0 psi
0.05 psi
0.02 psi
0.01 psi
Length of Tubing; feet
Flow; gallons per minute
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
2
3
4
5
6
7
8
9
10
20
30
40
50
60
80
100

Figure 1

approximately 1.8 for "T"s, 0.9 for 90° elbows, and 0.4 for 45° elbows (1). The expression may be converted to pressure drop, for "T"s, in the more convenient form:

$P_L = .0126\ V^2$ P_L = Pressure drop, psi

Figure 2 shows the pressure loss characteristics for "T"s of various inside clearances, both from "form" sources and wall friction. For example, with an internal clearance of 1/5 inch (.200") and a flow of 4.0 liters per minute, the form loss is 1.3 psi, while the friction loss is only .067 psi (less by a factor of approximately 20).

Flow Rates Considered

The median basal cardiac output of seven adult males (median weight 64 kg) was 5.25 liters per minute, based on oxygen consumption measurements (2). Data obtained on a runner during a two mile race for a world's record indicated cardiac outputs of up to 27.5 liters per minute. During perfusion, it is possible that flow rates might surpass basal values but these probably will not approach maximum cardiac output.

In the cases of ten dogs perfused with "artificial bloods", perfusion flow rates (corrected to a standard weight of 64 kg, equivalent to the "mean subject" above) ranged from 2.43 to 14.9 liters per minute, and averaged 7.78 liters per minute (3). Mean arterial pressures ranged from 85 to 140 mmHg (1.64 to 2.71 psi). These values are similar to those in humans above. While the perfusion was accomplished in well oxygenated living tissues, it was accomplished with a foreign fluid containing sizeable particles. Three of ten dogs were on total bypass.

The viscosity of water at 4°C is approximately one half that off blood at normal body temperatures, and perfusion at static pressures should be mechanically more efficient than the pulsatile perfusion developed by the heart. The body does not conform to mechanical principles as a circulatory system, however. Additionally, effects associated with clinical death can further impede circulation. Loss of osmotic pressure with either withdrawal of large proteins or lack of oxygenation can produce edema in tissues surrounding capillaries, restricting throughout. Post mortem clotting can constitute a significant obstruction.

Based on the preceding discussion and assumptions, maximum flow rates of 8.0 liters per minute will be considered in the discussion to follow. This figure does not indicate that such flow rates are required or even desireable (for this is currently unknown). It indicates only

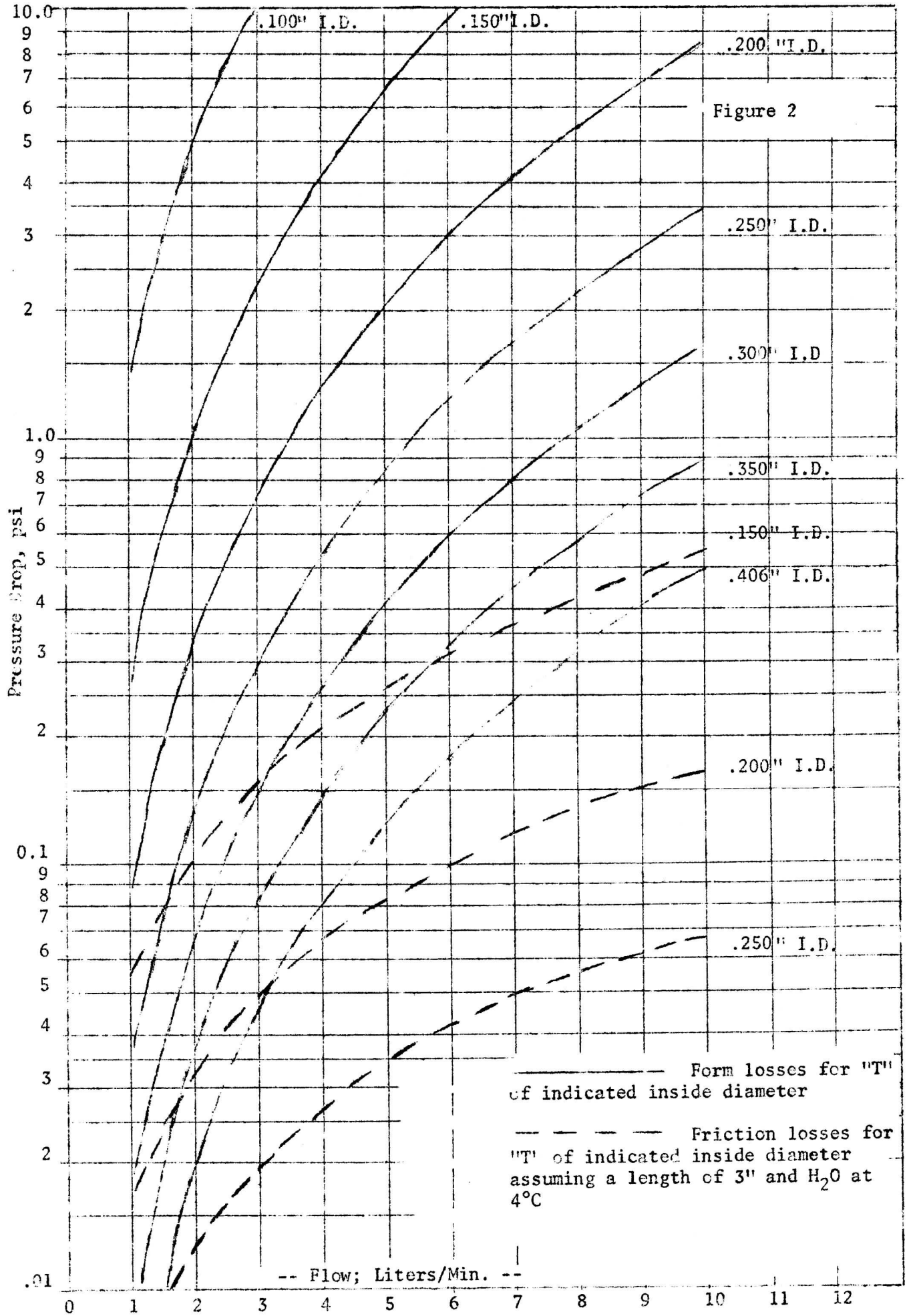
.100" I.D.
.150" I.D.
.200" I.D.
.250" I.D.
.300" I.D
.350" I.D.
.150" I.D.
.406" I.D.
.200" I.D.
.250" I.D.
Pressure Drop, psi
10.0
9
8
7
6
5
4
3
2
1.0
9
8
7
6
5
4
3
2
0.1
9
8
7
6
5
4
3
2
.01
0
1
2
3
4
5
6
7
8
9
10
11
12
-- Flow; Liters/Min. --
Form losses for "T" of indicated inside diameter
Friction losses for "T" of indicated inside diameter assuming a length of 3" and H_2O at 4°C

Figure 2

that such flow rates are theoretically possible and that cannula performance in that range is of interest.

Significance of Pressure Drops in Cannulas

Perfusion pressures are limited by the possibility of damage to the circulatory system. The pressure range of 150-200 mm Hg (corresponding to 2.8 - 3.9 psi) is currently considered to be the upper limit of safe pressures. As earlier observed, a 1/5" T-shaped cannula passing 4.0 liters per minute develops a 1.3 psi drop due to form factors alone. At 8.0 liters per minute the loss would be 5.3 psi.

Suppose for example that a 3.9 psi perfusion pressure would result in a flow of 8.0 liters per minute, and that a "T shaped" cannula of 1/5" inside diameter is used. In order to develop 3.9 psi residual pressure after passing the cannula, perfusate must be injected into the cannula at 9.2 psi (since 5.3 psi will be lost by 8.0 liters/min. flowing through the cannula). The danger in that case might be that a circulatory blockage would develop and the flow would slow up, so that less pressure drop would be developed in the "T", and an increased pressure drop applied to the circulatory system itself. If flow stopped entirely due to internal blockages, the full 9.2 psi would then be developed in the vessel injected, possibly resulting in damage. To be more explicit, a pile-up of solid blood constituents at the outlet cannula might result in the 9.2 psi pressure being expressed throughout the body.

Conclusions

"T-shaped" cannulas have significant pressure losses if high perfusion rates are induced. Means for protection of the circulatory system (pressure relief or reduction devices) may be appropriate if "T" cannulas are used in this way. Conversely, if relatively low perfusion rates are used, with minimal pressure drops in the cannula, "T-shaped" cannulas offer many advantages. They are not yet readily available for use in cannulation, but adaptations of existing hardware may be possible.

General formulas are available for calculating both wall friction and form losses of pressure in tubing and "T" connectors, so that the specific pressure-flow characteristics of any proposed cannula and its supply system may be analyzed and evaluated.

References

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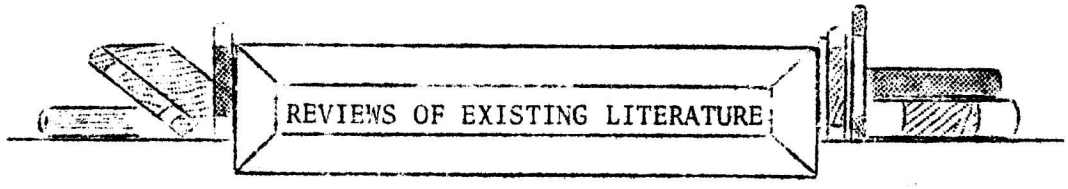
2. "The Circulation", by H.C. Bazett and Philip Bard, Part I of *Medical Physiology*, Mosby, 1956.
3. "Perfusion of Whole Animals with Perfluorinated Liquid Emulsions Using the Clark Bubble-Defoam Heart Lung Machine", by L.C. Clark, S. Kaplan, F. Becattini, and G. Benzing, in *Federation Proceedings*, v. 29, n. 5, Sept-Oct, 1970.

Correction to the chart on page 13 of MTR, volume 1, number 1:
Component "6." should indicate "Heparin (5000 u/l)"



requirements for prolonged low-resistance perfusion

In the perfusion of isolated organs, particularly kidneys, researchers have repeatedly encountered the problem of vascular resistance. This condition is evidenced by rising perfusion pressure and falling venous output. The result is increasing edema, i.e., swelling due to the accumulation of abnormal amounts of fluid (perfusate) in intercellular tissue spaces. This study suggests that thorough blood wash-out prior to recirculation, and continuous filtration using a pore diameter smaller than one micron, are useful in reducing these problems.

["Vascular Resistance of the Isolated Rabbit Kidney", by D. E. Pegg, *Cryobiology*, 8:431-440, 1971.]

A causal relationship between vascular resistance and edema was suggested. As pressure within the capillaries increases, more fluid is forced into the extracellular spaces. The edema, in turn, will cause tissue pressure which results in the further constriction of the capillaries and an increased rise in resistance. The purpose of this study was to determine the factors involved in the recurrent problems of vascular resistance and edema experienced in the perfusion of isolated organs.

Among the factors studied were recirculation, filtration and method of perfusate oxygenation (this latter factor being of little significance in the application of SSH, at this time, as it relates to perfusion with blood constituents). The possible effects of pH and osmolarity were

briefly discussed. A solution (Na^+, K^+, Ca^{2+}, Mg^+) containing dextrose, dextran 70, and papavarine, was used to perfuse isolated kidneys to 37°C.

The effect of the filter pore diameter on the degree of resistance during recirculation was tested on six kidneys. Two were perfused using each filter pore size in the Millipore series (5.3, 1.2, 0.65, 0.3, 0.22 microns). In addition to this, two kidneys were perfused without any filter and two others were perfused without recirculation. The article states that "It was found that all the experiments in which the resistance was more than 1.0* were obtained with a filter pore diameter of more than one micron. Conversely, all those kidneys perfused with a filter pore diameter of less than one micron had a resistance less than 1.0. However, there were six instances in which the resistance was less than 1.0 although the pore diameter had been more than one micron".

The effect of perfusion pressure on blood wash-out was tested on fifteen kidneys. Perfusion pressures of 20, 40, or 60 mm Hg, respectively, were applied to a group of five kidneys. The venous effluent was collected at separate, one minute intervals. Each sample was tested to determine hemoglobin content. The residual hemoglobin in the kidney was determined after 30 minutes of perfusion. Thus it was possible to plot a curve of the amount of hemoglobin remaining in the kidney at each minute after perfusion was begun. Comparison of the three curves (plotted for the above perfusion pressures) reveals that "both the rate of blood removal and the ultimate completeness of wash-out were increased by raising the perfusion pressure, but that stable values were obtained after 15 min at 40 mm Hg or 10 min at 60 mm Hg".

The work reported in this paper showed that rising vascular resistance is partly due to precipitates which arise with recirculation of the perfusate. Resistance also increased when mixed blood cells or platelets were added to the perfusate without oxygenation. It was concluded that preliminary blood wash-out should be as thorough as possible and continuous filtration with a pore diameter not greater than one micron should be used to minimize vascular resistance and edema in prolonged perfusion.

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* Unity (1.0) resistance corresponds to the "physiological value" of vascular resistance.

cooling rates as a function of cell survival

Cells that are cooled at reduced rates are affected less by the warming rates than are cells which are cooled at rates equal to or greater than a predetermined optimum. This article points out the dilemma of choosing (difficulties of attainment neglected) an optimum cooling rate for a highly differentiated organism such as a mammal.

["The Role of Cooling Rates in Low Temperature Preservation", by S. P. Leibo and Peter Mazur, from *Cryobiology*, 8:447-452, 1971.]

This study was based on the comparison of the effects of cooling rates, warming rates, and additive concentrations at temperatures less than 0°C (on the survival curve of mouse marrow stem cells and Chinese hampster tissue-culture cells). It is suggested that a search for optimum cooling and warming rates should receive high priority as an approach to maximizing low temperature preservation of mammalian cells.

The article states"...it appears that the obstacle is encountered during the cooling to and/or warming from the storage temperature. The problem of preservation of a given cell type, then, becomes essentially a search for an optimum cooling rate and an optimum warming rate". It is proposed that cells which are cooled slowly undergo dehydration and are damaged by prolonged exposure to the solutions which remain within the cell. Cells frozen at higher rates experience a lesser degree of "solute damage" and a greater degree of survival. This is due to the phase change taking place before dehydration is excessive.

A certain "optimum" cooling rate is reached, after which increased cooling rates cause more extensive "intracellular freezing" (crystalization within the cells) and the survival curve begins to once again decrease. The authors conclude, "Therefore, it would not be surprising if the response of the rapidly cooled cell to warming differed from that of the slowly cooled cell, as is indeed the case".

Results of the study indicated that there is no one optimum cooling rate which applies to all cell types. The search for optimum cooling rates is further complicated by the fact that these rates appear to be affected by type and concentration of the protective additives used. In studying the effects of the type and concentration of

protective additives, the following information was obtained regarding DMSO vs. PVP (dimethyl sulfoxide vs. polyvinylpyrrolidone) in a Hanks balanced salt solution.

Additive and Concentration	Survival (%)
None	0
0.004 m PVP	32.0
1.0 m DMSO	58.2
0.004 m PVP + 1.0 m DMSO	97.1

The above reflects the survival of hampster cells cooled at 1.5°C per minute to -196°C and rapidly warmed.

The article concludes by suggesting that further measurements of cell survival as a function of cooling rate will contribute to a better understanding of the mechanics of freezing damage, if methods are employed which yield both theoretical and practical information.

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neurologic cooling study in "Ph. D. monkeys"

Primates were used to test brain tolerance to extended ischemia at 10°C. The results of this study demonstrate that increased prospects of survival could be achieved by early low temperature perfusion of the brain, in addition to external cooling, during Phase I procedures.

["Monkey brain cooling study may permit longer surgical procedures", Medical News, in the *Journal of the American Medical Association*, 218:1507, 6 December 1971.]

Due to time requirements, some neurosurgical procedures are considered impossible (i.e., too complicated and time-consuming) by the present level of technology. Local, extracorporeal cooling is already being employed, extending the time for certain procedures. This is inadequate in other cases. Lengthened operative time would enable more difficult and advanced procedures to be used.

Toward this end, Robert J. White, M.D., professor of neurosurgery at Case Western Research University and director of neurological surgery at Cleveland Metropolitan General Hospital, studied 17 highly trained monkeys to determine the effect of lowering the brain temperature to 10°C by extracorporeal perfusion. 13 monkeys survived 30 minutes of circulatory arrest at this temperature. The surviving animals were retested on the skills they had learned prior to the induction of hypothermia. No evidence was found of any brain damage or psychological impairment.

Dr. White's work also included the perfusion of isolated monkey brains. He found that the EEG from a brain which is receiving no input from the usual sensory neurological circuits is very similar to the EEG taken before the brain was removed. This is in contrast to the notion that no EEG, or at the very least, a markedly abnormal EEG, would be produced without the usual inputs from external sources.

In conclusion, Dr. White states that "the data support the concept that both 10°C and one half hour of circulatory arrest to the brain are safe".

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emergency ambulance services

The Oregon Board of Medical Examiners has recently made it possible for specially trained ambulance personnel in telemetry contact with a physician to administer cardiac defibrillation (electric shock to restore coordinated heartbeat) and certain injections which can make the difference between death and survival to victims of heart failure. Before these rulings, the above techniques were limited (and still are in most states) to physicians and nurses. The precedent set by Oregon is of value to SSH Donors since its gradual extension into general practice will improve chances for effective rescue by paramedical professionals of the future.

["Cardiac Defibrillation by Ambulance Attendants", by Leonard B. Rose, M.D., and Edward Press, M.D., M.P.H., in the *Journal of the American Medical Association*, 219:63-68, 3 January 1972.]

The most important advancements in modern medical science can only be beneficial if they are readily available. All the technology of today will not help a heart patient who does not reach the hospital in time to take advantage of advanced equipment and knowledge. Approximately 1000 persons per day die from acute myocardial infarction (heart failure). 70 percent of these die before they reach a hospital. The availability of cardiac resuscitative measures en route can often mean the difference between survival and death.

Mobile intensive care units to bring emergency care to patients have been established in many cities. In particular, a project begun in Oregon in 1969 (a joint project involving the State Board of Health, a large community hospital, and a commercial ambulance company) has accomplished several important things. It has demonstrated the possibility of incorporating mobile services for emergency cardiac compression, including electrical defibrillation and administration of certain medications (under radiotelemetry supervision and direction) by the ambulance attendants, with a nominal dependence on public funds and very little increase in costs to the patient.

Two important medico-legal problems were surmounted. The first regarded the question of whether or not trained ambulance personnel could use cardiac defibrillation equipment without the presence of physicians or nurses. In 1969, the Oregon Board of Medical Examiners ruled that the use of electric cardiac defibrillation could be used by paramedical personnel in cases of emergency. The decision was based on the fact that cardiac defibrillation, like mechanically aided pulmonary resuscitation, would be considered an emergency procedure rather than the practice of medicine.

The second question was the legality and appropriateness of the administration of medications, such as atropine, by specially trained paramedical personnel who were under the radio direction of a physician and in response to the physician's order. In 1970, the Oregon Board of Medical Examiners ruled that under the specified conditions, specially trained ambulance attendants could administer certain medications by injection.

The effectiveness of mobile coronary units under this project is well documented in the article. Programs like this, in cities throughout the country, could greatly reduce the rate of prehospital deaths due to myocardial infarction. More important, in relation to SSH, are the precedences set by the Oregon Board of Medical Examiners which may be useful to those coordinating rescue and resuscitation attempts.

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better prehospital emergency care

In August of 1971 the Engineering Foundation sponsored a conference on "Engineering in Medicine -- Biotelemetry". This article summarizes the conclusions of doctors and engineers regarding the application of specialized communications in emergency health care vehicles.

["Where would you like to have your heart attach?", a Special Staff Report, in the *IEEE SPECTRUM*, Vol. 8, No. 10, pp. 44-50.]

The function of an ambulance or other emergency vehicle is defined as the ability to deliver the patient to the hospital alive. An increasing awareness exists that the majority of deaths from acute myocardial infarction occur within an hour of the onset of symptoms, and usually before admission to a hospital. This awareness finally led to the first mobile coronary care unit in Belfast, Ireland in January of 1966.

The dramatic success of the Belfast program prompted similar projects to spring up around the world. The article discusses several domestic programs in some detail. The emergency vehicles are manned with highly trained paramedical personnel who have at their disposal advanced medical and communications-telemetry equipment. Such emergency vehicles are as important in caring for victims of vehicular accidents and drug overdoses as in cardiac disabilities.

Basically, the emergency vehicle is only one element of a two part (interacting) system. First, the paramedical personnel make a preliminary diagnosis, based on the observation of an ECG oscilloscope trace. Then a cardiologist is contacted via a communications system and these observations are reported to him. The cardiologist may view a telemetered ECG pattern, so that he may confirm the diagnosis, and order appropriate actions, often including defibrillation and administration of suitable drugs. This procedure has been given legal sanction in states where these programs have been initiated.

The article also discusses some of the problems connected with the widespread deployment of emergency vehicle programs. In general, technical (medical and engineering) as well as economical and organizational problems still need to be tackled. But there is no question as to the effectiveness of emergency vehicles in cases where prehospital care is required.

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advances in medical sciences

The Science News section of the American Medical Association recently released its "Medicine in Review - 1971". Among the eight advances it finds most significant in 1971 are areas of research which also are of interest to those in life extension.

["AMA GRAMS", in the *Journal of the American Medical Association*, 210:570, 31 January 1972.]

Artificial and transplanted organs. Of the 7,581 kidney transplants performed worldwide since 1953, 3,000 patients have survived. Twenty-eight heart transplant recipients are currently alive. Nine of these transplants were performed more than three years ago. Heart transplants are declining in number (from 101 in 1968 to 17 in 1970) partly due to advanced techniques in open heart surgery.

Transplant techniques have not been confined to the heart and kidneys. Attempts at transplantation have been extended to the liver, lungs, pancreas, ovary, and bone marrow. Success ratios were not given, but it was stated that the increase in success is due primarily to better understanding of the immunological processes.

Research has been extended toward the development of artificial organs such as the pancreas. Silicone rubber heart pumps and externally rechargeable battery systems for pace-makers have also been developed.

Reproductive processes. New tests for predicting ovulation, sperm storage and reproduction of mammals "outside the body" were discussed. Close to 70 women have now had babies by means of arificial insemination of semen which had been "deep frozen". Techniques for ex vitro reproduction of whole mammals have been improved, making possible the study of prenatal growth.

Cancer research. In 1930 only 20 percent of the victims of cancer survived. Today 33 percent survive. Marked improvements have been made in the treatment of cancer of the colon, rectum, bladder, breast, uterus, and ovary. The improvements in lung and stomach cancer therapy have been less encouraging. Viruses have been discovered in several forms of cancer and a new discovery, the viroid, may prove to be responsible for some forms of cancer.

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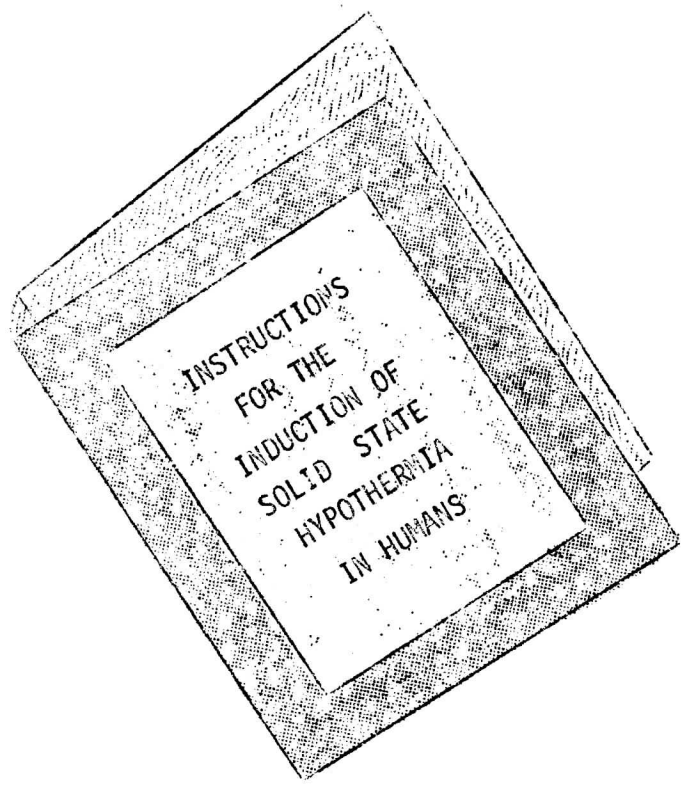

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