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Don Weller Cline
University of Nebraska Medical Center

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THE EFFICACY OF ALCOHOL IN STERILIZING AND PRESERVING ARTERIAL GRAFTS

I. GROSS AND MICROSCOPIC CHANGES IN ARTERIAL SEGMENTS STORED IN ALCOHOL

Donald Weller Cline

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College of Medicine, University of Nebraska

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If alcohol could be used to sterilize and preserve arterial segments for homologous grafts, it would simplify the repair of diseased and injured arteries. Methods now in use for taking and preserving arterial segments are somewhat complicated, time consuming, and often expensive. We could save time and trouble if we could take arterial segments from the donor without using sterile technique. We thought placing these segments in 70% alcohol should sterilize them. Next, it would simlpify things if we could then keep the segments in a solution of alcohol until ready for use.

Reports in the literature (7,8,9,10) suggest that arterial grafts may be preserved as well by storage in alcohol as by any of the other methods now in use. Berman (1) however states that arterial grafts may be preserved successfully in alcohol for as long as two weeks but no longer. Berman indicated to us that on microscopic examination he and his coworkers had observed fragmentation of elastic fibers in aortas that had been preserved in 70% alcohol for longer than two weeks. Changes in tissue hydration might account for this. We speculated that this might be avoided or aggravated by changing the

concentrations of alcohol in the solution.

We have studied the effects of varying periods of storage in varying concentrations of alcohol on the sterility and on the gross and microscopic structure of segments of dog aortas. Our studies to date suggest that arterial grafts may be successfully sterilized and stored in solutions of alcohol.

Materials and Methods

The descending portions of the aortas of two healthy mongrel dogs were obtained, without sterile precaution, for study. These were divided into three millimeter segments and placed in a solution of 70% alcohol in water. After 24 hours, eighteen segments were transferred into 50% and eighteen into 90% alcohol. After 24 hours, twelve segments were transferred from the 90% solution into a 95% solution, and twelve segments were transferred from the 50% solution into a 40% solution. Likewise, after a similar period, six segments were transferred from the latter solutions into 30% and 100% solutions. The stepwise transfer of the segments was utilized in order to gradually hydrate or dehydrate the tissue, rather than to subject the tissue to sudden change in state of hydration.

Segments were taken for gross and microscopic examination at two, three, six, and thirteen weeks from the time of their placement in the original solution of 70% alcohol. A segment was also taken at thirteen weeks from each solution and reconstituted for two hours in normal saline solution. In addition, cultures were taken from each solution at the two, three, and six week intervals. Histologic staining was carried out by the Hematoxylin and Eosinol, and Verhoeff's Elastic Tissue techniques.

Results

The microscopic and bacteriologic findings are listed in table 1. Although several isolated variations appear, no consistant variation was found in the microscopic appearance of the segments, either in the direction of alcohol concentration or of the length of time of storage. The only consistant change noted was the appearance of moderate edema following reconstitution in saline. Bacteriologically no consistant pattern was obtained. Growth appeared twice in the 30%, 70%, and 100% solutions, and once in the 40%, and 50% solutions. No growth was seen in the 90% and 95% solutions. The only organism isolated from the culture tubes was Bacillus Subtilus.

It was thought that this was introduced as a contaminant at the time of innoculation of the culture media.

The gross variation followed a consistant pattern of progressive hardening of the segments in all solutions, reaching a constant stage at about six weeks. The degree of hardness varied directly with the concentration of alcohol. However, when reconstituted after thirteen weeks, segments from all concentrations regained a considerable degree of pliability, sufficient to permit them to be easily handled surgically. A study of the tensile strength of the stored segments is at present underway in an effort to correlate histologic and gross characteristics with usable strength.

Discussion

At the turn of the century, concomitant with investigations of means of anastomosis of arteries and veins, the first efforts were being directed towards the possible use of free grafts in the bridging of arterial defects. Alexis Carrel, in 1912, (2) was one of the first to achieve success in the preservation of vascular grafts. Despite his early success, clinical trial during World War I, in an

effort to preserve injured extremities, met with failure. Failure largely resulted from thrombosis and infection. After this interest waned. Little more was done until the great number of arterial injuries in World War II initiated renewed interest in arterial repair. Despite relatively poor results in World War II several factors have promoted continued interest in the grafting of arteries: recognition of the need for reliable methods for repairing vascular defects, new means for combating thrombosis and infection, and the development of surgery of the heart and great vessels.

A major problem in bridging arterial defects is obtaining the bridge. In many areas the need for grafts is not great enough to warrant the use of any method of graft storage which is not simple, inexpensive, and trouble free. One of the prime thoughts leading to this investigation was that it might provide a simple method whereby a surgeon or a small hospital might be able to maintain a small but adequate graft bank without a prohibitive expenditure of time or money.

To be useful in arterial repair, grafts must fulfill several criteria. These fall into three groupings. 1) Are they available, and may they be

easily prepared, stored, and maintained in a sterile state? 2) Are they workable at time of operation, ie; pliable, and able to hold sutures? 3) In the host, do they show flexibility, strength, a low degree of foreign tissue response, little tendency towards thrombosis, and longevity?

Grafts may be of several types: fresh, preserved in a viable state, or fixed. They may also be classified as autografts, homografts, or heterografts; or, further, as arterial or venous grafts. In an excellent review of the literature Schloss (11) compared each type in terms of their eventual fate in the host. The fresh autograft appeared to be retained as a live functioning tissue at the host site. Fresh homo- and heterografts, however, after a brief interval underwent degenerative change and fibrous tissue reinforcement. They may still be retained as an inert conducting channel. Preserved grafts appeared to behave in the same manner as fresh homo- and heterografts. Fixed grafts, being antigenically inert, appeared to be retained as an inert channel with fibrous tissue reinforcement. They appeared less likely to undergo sloughing than preserved or fresh homo- and heterografts.

The use of fresh grafts does not meet the first criterion, availability; and also, they can only occasionly be used in the repair of a smaller artery. Because of this, most attention has been given to the study of preserved and fixed grafts. Opinions vary as to the efficacy of preserving grafts in a viable state. There is agreement, however, as to the relatively short period for which they may be kept and the problems inherent in the technique, which is essentially that of tissue culture. (5,6.) There is relatively little to be found in the literature on the use of fixatives for the storage of grafts, particularly the use of ordinary ethyl alcohol. Nagoetti (9) first described the use of alcohol for preserving arterial grafts. He obtained fair to good results in animal experiments. Kimoto (7) reported excellent results in both animals and man with homo- and heterografts stored for periods up to and exceeding one year in 70% and 100% alcohol. Paolucci (10) reported good results in dogs in a small series. Moore (8) reported good results in comparison with freeze-drying in the preservation of small canine arteries. There have been recent reports (3,4,7,8) that the fixed tissue graft, save for the elastic fibers which are retained, is replaced by connective

tissue over a period of from one month ta a year. It would appear then that the important factor in the use of fixed grafts is that the integrity of the elastic fibers be preserved in the fixing media.

Within the limits of our study, the only consistant variation noted microscopically was the appearance of a moderate amount of edema of the muscle and connective tissue of the aorta following re-constitution in saline. Whether or not this will be of any significance will be determined by later studies in tensile strength. No consistant change was noted in the elastic fibers. The gradual hardening, which appeared grossly, seems to be reversible, by reconstitution, to an extent satisfactory to permit suitable workability. There is, so far, nothing to indicate a later radical change in the gross and microscopic appearance.

Bacteriologically, despite growth in a few instances, found to be a contaminant, Bacillus Subtilus, It would appear that growth is inhibited sufficiently to consider the grafts sterile.

This preliminary study indicates that the use of an alcohol solution provides a simple method of storage requiring no care beyond the initial procurement of

the graft. However, before final opinion can be made, further study must be made in regard to the acceptance of the graft by the host, and the functional adaptation and longevity of the graft in the host. This study is at present underway.

Summary

1) Segments of dog aorta were stored for periods of from 1 to 13 weeks in solutions of alcohol ranging from 30% to 100%.

2) Gross and microscopic examination reveal a gradual hardening of the tissue grossly, more pronounced in the more concentrated solutions, but no significant microscopic changes.

3) Reconstitution for two hours in normal saline solution restores pliability while producing moderate edema of the muscle and connective tissue of the aorta.

4) Sterilization by the various concentrations of solutions proved adequate.

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TABLE 1.

Summary of the Effects of Varying Periods of Storage in Varying Concentrations of Alcohol on the Histologic Character of Aortas from Dogs

Spec. no.	Alc. conc.	Storage time	Histology		Culture studies
			General	Elastic Tissue	
1	fresh	0	Some evidence of edema with separation of elastic fibers	Scattered areas of thinning and fraying of elastic fibers	--
2	30%	2 wks	Scattered areas of fiber separation	Elastic fibers normal in appearance	growth
3	30%	3 wks	Blue cast to elastic fibers, otherwise normal	Normal in appearance	growth
4	30%	6 wks	Appears somewhat edematous	Separation of fibers by edematous tissue	no growth
5	30%	13 wks	Loss of ground substance and separation of fibers	Normal in appearance	no growth

Spec. no.	Alc. conc.	Storage time	Histology		Culture studies
			General	Elastic Tissue	
6	30%	13 wks reconst.	Moderate edema of muscle and connective tissue	Distortion of fibers by edematous tissue	no growth
7	40%	2 wks	Blue cast to elastic fibers, some edema	Normal in appearance	no growth
8	40%	3 wks	Moderate edema of connective tissue	Normal in appearance	no growth
9	40%	6 wks	Normal in appearance	Amount of elastic tissue decreased (terminal aorta)	growth
10	40%	13 wks	Normal in appearance	Normal in appearance	no growth
11	40%	13 wks reconst.	Moderate edema of muscle and connective tissue	Normal in appearance	no growth
12	50%	2 wks	Blue cast to elastic fibers	Normal in appearance	growth
13	50%	3 wks	Moderately edematous in appearance	Elastic fibers intact, reduced in mumbers (terminal aorta)	no growth

Spec. no.	Alc. conc.	Storage time	Histology: General	Histology: Elastic Tissue	Culture studies
14	50%	6 wks	Normal in appearance	Normal in appearance	no growth
15	50%	13 wks	Normal in appearance	Normal in appearance	no growth
16	50%	13 wks reconst.	Moderate edema of muscle and connective tissue	Fibers intact, separated somewhat by edema	no growth
17	70%	1 wks	Blue cast to elastic fibers, with scattered areas of separation	Normal in appearance	no growth
18	70%	2 wks	Blue cast to elastic fib- ers, otherwise normal in appearance	Normal in appearance	growth
19	70%	3 wks	Normal in appearance	Normal in appearance	growth
20	70%	6 wks	Normal in appearance	Normal in appearance	no growth
21	70%	13 wks	Normal in appearance	Normal in appearance	no growth

Spec. no.	Alc. conc.	Storage time	Histology		Culture studies
			General	Elastic Tissue	
22	70%	13 wks reconst.	Moderate edema of muscle and connective tissue	Separation of fibers by edematous tissue	no growth
23	90%	2 wks	Normal in appearance	Normal in appearance	no growth
24	90%	3 wks	Blue cast to elastic fibers, otherwise normal in appearance	Normal in appearance	no growth
25	90%	6 wks	Normal in appearance	Normal in appearance	no growth
26	90%	13 wks	Normal in appearance	Normal in appearance	no growth
27	90%	13 wks reconst.	Moderate edema of muscle and connective tissue	Separation of fibers by edematous tissue	no growth
28	95%	2 wks	Normal in appearance	Normal in appearance	no growth
29	95%	3 wks	Normal in appearance	Normal in appearance	no growth

Spec. no.	Alc. conc.	Storage time	Histology		Culture studies
			General	Elastic Tissue	
30	95%	6 wks	Normal in appearance	Normal in appearance	no growth
31	95%	13 wks	Normal in appearance	Occasional splitting of elastic fibers	no growth
32	95%	13 wks reconst.	Normal in appearance	Normal in appearance	no growth
33	100%	2 wks	Normal in appearance	Normal in appearance	growth
34	100%	3 wks	Normal in appearance	Normal in appearance	no growth
35	100%	6 wks	Normal in appearance	Normal in appearance	growth
36	100%	13 wks	Normal in appearance	Normal in appearance	no growth
37	100%	13 wks reconst.	Moderate edema, muscle and connective tissue	Normal in appearance	no growth

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