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SOME STUDIES ON FREEZE-DRIED ARTERIES.

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While gaining experience in the management of artery bank and the technique of preservaion by freeze-drying, I have carried out experiments which concern freeze-drying methods and vessel reconstitution.

These expcriments are divided into three groups:

(1) The value of the secondary stage of freeze-drying;

(2) Residual moisture determinations;

(3) Reconstiution of freeze-dried essels prior to use.

Primary Stage of Freeze-Drying (FIG I)

After removing arterics from the cadaver and trimming them, they are sterilised by being ineubated in a 1% solution of beta-proiolactone for three hours After that, freeze-drying takes place in two stages. In the first stage. using a vacuum of 1 to 0.5 mm. Hg. most of the watar is removed. A cold condenser is used to avoid swamping of the pump. This stage lasts for two days.

Secondary Stage of Freeze - Drying (FIG II.)

In the second stage, as is shown in the figure, the arteries are placed in separate containers. The system is evacuated with a greater vacum and the small amount of remaining water is removed by P_2O_5 trap. This stage also lasts for two days.

EXPERIMENTS

A. Concerning Freeze - Drying Techniques.

The first step in the experiment was to check the amount of water removed in any of the stages. The arteries were removed as soon as possible after death, and after trimming were weighed with the accuracy of 1/10,000 of a gramme. They were incubated in 1% sol. of B. P. L. for three hours and were then weighed again.

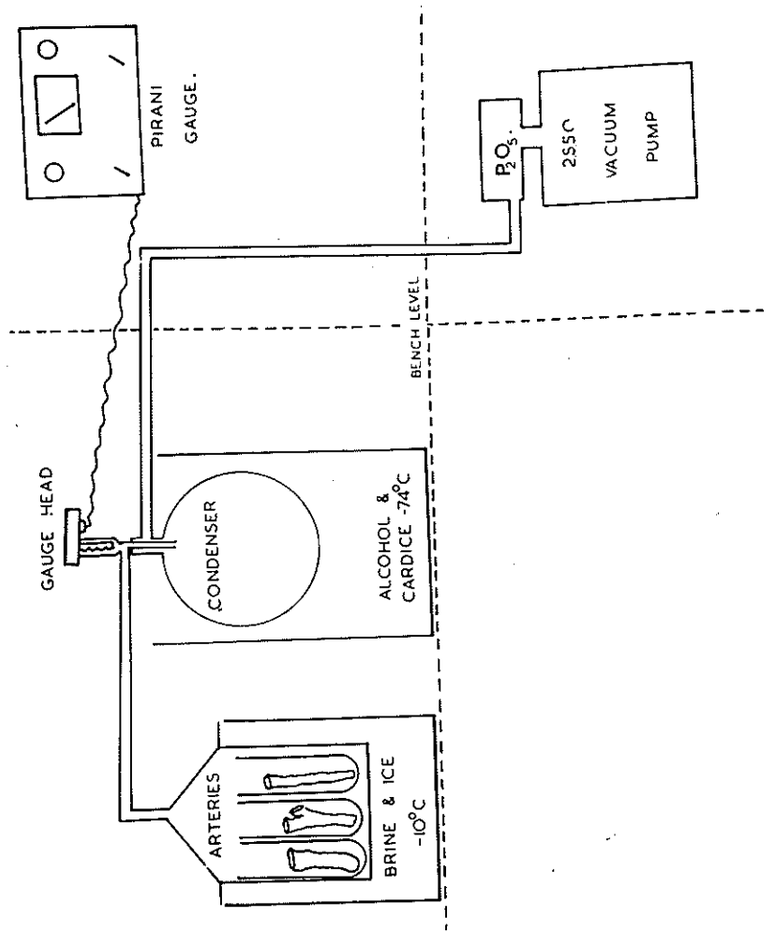


FIG : I – Primary Stage of Freeze Drywig

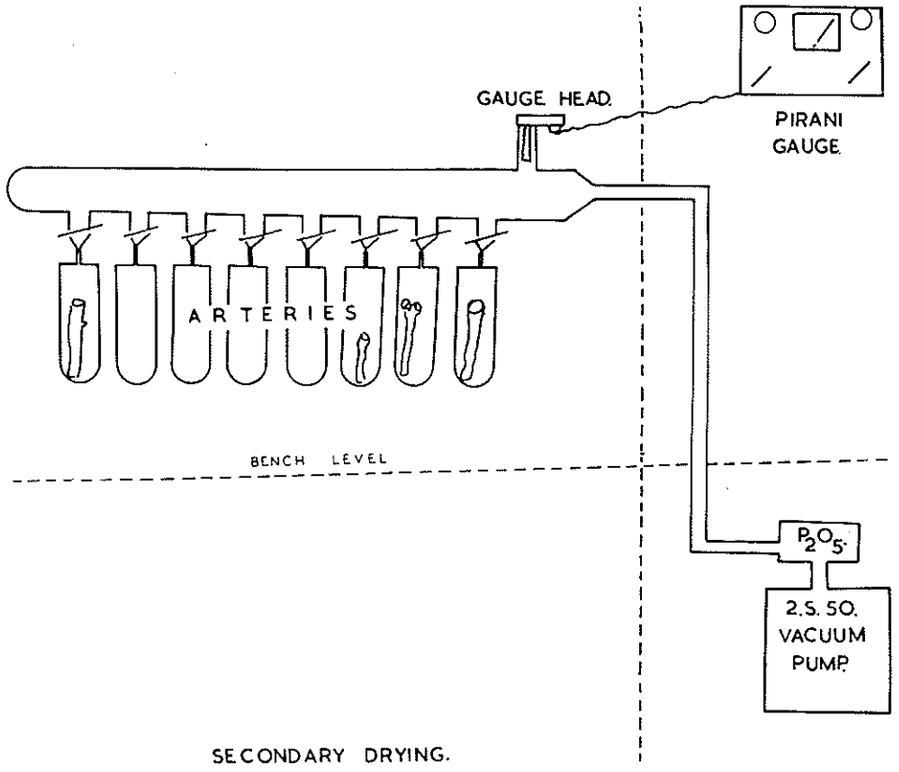


FIG : II Secondary Stage of Freeze - Drying in

During the freeze-drying process the weights were checked every twenty four hours.

The sort of graph which you got in freeze - drying. is shown in FIG III. Usually there is a gain in weight after treatment with B. P. L. What is most striking, however, is that loss of weight in the second stage of freeze - drying, especially in the last day, is so small, 0.5% to 0.2%

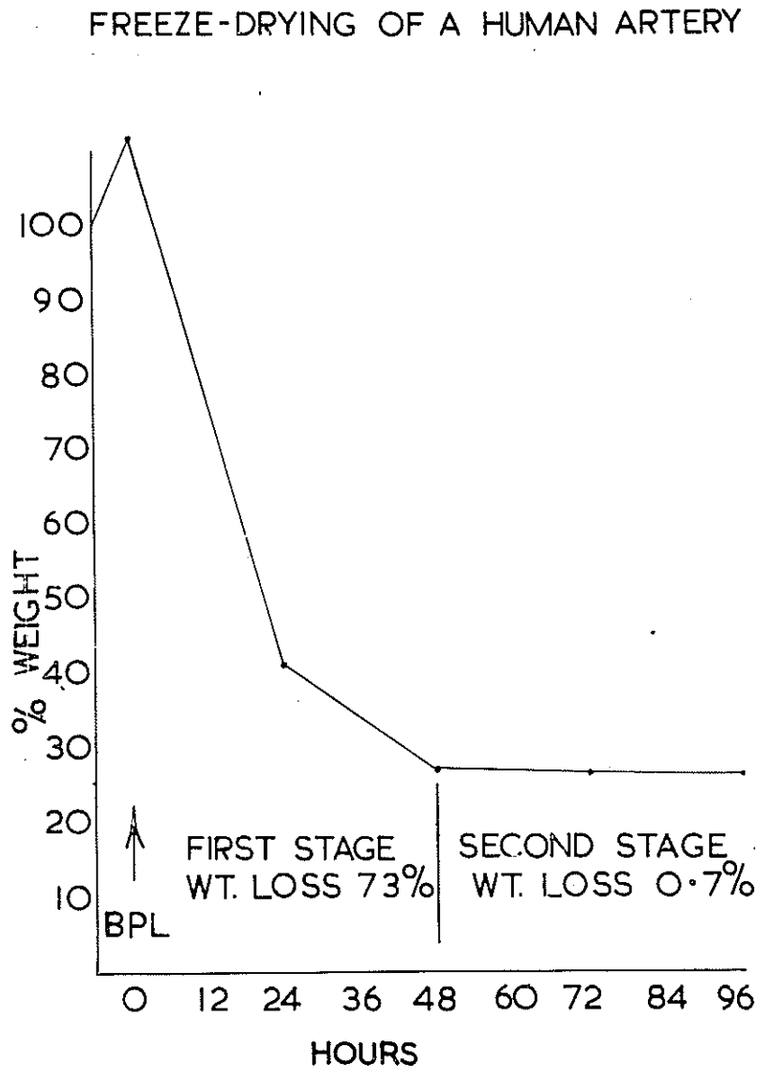
Charl I Shows the result of freeze_drying a series of eight arteries from one donor under the same conditions. It shows that an average of 80% ofthe weight it reduced, Seven of these gained weight after B. P. L. The lose less weight than smaller vessels.

Chart I

FREEZE - DRYING OF HUMAN ARTERIES.

No.	Kind	Wt. after B. P. L.	Wt. after 24 hrs. Freeze-drying	Wt. after 48 hrs. Freeze-drying	Wt. hrs. 72 hrs. Freeze-drying	Wt. after 96 hrs. Freeze-drying
1.	Aorta	112	40 8	27.0	26.5	26.3
2.	Aorta	108	44.0	24.8	23.5	23.2
3.	Aorta	93.5	45.1	25.4	23.7	23.4
4.	Bifurcation and commonlliahs	114	45.8	45.8	21.6	21.5
5.	Iliac	122.6	48.0	19.9	18.8	18 7
6.	Iliac	124.0	36.7	19.5	18.2	18.0
7.	Iliac	123.0	48.9	19.3	18.2	18.4
8.	Iliac	115.6	27.1	17.2	15.9	15.8
Aver_age	Ma-e Vessels	114.1	42.1	22.0	20.8	20.6

FIG III



Regarding the low amount of water removed in the second stage, especially the last day, and knowing that in some centres, including. St. Mary's Hospital in london don't use the second stage. using a vacuum dessicator instead, I decided to try that method and see if there is any difference. The arteries which had already been freez-dried by our own method were placed in a vacuum dessicator with $p_2 0_5$ for a period of 48 hours. The result showed that weight loss is between 0.4% and 0.5% which is rather similar to the last day of freeze drying by our own method (0.1% to 0.15% every day)

Conclusion.

The second stage of freeze-drying, especially the last 24 hours, is not important in the preservation of vessels. Dessication over $P_2 0_5$ in a vacuum has the disadvantage that it takes time and that the grafts may be more easily contaminated. I believe that the last day of freeze-drying could be omitted, so the technique could be limited to two days primary stage. This alteration, apart from the economic point of view, enables the apparatus to be run twice instead of once a week, so doubling the output.

B. Residual Moisture Determinations.

These series of experiments have been done to estimate the amount of water that remains in vessels after freez-drying. The melhod applied was to put arteries in an oven of 105° C and weigh them regularly until getting to a constant weight, It usually takes 20 to 30 days.

FIG IV Shows an example of the results obtained. The arterywhich had already lost some of it's weight in freez-drying, lost another 3% by dessication. The residual moisture is rather higher than usual. The column on the right side of the graph shows the amount of water removed by freeze-drying, with residual moisture and solids.

Chart II. shows the amount of residual moisture in eight freeze-dried arteries from one donor. Here is shown that the residual moisture is on an average about 1.2% of the original, which is about 1 5% of whole water content. It is relatively the same in larger and smaller vessels.

FIG IV

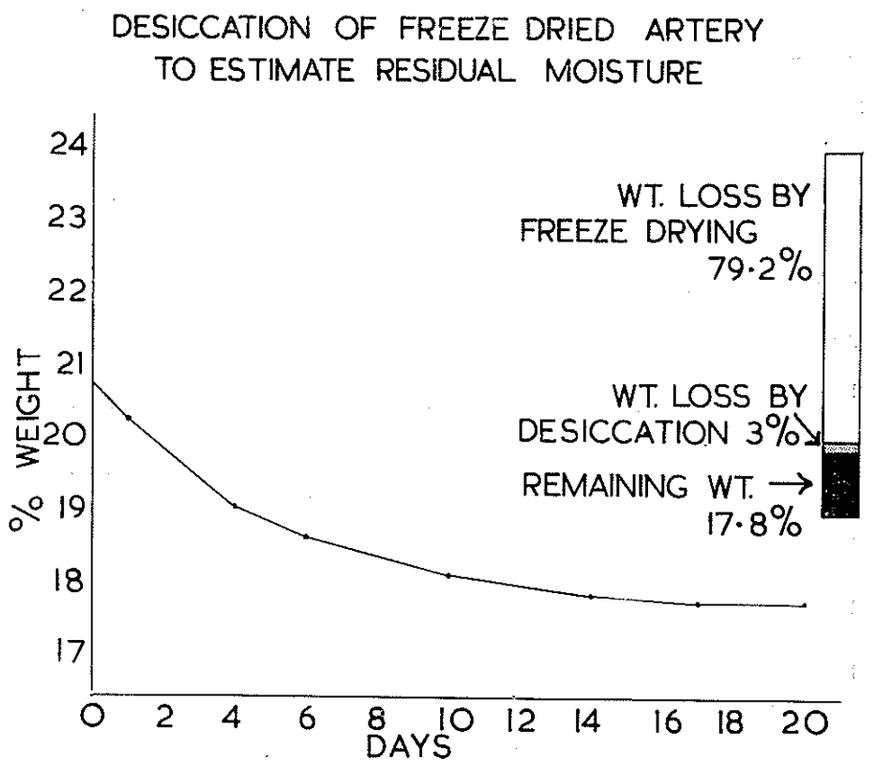


Chart II

RESULTS OF DESSICATION OF EIGHT FREEZE-DRIED ARTERIES FROM ONE DONOR.

Kind	% Weigt loss lst stage frenz-drying	% Weight loss 2nd stage freeze-drying	%weight loss dessication	Remaining weight. %
Aorta	73.0	0.7	1.3	25.0
Aorta	75 2	1.6	1.6	21.6
Aorta	74.6	2.0	1.6	21.8
Bifurcation and common iliacs	77.2	1.3	1.3	20.2
Iliac	80.1	1.2	1.4	17.3
Iliac	80.5	1.5	0.7	17 3
Iliac	80.7	0.9	1.3	17.1
Iliac	82.8	1.4	0.8	15.0
Average	78.2	1.2	1.2	19.4

In Chart III. is the result of estimating residual moisture in eighteen freeze-dried arteries. As these vessels were freez-dried for other experimental purposes, the original weight was not measured and the figures given start with the original weight after freeze-drying (which is about 1/5 of the original). It shows that an average of 9.8% of weight is reduced by dessication (2% original). Il also shows that residual moisture in arteries treated with B. P. L. is higher than those which were not sterilised.

Conclusion.

These investigations prove that on the average, after freeze-drying the residual moisture is less than 2% of the original weight. and also water content. This fits in with results obtained in other centres and is quite satisfactory.

RESULTS OF DESSICATION OF 18 FREEZE-DRIEC ARTERIES.

Chart III

Average weight loss.	9.8% of weight after freeze-drying.
Average weight loss of arteries treated with B. P. L.	10.6% of weight after freeze-drying.
Average weight loss of arteries not treated with B. P. L.	7.9% of weight after freeze-drying.

C. Reconstitution of Vessels Prior to Use.

This process has received relatively little space in the literature. It seems to be most logical to rehydrate with distilled water as it is pure water that is removed in freeze-drying. However, most workers recommend 20 to 30 minutes reconstitution with normal saline solution. No reason is given for this choice, though Hallen (1955) says that perhaps it is the easiest solution obtainable in surgical theatres.

I did a series of reconstitution with saline and another with distilled water. The weights were checked every 15 minutes for the first hour, every 30 minutes in the second hour and after three hours.

Fig V Shows the result of reconstitution of artery with normal saline solution. The original weight had been achieved after 90 minutes and in half an hour only 70.8% of weight was regained.

Reconstitution of a piece of artery from the same donor with water. is shown in **FIG VI.** After 30 minutes 84. 5% of the weight is regained and original weight is obtained after 50 minutes.

We tried nine reconstitutions with normal saline. Four of these arteries did not regain their original weight in three hours and one

FIG V

RECONSTITUTION OF FREEZE-DRIED ARTERY WITH SALINE

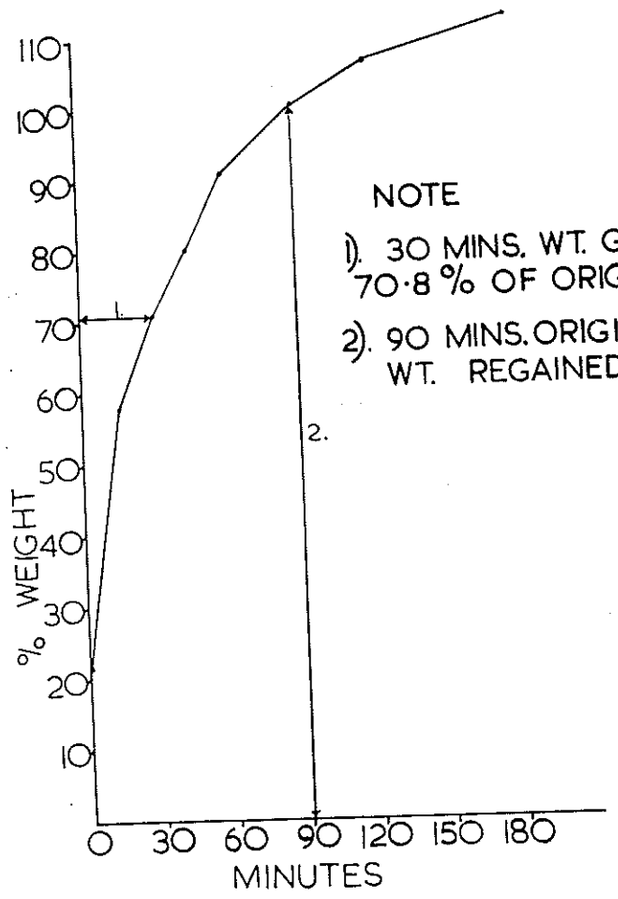


FIG VI

RECONSTITUTION OF FREEZE-DRIED ARTERY WITH WATER

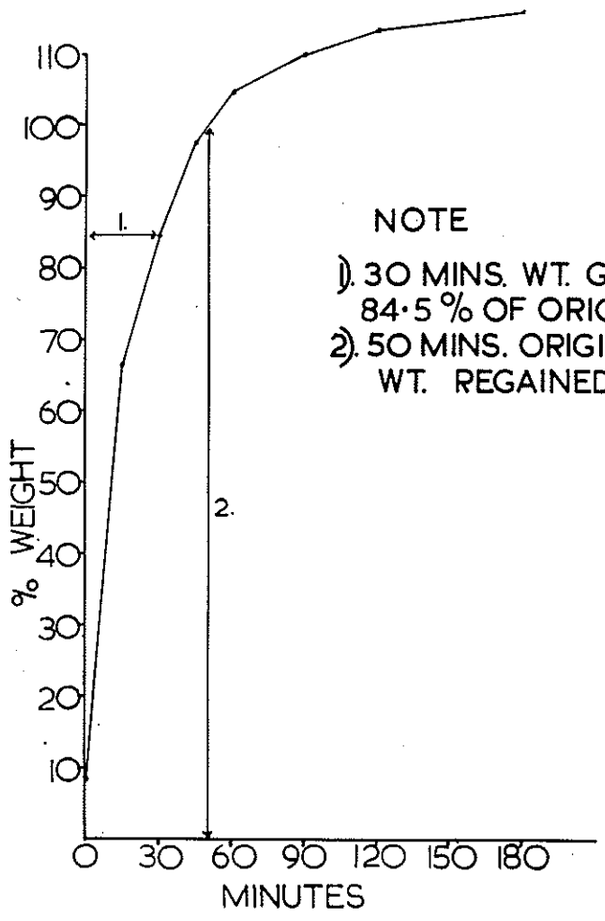


Chart (IV)

RECONSTITUTION OF FREEZE-DRIED ARTERIES WITH 0.9% SOLUTION OF Na Cl.

Wt. beforh Reconstit.	Wt. after 15 mins. of Reconstit.	Wt. after 30 mins. of Reconstit.	Wt. after 45 mins. of Reconstit.	Wt. after 60 mins of Reconstit.	Wt. after 90 mins. of Reconstit.	Wt. after 120 mins. of Reconstit.	Wt. after 180 mins. of Reconstit.	Time required to gain original wt.
27.5	61.4	72.8	79.0	89.6	93.0	99.6	99.6	180 mins.
17.8	57.1	66.8	77.0	77.4	84.0	85.4	93.0	
21.8	57.8	70.7	80.0	90.7	101.1	106.3	113.4	90 mins.
22.6	56.0	67.0	74.0	81.0	84.0	90.5	97.0	
29.5	53.5	65.0	67.5	76.0	78.5	87.5	92.0	
28.8	70.4	79.0	87.2	92.0	96.3	96.6	105.0	125 mins.
20.6	66.4	76.4	83.8	87.5	93 6	96.0	100.5	175 mins.
27.8	70.4	74.2	94.0	96.4	103.5	105.0	127.0	75 mins.
29.0	57.0	66.0	69.0	71.0	77.0	79.9	84.0	
AVERAGE:								
23.9	61.1	76.9	84.5	84.5	90.1	93.7	101.3	150 mins.

RECONSTITUTION OF FREEZE-DRIED ARTERIES WITH WATER.

Wt. before Reconstit.	Wt after 15 mins. of Reconstit.	Wt. after 30 mins. of Reconstit.	Wt. after 45 mins. of Reconstit.	Wt. after 60 mins. of Reconstit.	Wt. after 90 mins. of Reconstit.	Wt. after 120 mins. of Reconstit.	Wt. after 180 mins. of Reconstit.	Time reqnired to gain original wt.
19.4	60.8	73.8	82.6	89.0	101.5	107.1	108.0	85 mins.
20.6	71.3	80.2	92.3	99.6	108.1	115.5	112.9	63 mins.
18.5	66.6	84.4	97.4	104.5	109.9	113.5	116.6	50 mins.
22.6	65.5	78.3	87.0	94.4	100.8	104.6	108.3	88 mins.
AVERAGE:								
20.3	66.05	79.2	89.8	96.9	105.1	108.9	111.5	66.5 mins.

only just. The average time required to regain original weight was 150 minutes. (Chart 4)

Chart V Shows reconstitution with water in four vessels. Each one regained it'soriginal weight. The average time required to regain original weight was 66.5 minutes, less than half the time taken when saline is used for reconstitution.

Conclusion

In these experiment reconstitution with water is much quicker than with saline and as there is nothing against it I think it should be tried. In any case half an hour is not enough to regain original weight and I tkink it is better to wait longer. one hour, to let the vessel return as near to the original weight as possible.

SUMMARY.

(1) The secondary stage of freeze - drying, particularly the last day, is not important and I suggest the whole procedure is reduced to three days, the primary stage occupying two of these.

(2) The mothod used obtains the satisfactory low level of residual moisture.

(3) Experiments on reconstitution with saline and distilled water show that distilled water is more satisfactory and I suggest that saline should not be used.

RESUME

1- Le second stade de congélation à sec, particulièrement le dernier jour n'est pas important et je suggère que toute l'opération soit réduite à 3 jours, le premier stade en comprenant deux.

2- La méthode utilisée donne un bas niveau satisfaisnt d'humidité résiduelle.

3- Les expériences pour la reconstitution avec de l'eau distillée et salée montrent que l'eau distillée seule est plus est plus satisfaisante et, je suggère que l'eau salée ne soit pas utilisée.

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