TISSUE-CULTURE EVALUATION OF THE VIABILITY OF BLOOD VESSELS STORED BY REFRIGERATION*

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For several decades experimental surgeons have been studying methods for the storage of vascular segments and have been working toward the goal of using such stored vessels as grafts whenever it is necessary to bridge a large gap in the arterial or venous system. Vascular segments have been stored in vaseline, moist air, saline, Ringer's solution, and other media, at temperatures slightly above freezing.[6, 7, 8] Vessels have also been stored in a frozen state.[2, 3, 16] To evaluate the viability of a stored vessel, examinations have generally been made by studying its histological detail or by implanting a portion of it as a graft and observing it during a survival period in a living animal. The viability of stored tissues destined for grafts has been tested by tissue-culture methods on only a few occasions,[8, 23] but such tests have not been applied to the study of blood vessels. It is the purpose of the present communication to record our observations on tissue-culture studies which were made on arterial segments (animal and human) which were stored by various methods.**

METHODS EMPLOYED FOR VESSEL STORAGE

First Method—Storage in 10 Per Cent Homologous Serum and Balanced Salt Solution

Portions of aorta or its large branches were obtained under aseptic conditions within five hours after death from mongrel dogs of various ages, young adult human beings, a young baboon, and a young pig. Following ligation of branches with 0000 silk the vessels were divided into segments 2 to 6 cm. long which were suitable for grafting and into control segments 0.3 to 1 cm. long. The vessel sections were held temporarily in sterile "Ringer's"† or balanced

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** This study has been greatly facilitated by the generous assistance of Dr. John F. Enders, The Children's Hospital, Boston, Mass., and Dr. John H. Hanks, Department of Bacteriology, Harvard Medical School, Boston, Mass., who suggested the methods of storage and of tissue culture and guided the project at many stages.

† The "Ringer's" solution had the following composition per liter: NaCl 7 Gm., Na Lactate 2.7 Gm., KCl 0.4 Gm., $CaCl_2$ 0.2 Gm.

salt solution (hereafter called "BSS"),* and as soon as possible thereafter (generally within 30 minutes and always within 2 hours) were placed individually in sterile 25 or 50 cc. Erhlenmeyer flasks containing 10 or 20 cc. of the following nutrient medium: BSS 85 per cent, homologous serum** 10 per cent, penicillin and streptomycin (1000 units each per cc.) 5 per cent. During most of this study, flasks were stopped with cotton and the volume of fluid was so chosen that it just covered the tissue (Fig. 1.). Segments for tissue-culture or histological control were generally included in the flasks, with the graft segments but occasionally in the earlier part of the work they were placed in separate flasks. Recently it has seemed better practice to close the flasks with a tightly-fitting skirt type rubber stopper. Most of the flasks were stored at 1° to 4° C. in the hospital blood bank refrigerator; a smaller number were held in a common domestic refrigerator, the temperature of which varied between 6° and 11° C. In some instances the nutrient medium was renewed at intervals, generally every two weeks. During storage the flasks were carefully observed for cloudiness, growth of fungi, or changes in pH. In a few instances bacteriological studies were made. These included culture of the centrifuged and washed sediment from the storage media and measurement of

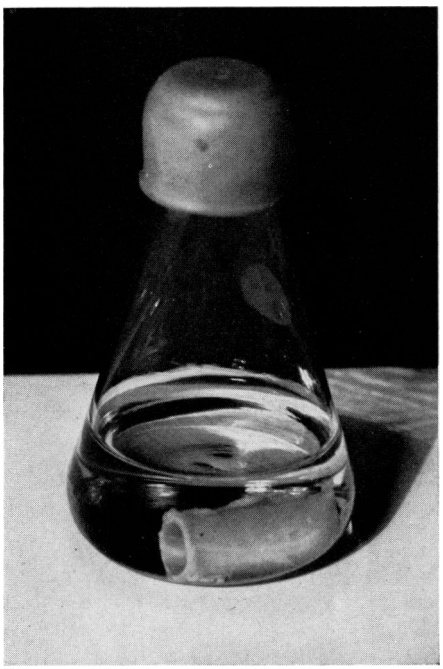

FIG. 1.—Blood vessel stored in flask containing 10% homologous serum and balanced salt solution.

* BSS solution was supplied by Dr. J. H. Hanks. It resembles "Tyrode's" solution and was prepared as follows:

Stock solution, contents per 250 cc.: NaCl 20 Gm., KCl 1 Gm., $MgSO_4 .7H_2O$ 0.2 Gm., $MgCl_2 .6H_2O$ 0.2 Gm., $CaCl_2$ 0.35 Gm. (dissolved separately), Na_2HPO_4 0.15 Gm. (0.38 Gm. of $Na_2HPO_4 .12H_2O$), $KHPO_4$ 0.15 Gm., glucose 2.5 Gm., 0.4% phenol red 12.5 cc.

Buffer: 1.4% $NaHCO_3$.

The stock solution was stored at room temperature with 1 cc. chloroform. The final solution was made by diluting the stock 1:10, autoclaving, and adding 0.5 cc. (previously autoclaved) buffer per 20 cc. This was stored in cotton-stoppered containers in the ice-box, which caused pH equilibration at about pH 7.6.

A somewhat similar solution prepared according to Simms' formula is available commercially from Microbiological Associates, Flemington, New Jersey.

** Baboon and pig vessels were stored in dog serum. Fresh or frozen serum was employed.

the antibacterial titer of the storage fluid from time to time.*

Second Method—Storage in Helium under Pressure

Segments of dog vessels were washed in BSS, placed in test tubes, and then stoppered tightly. The air was replaced with medicinal-grade helium at 76 cm. Hg. above atmospheric pressure and the tubes were stored at 0° C.

Third Method—Storage in Salt Solutions

Several experiments were carried out, placing sections of dog arteries in cotton-stoppered 50 cc. flasks in normal saline, "Ringer's" solution, or BSS. In each instance penicillin and streptomycin solution was added (giving a final concentration of each drug of 50 units per cc.). These were stored at 6° to 11° C.

Fourth Method—Storage in 100 Per Cent Serum

Dog vessel segments were placed in 10 cc. of autologous serum in 25 cc. cotton-stoppered flasks. Their refrigeration temperature was 1° to 4° C. No antibiotics were added.

Fifth Method—Storage in Serum Ultrafiltrate, With and Without 10 Per Cent Homologous Serum

Dog vessel segments were placed in 50 cc. flasks containing 20 cc. of fluid which was composed of serum ultrafiltrates** 33 per cent and BSS 67 per cent. To each flask was added penicillin-streptomycin (final concentration 50 units per cc.), and to about half, 2 cc. homologous serum. Approximately half of the tubes were closed with sterile cotton and the remainder with skirt type rubber stoppers. Some of the flasks were stored at 1° to 4° C. and some at 6° to 11° C.

Sixth Method—Storage of Vessels in Frozen State

Segments of dog vessels of varying size were placed individually in sealed 15 mm. Pyrex test tubes or sealed one-half inch heavy copper tubes and frozen in a variety of ways to approximately —70° C. They were all then stored at about —76° C. in a solid carbon dioxide deep-freeze.† When specimens were to be used for grafting or for tissue-culture studies, they were thawed by immersing the tubes for 3 to 10 minutes in a 37° C. water bath.

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* The bacteriologic examinations were kindly performed by George E. Foley, Department of Pathology of The Children's Hospital.

** Serum ultrafiltrate is obtainable commercially from Microbiological Associates, Flemington, New Jersey.

† Most of these experiments were carried out in conjunction with Dr. C. A. Hufnagel, Department of Surgery, Peter Bent Brigham Hospital, Boston, who shared the vessels stored for grafting.

The methods of freezing were as follows:

A. Glass tubes containing air at atmospheric pressure were immersed in a mixture of alcohol and solid carbon dioxide.[4]

B. Sealed glass tubes were filled with helium at 20 to 152 cm. Hg. above atmospheric pressure and frozen by immersion in an alcohol-carbon dioxide mixture.[16]

C. Glass tubes with helium at 76 cm. Hg. above atmospheric pressure were slowly brought to —70° C. in about 30 minutes rather than rapidly as in A and B.

D. Copper tubes flushed with sterile mineral oil and filled with helium at about 152 cm. Hg. above atmospheric pressure were immersed in an ether-carbon dioxide mixture for very rapid freezing. To ascertain if the copper *per se* was toxic to the tissue, several tubes were not frozen but were stored at 6° to 11° C. instead.

FIG. 2

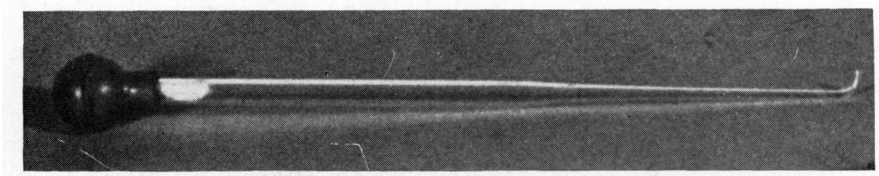


FIG. 3

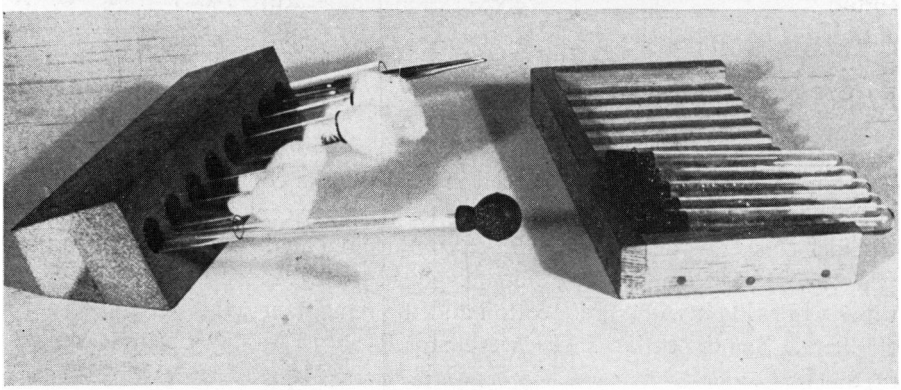


FIG. 2.—Right angle pipette for transferring tissue to culture tube. Made by drawing out 8 mm. soft, glass tubing.

FIG. 3.—*Left*—Wood block used for holding reagents and Bard-Parker knives. *Right*—Test tube holder employed for setting up cultures and for slanting them in the incubator (after Hanks).

METHODS EMPLOYED FOR TISSUE-CULTURE STUDIES

When a portion of vessel was to be cultured, it was placed in a sterile Petri dish, washed with BSS, and cut into 1 to 2 mm. squares the full thickness of the vessel wall with two No. 11 Bard-Parker knives which had been steril-

ized by storage in 95 per cent ethyl alcohol and ignition of the alcohol. The BSS was then pipetted off and the following transfer medium substituted: Homologous serum* 2 cc., BSS 2.5 cc., beef embryo juice** 0.5 cc., penicillin and streptomycin solution (1000 units each per cc.) 0.5 cc. Sterile acid-cleaned 125 x 15 mm. test tubes were prepared by rinsing their entire lower half with one drop of chicken plasma.† Four pieces of a given tissue were then transferred to each of two tubes with a small right angle pipette (Fig. 2.). After aligning these fragments in the lower one-half of the tube, 4 to 6 drops of the transfer medium were added and the tubes rotated and tilted to mix the medium and the chicken plasma thoroughly (Fig. 4). The tubes were then placed flat, with the tissue down. When firm clotting had occurred (usually after 5 to 10 minutes) 10 to 15 additional drops of medium were added with care to wet the entire surface of the clot. The tubes were then closed tightly with rubber stoppers, slightly slanted (Fig. 3), and place in a 37° C. incubator. Readings were taken at intervals for one, and occasionally two weeks, by examining the edges of the tissue explants for cell growth under low power and by observing changes in pH. Any increase in acidity due to tissue metabolism was shown by change of color from pink (pH 7.4–7.6) to orange (pH 7.2–6.8) to yellow (below pH 6.8). The fluid in the tubes was not renewed but one drop of bicarbonate buffer was added if the fluid became yellow. When growth occurred, a rough index of its extent was obtained by recording the proportion of explant circumference supporting cells. Growth consisted of fibroblastic proliferation. Since endothelium is difficult or impossible to differentiate from this[21] and may actually transform into fibroblasts[27] no special effort was made to distinguish it.

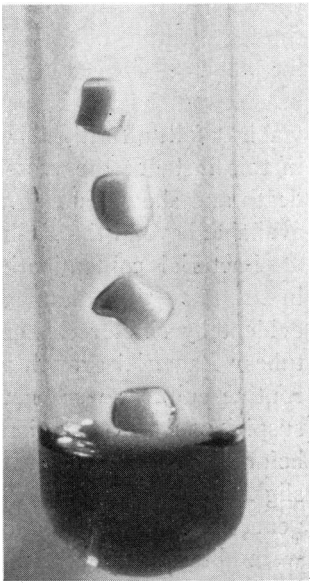


FIG. 4. — Tissue cultures showing tissue fragments in proper position and correct supernatant.

* Dog serum was used for the baboon and the pig vessels. The serum was centrifuged twice and stored in 2 cc. amounts at —20° to —25° C.

** Supplied by Dr. J. H. Hanks. Three to 5-inch beef embryos and an equivalent volume of BSS solution were mixed in a Waring blender for 1.5 minutes. The suspension was centrifuged for 30 minutes at high speed and the supernatant fluid collected and respun. The resultant fluid was tested for sterility and stored in small amounts at —20° to —25° C. Before use, the unfrozen material was re-centrifuged.

† Supplied by Dr. J. H. Hanks. Chicken blood was taken in a 10 cc. syringe containing 0.2 cc. of 10% sodium citrate, centrifuged twice, and stored in 1 to 2 cc. amounts in cotton-stoppered tubes at —20° to —25° C.

Some tissue cultures were photographed fresh, while others were fixed in Zenker's solution, cut at 3 to 5 mu., and stained with hematoxylin and eosine.

OBSERVATIONS ON VESSELS STORED BY THE VARIOUS METHODS

I. Tissue Preserved in 10 Per Cent Serum in BSS

Vessels stored within six hours following death (of animal or human) showed no consistent diminution in vitality due to this delay. Most of the tissue taken near six hours, however, grew less luxuriously than tissue taken within two to three hours. Variations in body size (of animal or human), the temperature of the room or icebox in which a body had been kept before the blood vessels were removed obviously presented factors which had a direct bearing on the viability of the removed tissues. The probability that vessel viability falls off rather sharply after death is supported by one human example; in this instance the vessels were obtained between 6½ and 7½ hours after death and showed no growth on tissue culture. Except for this, data are not available beyond the six-hour period.

Bacterial contamination is manifested by the appearance of cloudiness in the storage medium or by an abrupt change in its pH. It is also made evident by similar changes plus the growth of colonies in the tissue-culture tubes. Bacterial growth was not observed in stored dog tissue and although routine cultures were not taken, the culture of spun sediment from several suspicious flasks on various occasions yielded no growth. Human tissue stored before six hours was without demonstrable bacterial contamination. When slight cloudiness appeared after several weeks of storage, the spun sediment generally consisted of amorphous debris. In the one case where human vessels were obtained after six hours, cloudiness and demonstrable bacterial contamination became evident in each of eight flasks starting at 15 days. The contaminant (probably present when the tissue was first preserved) was *Ps aeruginosa.* Once it had made its appearance, it could not be suppressed with eight times the original antibiotic concentration.

In contradistinction to bacterial contamination, growth of fungi occurred sufficiently frequently (under 5 per cent) in stored dog tissue to constitute a nuisance, but was not observed in stored human tissue. Such contamination was generally not evident until three or four weeks of storage. Its appearance was unrelated to the type of stopper used. It was easily recognized by inspection of the flasks, all of which were immediately discarded.

The results of antibiotic titer tests were surprising and gratifying (Table I). Although the very high effective antibacterial activity cannot readily be explained, it probably results from the combined action of the specific agents, the serum, and possibly even the stored tissue. This high antibacterial titer, together with the low storage temperature, probably explains the very low incidence of bacterial contamination which was encountered.

Dog aorta maintained in cotton-stoppered flasks at 1° to 4° C. showed growth comparable to that of the fresh controls for approximately 20 days. After this time both the per cent of tissue pieces exhibiting growth and the

extent of growth apparently fell slowly, but substantial growth was demonstrated after storage periods of 50 days (Figs. 5 and 7). Tissue cultured during the first three weeks of storage frequently showed earlier and more luxuriant growth than the controls, suggesting a diminution of the usual adult growth inhibition.[28, 29] Tissues stored for more than three weeks generally exhibited a lag in the appearance of growth. For all storage intervals, the extent of growth paralleled the percentage of viable tissues.

Dog aorta stored at 6° to 11° C. in cotton-stoppered flasks grew more luxuriantly than that stored at 1° to 4° C. for two weeks but did no better at four weeks.

TABLE I.—*Antibacterial Titer of 10% Serum and BSS Storage Medium and Its Components*

Material Studied	Length of Use	Oxford Units/cc. Equivalent Penicillin Titer*	Mcg./cc. Equivalent Streptomycin Titer**
Dog serum	Fresh-frozen	0	640
Human serum	Fresh-frozen	0	640
BSS	Fresh	0	0
BSS plus dog serum	Fresh	0	640
BSS, dog serum, penicillin and streptomycin, (standard storage medium)	Fresh	1280	2000
	Fresh	512	6400
BSS, dog serum, penicillin and streptomycin, blood vessel	14 days	640	1000
BSS, human serum, penicillin and streptomycin, blood vessel	23 days	640	2000
	25 days	512	6400
	26 days	512	5120

* Assay organism *B. sublilis*, strain S.D.[26].

** Assay organism B. circulans[25].

The antibiotic assays were done according to the standard methods of the Food and Drug Administration.

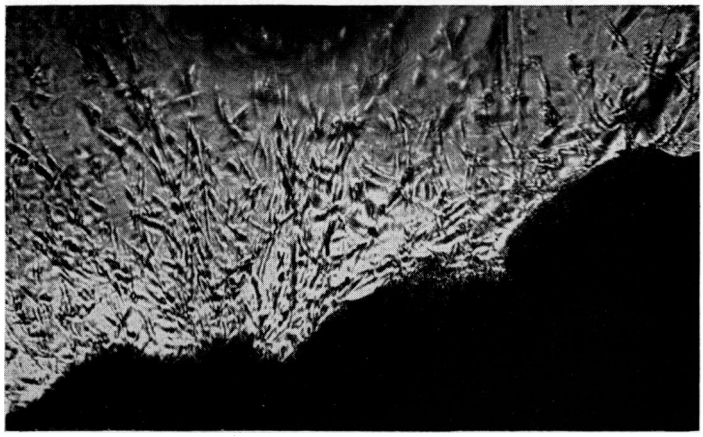
A smaller series of cultures of human vessels stored at 1° to 4° C. showed roughly comparable results (Table II, Fig. 6).

Pig and baboon aortas stored in 10 per cent dog serum and BSS maintained viability for about two weeks.

Renewing the nutrient medium in the storage flasks at two week intervals produced no definite growth improvement, and since it was shown that the original antibiotic titer was well maintained, the practice was discontinued.

In all of the cotton-stoppered series the storage of small (less than 1 cm.) portions of tissue alone adversely affected their chance of survival. Larger pieces of tissue produced an appreciable drop in pH of the storage medium within a few days to a week; the flasks containing only small tissue fragments at best maintained a constant pH but generally became more alkaline with time. Small fragments which had been stored alone regularly failed to show growth after short intervals. In order to give an overall picture, results obtained when small pieces of tissue were stored alone have not been excluded

FIG. 5



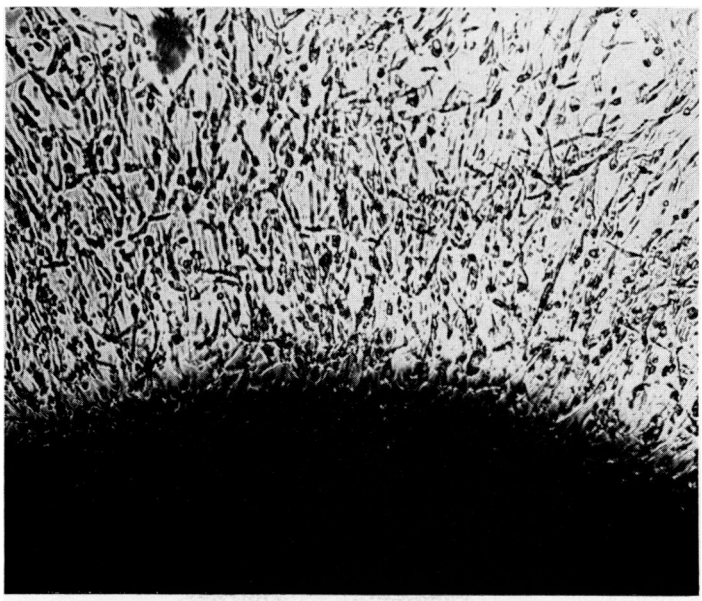


FIG. 6

FIG. 5.—Photomicrograph, unstained x55, of dog aorta tissue-culture. The aorta had been stored for 50 days in 10% homologous serum and BSS at 1° to 4° C. and had then been cultured for 7 days. There is active growth of fibroblasts.

FIG. 6.—Photomicrograph, unstained x55, of human aorta tissue-culture. The aorta had been stored 28 days in 10% homologous serum and BSS at 1° to 4° C. and had then been cultured for 7 days. There is active growth of fibroblasts.

TABLE II.

Results of tissue-cultures of human aorta stored in 10% serum and BSS at 1° to 4° C.
Three fresh controls showed 80% growth.
Each percentage represents studies on 8 pieces of tissue.

Duration of Storage	Percentage of Pieces Growing	
	Cotton-Stoppered Flask	Rubber-Stoppered Flask
1 day	75.	...
2 days	37.5	...
3 days	62.5	100
7 days	87.5, 0, 0	...
14 days	87.5, 12.5, 0, 0	100
16 days	37.5	100
21 days	75, 0,0	...
26 days	...	87.5
27 days	62.5, 0	87.5
36 days	25	...
37 days	...	75
42 days	0	...
49 days	0	...

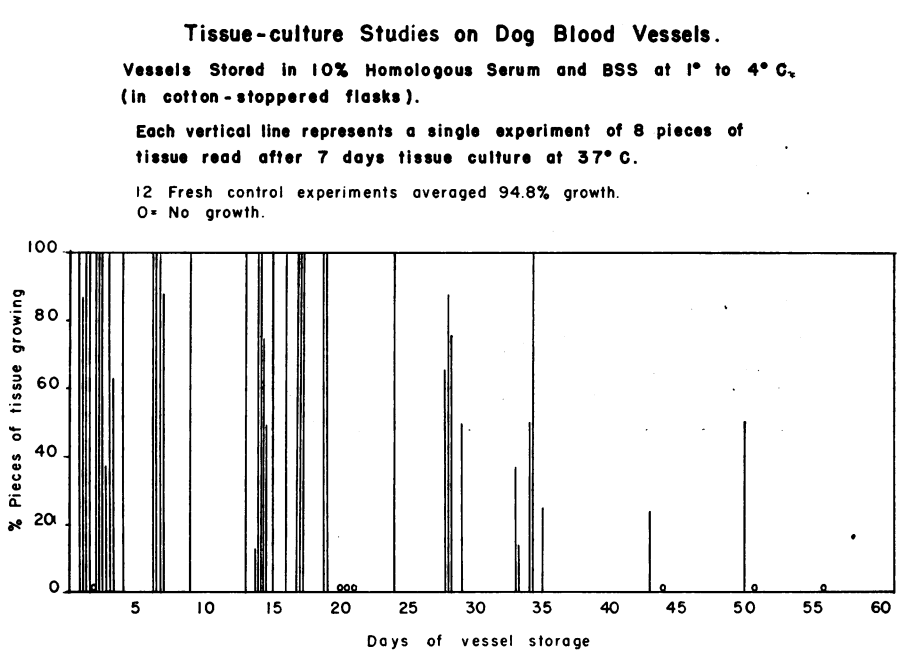


FIG. 7.—Results of culture of specimens of dog aorta which had been stored for varying periods of time. The vessels could be grown in a high percentage of cases after 35 days of preservation, and in one instance could be grown after 50 days of storage.

from Fig. 7 and Table II. A breakdown of some of the human data, however, shows how important this factor of small size is in influencing viability (Table III). For this reason, whenever it has been necessary to store small tissue pieces in cotton-stoppered flasks, the viability of the small piece can be enhanced by including a large piece of tissue in the flask.

TABLE III.—*Effect of Tissue Size on Survival. Blood Vessels, Human, Stored in 10% Serum and BSS at 1° to 4° C. as Large and Small Pieces and Then Cultured at 37° C. for 7 days.*

Length of Vessel Storage	Stored as Large pieces (3–4 cm.)		Stored as Small pieces (less than 1 cm.)	
	Number Pieces Cultured	Percentage Growth	Number Pieces Cultured	Percentage Growth
1 day	..	....	8	75.
3 days	..	....	8	62.5
7 days	..	....	24	16.
14 days	8	87.5	16	6.3
21 days	8	75.	16	0
28 days	8	62.5	8	0

TABLE IV.—*A Comparison of the Effects of Storage Temperatures and the Method of Sealing of Storage Flasks on Growth of Dog Arteries Preserved in 10% Serum and BSS. The Various Groups of Tissue-Cultures Were Carried Out Concurrently.*

Duration of Storage	Storage Temperature	Percentage of Pieces Growing	
		Cotton-stoppered	Rubber-stoppered
14 days (M)	1°–4° C.	100	100
17 days (L)		100	100
17 days (M)		100	100
17 days (S)		100	100
28 days (M)		62.5	100
33 days (L)		37.5	100
33 days (S)		12.5	100
44 days (M)		0	50
57 days (M)		...	0
17 days (M)	6°–11° C.	100	100
33 days (M)		Contaminated	100

Four fresh controls showed 100% growth.
Each percentage represents studies on 8 pieces of tissue.
L—approximately 6 cm. vessel segment.
M—2 to 3 cm. vessel segment.
S—1 cm. or smaller vessel segment.

In contrast to the results observed on tissues stored in cotton-stoppered flasks, blood vessels stored in rubber-stoppered flasks either at 1° to 4° C. or at 6° to 11° C. showed more reliable growth (Tables II and IV). When stored in rubber-covered flasks, the size of the tissue had very little effect on viability and in one instance 1 cm. sections of dog carotid artery grew as well after 33 days' storage as at 17 days. A cotton-stoppered control grew well at 17 days but very poorly at 33 days. The data on vessels stored at 6° to 11° C. is limited but indicates that this storage temperature is satisfactory.

Vessels in sealed flasks produced an appreciable drop in pH much more quickly than did similar vessels in cotton-stoppered flasks. Generally, the original pink color (pH 7.6) gave way to an orange (pH 7.2–6.8) within 12 to 24 hours. When this drop failed to occur, tissue-culture produced no growth. This early pH drop is therefore suggested as an important gross indication of viability. Once the pH had fallen to 6.8–7.2, it changed very slowly thereafter.

Blood vessels stored in 10 per cent serum and BSS were slightly yellowish in color, but showed no important edema. They retained their normal con-

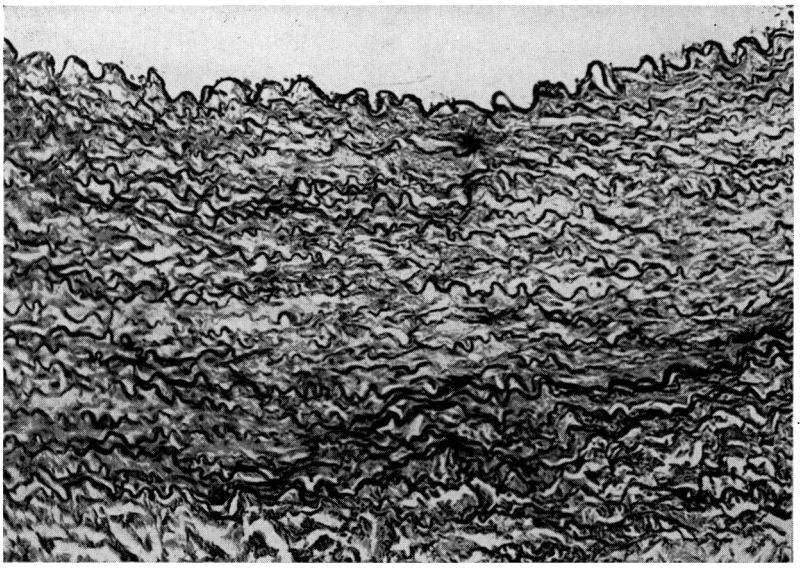


FIG. 8.—Section of stored dog aorta, showing excellent preservation of architectural detail. Hematoxylin and eosine. Vessel had been stored in 10% homologous serum and BSS at 1° to 4° C. for 56 days.

sistency and were indistinguishable from fresh vessels in suturing properties. Histological sections of tissue stored as long as 56 days showed preservation of architecture and cell detail (Fig. 8). Aortic grafts of stored tissue were generally successful; dehiscence and thrombosis rarely occurred (Fig. 9).

II. Tissue Preserved in Helium under Pressure at 0° C.

Tissue preserved by this method showed rapidly diminishing viability with no growth after one week and the segments were strongly acid in reaction indicating an excess accumulation of metabolites.

III. Vessels Preserved in Salt Solutions

Dog aortas held in BSS, normal saline, or "Ringer's" solution at 6° to 11° C. for periods up to four days grew almost as well as fresh controls.

After this time, there was a constant loss of viability and by 14 days' storage only a small proportion of the explanted tissue pieces grew. For this particular set of experiments, the normal saline was the most favorable and the "Ringer's" solution the least favorable storage medium.

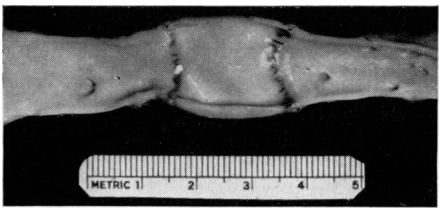


FIG. 9.—Graft of abdominal aorta (dog to dog). The grafted section had been stored in 10% homologous serum and balanced salt solution for 6 days and then had been implanted into a recipient animal which was kept for 6 months before sacrifice.

IV. Vessels Preserved in 100 Per Cent Serum

Tissue stored in whole autologous serum at 1° to 4° C. exhibited no growth or tendency to reduce the pH of the medium after three days' storage. Material stored for shorter periods was not studied.

TABLE V.—*Results of Tissue Cultures on Dog Aorta Stored in Serum Ultrafiltrate and Serum Ultrafiltrate with 10% Serum and BSS. Medium-sized Pieces of Tissue Were Stored.*

Storage Medium	Storage Temperature	Storage Time	Percentage of Pieces Growing: Cotton-stoppered	Percentage of Pieces Growing: Rubber-stoppered
Serum ultra-filtrate.....	1°– 4° C.	14 days	75.	...
		17	87.5	100
		29	62.5	...
		44	25	..
		57	0	...
	6°–11° C.	17	100	100
		33	...	100
Serum ultra-filtrate with 10% serum and BSS..	1°– 4° C.	14 days	62.5	...
		17	100	100
		29	50	...
		33	25	87.5
		44	37.5	...
		57	12.5	...
	6°–11° C.	17	100	100
		33	...	25

Each percentage represents studies on 8 pieces of tissue.

V. Vessels Preserved in Serum Ultrafiltrate

In general, dog vessels preserved in serum ultrafiltrate or serum ultrafiltrate plus 10 per cent serum showed growth roughly comparable to that obtained when 10 per cent serum in BSS was employed. The series of experiments was small and there were a number of variations but the impression was obtained that storage in 10 per cent serum in BSS was a little better. The addition of 10 per cent serum in BSS to the serum ultrafiltrate did not appear appreciably to improve growth. As with the earlier studies, however, sealing the flasks with rubber stoppers led to a distinct improvement in

viability of the stored tissue. The effect of the higher temperature, 6° to 11° C., was again to produce more luxuriant growth at about two weeks but little difference at four or five weeks. pH changes were comparable to those encountered with 10 per cent serum in BSS but the solutions tended to remain more alkaline. The results are compared in Table V.

VI. Vessels Preserved by Freezing

Material stored at —76° C. showed positive growth on tissue-culture in only two of 34 experiments.* In one instance the tissue was frozen rapidly (Method B) and in another it was frozen slowly (Method C). The growth was poor in both instances. Of 12 dogs who received frozen aortic grafts, all but two died because of thrombosis or breakdown of the graft. The tissue appeared to be more friable and more difficult to suture than fresh tissue. These results are unlike those reported by Blakemore[2, 3] and by Hufnagel.[16]

DISCUSSION

Although a graft of dead bone, cartilage, or blood vessel, may provide a suitable framework and produce a functional result[12, 13, 19, 20] the evidence at hand indicates that in the case of arteries, at least, more reliable results are obtained when living tissue is employed. Consequently, efforts have been directed primarily to finding a method of preserving vessels in a viable state. Several modes of storage, including freezing, have been abandoned by us at least temporarily, since, when employed, viability could be demonstrated only sporadically and, in experimental animals results from grafts were poor. The method of storage recommended in this paper is not entirely new but is based on the previous work of many others.

Bert[1] refrigerated rats' tails in confined air at about +12° C. for several days and then successfully transplanted them. Carrel[6, 8] stored blood vessels, skin, fascia, and other tissues, in vaseline, moist air, normal saline, and "Ringer's" solution, at temperatures slightly above freezing. He evaluated his method of preservation after one to six days by cultivation of stored embryonic tissue and after intervals as long as seven weeks by grafting stored adult tissue in animals. The maximum duration of cell viability was not determined. Lambert,[17] Lewis and McCoy,[22] Hetherington and Craig,[15] Waterman,[32, 33] Walter *et al.*,[31] Hanks and Wallace,[14] Garry,[10] and Carpenter,[5] studied the survival of refrigerated embryonic and other tissues by tissue cultivation or oxygen consumption, but were either unable to demonstrate viability after more than short intervals or were concerned with comparatively brief periods of storage. Their work indicates that refrigerated tissue retains its viability for varying periods of time, depending primarily on the type of tissue,[32, 33] and secondarily on the size of tissue pieces,[22] available oxygen,[10, 14, 24] storage temperature,[15, 22, 33] and other factors. Irreversible changes took place quickly

* Tissue refrigerated but not frozen in the copper tubes grew well on tissue-culture, indicating that the presence of the metal itself did not account for the failure of the frozen tissues to grow.

in brain, kidney, liver, and other solid organs, while bone, cartilage, skin, fascia, and blood vessels, survived for comparatively long intervals. The degree of preservation was more or less dependent on the size of the fragments, very small pieces of tissue surviving for only brief periods. Oxygen demand was greatly diminished as the temperature fell. Tissue fragments at 37° C. required very high oxygen tensions to prevent necrosis,[24] while at 0° C. tissues were able to survive for a time even when oxygen was excluded.[10, 14]

Tissues for clinical grafting have been stored refrigerated for short intervals. La Roe[18] kept skin in "Tyrode's" solution for periods of one week, while Castroviejo[9] stored corneas for two to five days in "Ringer's" or normal saline. Both reported satisfactory clinical results. More recently, Matthews[23] preserved human skin wrapped in saline sponges, in air-tight containers at +3° to 6° C. He reported viability after eight days as measured by tissue cultivation, and stated that autogenous grafts at three months were successful.

It is thus clear that certain tissues can be preserved vitally by simple refrigeration. However, evidence concerning the limitations of storage methods is slowly accumulating. Skin[14] and blood vessels (present report) can be preserved alive for about a week in the absence of a buffered environment by storing at 0° C. with the exclusion of oxygen. As shown both by these workers and ourselves, however, there is an unfavorable accumulation of acid metabolites and a rapidly falling viability. Tissue stored in such media as balanced salt solution, Ringer's solution, or normal saline, at temperatures just above 0° C., will also remain alive for short periods, as demonstrated by our data, but the lack of suitable nutrient material is evidently unfavorable for lengthy preservation of viability. In addition, blood vessels so kept may become edematous and prove unsuitable for vascular grafts after as little as 24 hours' storage.[11] Preservation in serum or whole blood is also satisfactory for short periods of time,[14] but the large protein and fat molecules seem seriously to interfere with metabolism.[29] The preservation of tissue in 100 per cent serum was unsuccessful in one series of experiments performed by us.

Thus, it appears that an ideal storage medium should contain a physiological concentration of salts, buffer, glucose, and the small accessory molecules which are present in serum. The large protein and fat molecules should probably be in reduced concentration. The storage medium developed by Hanks and Wallace[14] fulfills these criteria. It was found by them to be entirely suitable for storage of rabbit skin for periods up to two weeks at 8° C. Their procedure was modified in our hands by employing somewhat lower temperatures and by the more liberal use of storage medium.

The use of serum ultrafiltrate as a storage medium is suggested by the above reasoning, since all of the large molecules have been removed from it, while the small molecules remain. The data accumulated to date indicates that this solution either alone or with 10 per cent serum is satisfactory but is not superior to 10 per cent serum in BSS.

In the early part of the work cotton stoppers were used in the storage flasks because we thought it was important to facilitate the metabolic exchange

of the blood vessels which was evident even at low temperatures. This proved fairly satisfactory except in the case of small vessel segments which retained viability for only relatively short periods of time.* Experiments conducted with tightly-stoppered flasks showed that the metabolic activity was not sufficient to require a free exchange of carbon dioxide and oxygen. In addition, it was found that small vessels preserved in this latter manner remained viable as long as did large ones. Consequently, tightly-fitting stoppers are now used routinely.

The present study indicates that blood vessels may readily be preserved alive and with normal physical properties for periods of at least seven weeks. They have been safely employed in dogs and humans during that time for homoplastic grafts. Proper exploitation of this method should improve the surgical treatment of coarctation of the aorta, Tetralogy of Fallot, arterial aneurysm, injuries to major vessels, *etc.*

The general method of storage with suitable modifications might be applicable to fascia, skin, bone, nerves, cartilage, and corneal tissue.

SUMMARY [1]

The vital storage of vascular segments in 10 per cent serum and a balanced salt solution at temperatures slightly above freezing is described. The temperature of storage is not critical. The method is simple enough to be readily useful. Viability of the tissue is readily shown by growth in tissue-cultures after storage for as long as seven weeks. The preserved segments closely resemble fresh vessels in physical qualities, and a large series of successful grafts in dogs has been completed. Vessels have also been collected from humans, stored, and have been employed successfully for alleviation of the Tetralogy of Fallot and in treatment of coarctation of the aorta. This method of blood-vessel preservation appears superior to any other yet tried by us, including freezing. The theoretical basis for the storage method is discussed.

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* It has been shown [28, 29, 30] that a growth substance necessary for preservation as well as growth (A-factor) is destroyed or inactivated by alkaline media. Since the media in which small tissue fragments were stored frequently became more alkaline the loss of A-factor may be the explanation for their failure to grow.

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