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STUDY OF THE WATER BINDING PROPERTIES OF FREEZE-DEHYDRATED MEAT IN RELATION TO PROTEIN COMPOSITION AND PROCESSING TREATMENT

by
B. J. Luyet and A. P. MacKenzie

American Foundation for Biological Research
Madison, Wisconsin

Contract No. DA19-129-AMC-63(N)

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UNITED STATES ARMY
NATICK LABORATORIES
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FOREWORD

The relationships between dehydration variables and protein stability of muscle tissue are of direct importance to the development of freeze-dried meats with optimum rehydration and texture properties. The present report deals with studies of the effect of the rate of freezing and temperature of dehydration on protein denaturation in beef muscle tissue. An effort was made to separate the effects of freeze-drying from those of high temperature. It was then possible to show that heat applied during the course of freeze-drying to accelerate dehydration may induce insolubility in the portion already dried, while the part still to be dried remains undamaged. Free-drying per se (chamber temperature of -10°C throughout the drying, and subsequent warming only to room temperature) did not impair protein stability, as measured by extractable protein and insoluble residue.

The principal investigator was Dr. Basile J. Luyet and the co-investigator Dr. Alan P. MacKenzie. Their collaborators were Dr. L. J. Menz, Dr. P. M. Gehenio, Dr. J. Nath and Messrs. C. Kroener, D. J. Schroeder, J. M. Dobson and D. B. Pribor.

The U. S. Army Natick Laboratories Project Officer was Dr. John G. Kapsalis and the Alternate Project Officer was Dr. Karl R. Johnson, both of the Food Laboratory.

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ABSTRACT

(1) Determination of the low- and high-salt-soluble protein fractions, and of the insoluble fraction in beef muscle frozen at 3 rates and freeze-dried at 3 final temperatures. (2) Establishment of the adsorption isotherms of the freeze-dried muscle tissues. (3) Measurement, by the mechanical pressure method of the water-holding capacity of the freeze-dried, cooked and noncooked tissues. (4) Evaluation of the rate and extent of rehydration of freeze-dried tissues subjected to various pretreatments. (5) Tests of tenderness of meat cooked after being freeze-dried.

INTRODUCTION

A. General Plan and Division of the Work

The general plan in this research was to study: (1) the water-binding ability of freeze-dried muscle tissue, as determined by the method of vapor pressure equilibrium in an atmosphere of known relative humidity (method of Briggs, 1931, 1932 and of Bull, 1944); (2) the water-holding capacity of such tissue, as measured by the amount of water not removable by mechanical pressure from rehydrated freeze-dried specimens (method of Grau and Hamm, 1957, modified by Wierbicki and Deatherage, 1958). (One may note that the plan involved the use of two of the many methods described in the literature for the study of bound water; Kramer, in a review published in 1956, mentions 14 such methods.)

The water-holding capacity was to be examined in whole pieces of tissue; the water-binding ability, in both whole pieces and extracted protein fractions. Then the research was to be extended to tissues treated by heat (cooked meat), and finally a test was to be made of tenderness.

More specifically, the project involved: (1) Freezing muscle tissue in three ways: rapidly, slowly, and at an intermediate rate; (2) Freeze-drying each of the tissues so frozen under three sets of conditions: (a) by maintaining the temperature at -10°C. throughout the operation, or (b) by bringing the temperature to +50° for the completion of dehydration, (c) by bringing it to +80°. The three freezing rates and three freeze-drying conditions gave nine combinations of variables. The material treated in the nine corresponding ways constituted the "piece de resistance" of the research; but, in addition, we investigated other particular conditions which will be described and of which the results will be analysed in Appendices at the end of some of the sections of the report.

The complete list of experimental operations to be performed were then as follows: (1) freezing, (2) freeze-drying, (3) rehydrating, (4) heating (cooking), (5) extracting the proteins, (6) determining the water-binding properties by the method of adsorption isotherms, (7) determining the water-holding capacity by the pressure method, (8) determining the rate of rehydration, (9) testing for tenderness.

The first four of these operations may be considered as preparatory treatments, the last five as tests of the properties of the material after the treatments. Which tests are to be made after which treatments are indicated in the following schedule and summarized in Table 1.

Schedule of Operations: (a) Extraction (E in Table 1) of the 9 freeze-dried tissues, of the 3 frozen (not freeze-dried) ones, and of tissue not previously submitted to any preparatory treatment. (b) Determination of the adsorption isotherms (I in the Table) of the 9 freeze-dried tissues, of the extracted fractions of them, of the non-treated tissue, and of the extracted fractions of it. (c) Test by the pressure method (P) of the 9 freeze-dried rehydrated and the 9 rehydrated cooked tissues, of the 3 frozen (not freeze-dried) ones, of the nonfrozen cooked one, and of the one which did not receive any preparatory treatment. (d) Determination of the rate of rehydration (R) in the 9 freeze-dried tissues. (e) Tenderness test (T) of the 9 freeze-dried rehydrated cooked tissues and of the nonfrozen cooked one.

The report will be divided, on the basis of these tests, into five parts: (I) Protein Extractability; (II) Water-Binding as Determined by the Adsorption Isotherms; (III) Water-Holding Capacity as Measured by the Pressure Method; (IV) Rehydration Rates; (V) Tests of Tenderness.

B. Material and General Procedure

Material. The material used in this research was postrigor beef muscle longissimus dorsi. One-to-two-pound pieces, graded U. S. Choice, were purchased, two to four days after slaughter, at a local supermarket, and transferred immediately to polyethylene bags. For further storage, they were kept in these bags at +2°C.

Freezing Procedure. The tissue to be frozen was cut into pieces of appropriate size and the pieces either immersed directly into a freezing bath or wrapped in aluminum foil (see details below) and exposed in cold rooms to the desired temperatures. We used three freezing rates: (1) a high rate, in which slices of muscle 1 to 2 mm. thick were frozen by immersion in an isopentane bath at -150°; (2) an intermediate rate, in which cubes of tissue measuring about a cm. on the side were frozen in a cold room at -30°; (3) a relatively low rate in which a piece of tissue 2 x 8 x 15 cm. was frozen in a cold room at -10°. The times during which the pieces of tissue remained at the freezing point (plateau of the freezing curve) were, respectively, of the order of one second, several minutes and several hours (see the three curves of Fig. 1).

Table 1

Schematic Presentation of Experimental Operations

(I, Adsorption Isotherm; R, Rehydration Rate; E. Extraction; P, Mechanical Pressure; T. Tenderness Test)

(A) Tissues Freeze-Dried

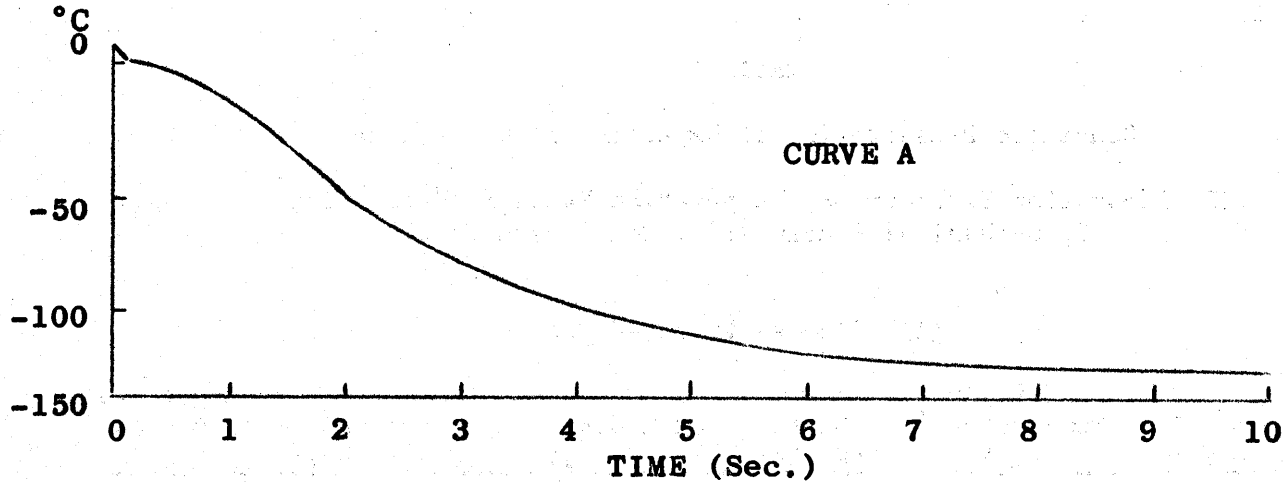
Mode of Freezing	Fr. Drying Temperature[1] (°C.)	In Dry State (Whole)[2]	Rehydrated (Whole)	Extracted (Fractions)[2]	Rehydrated Cooked (Whole)
Rapid -150°	-10	I,R	E,P	I	P,T
	+50	I,R	E,P	I	P,T
	+80	I,R	E,P	I	P,T
At Interm Rate -30°	-10	I,R	E,P	I	P,T
	+50	I,R	E,P	I	P,T
	+80	I,R	E,P	I	P,T
Slow -10°	-10	I,R	E,P	I	P,T
	+50	I,R	E,P	I	P,T
	+80	I,R	E,P	I	P,T

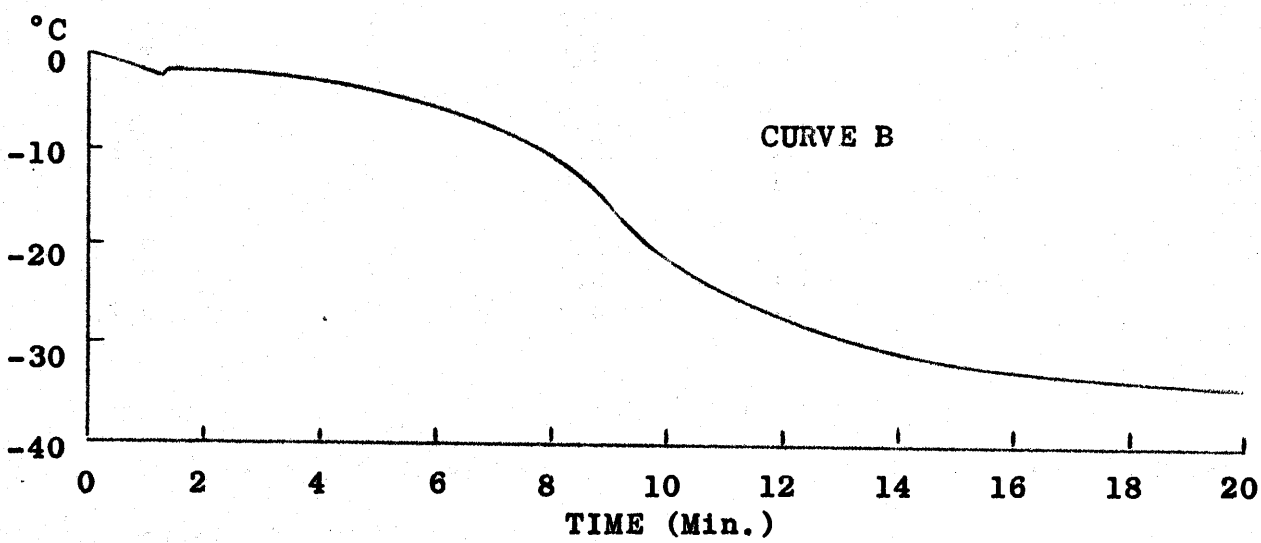
(B) Tissues Not Freeze-Dried

(a) Tissues Frozen		(b) Tissues Not Frozen[3]		
Mode of Freezing		Cooked (Whole)	Not Treated (Whole)	Extracted[3] (Fractions)
Frozen Rapidly	E,P			
Frozen at Interm. Rate	E,P	P,T	E,I,P	I
Frozen Slowly	E,P			

[1] The exposure to +80 was maintained for 24 hours (see text hereafter).

[2] "Whole" and "Fractions" refer, respectively, to pieces of tissues handled whole, or to the extracted fractions of the tissues.





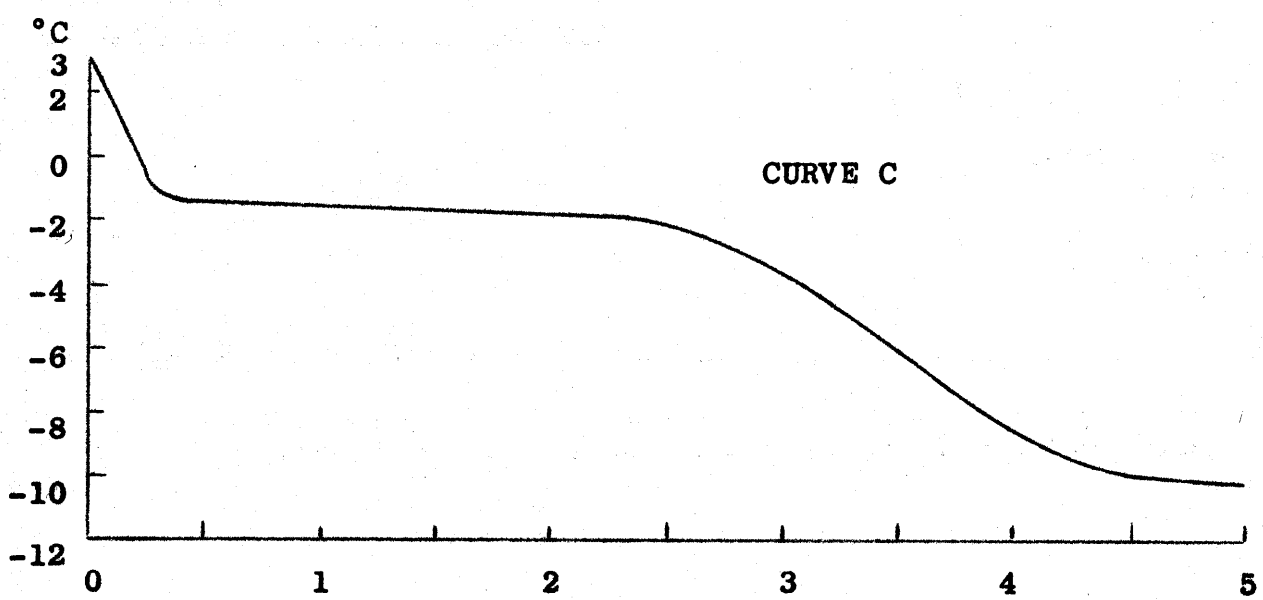


Fig. 1. Typical cooling curves for "rapidly frozen tissue" (Curve A), tissue frozen "at an intermediate rate" (Curve B), and "slowly frozen tissue" (Curve C).

In the case of freezing at the intermediate rate, the details of the procedure were as follows: The tissue was cut, in a cold room at +2°, into cubes one cm. on edge; the cubes were placed on a sheet of aluminum foil; the latter was folded over into an envelope of which the edges were replicated to prevent the evaporation of the contents. The envelope was then transferred to a cold room at -30° and placed in such a position that its two faces were exposed simultaneously to the cold air of the room. The pieces remained overnight in the cold room and were then stored, for convenience, in liquid nitrogen. A thermocouple had been inserted in the center of one piece of tissue to permit the recording of the freezing curve.

In the case of slow freezing, we followed essentially the same procedure, but used larger pieces (as indicated above) and exposed them in a cold room at -10°, instead of -30°. Then, after freezing was completed, these larger pieces were cut, at -10°, into cubes 1 cm. on the side.

Freeze-Drying Procedure. Freeze-drying was carried out in an apparatus constructed in this laboratory (cf. MacKenzie and Luyet, 1963) to permit (1) observation of the total loss in weight at any time, (2) measurement of the temperature within the tissue, (3) observation of the more or less gradual transition from primary to secondary drying ("pseudo freeze-drying" of Luyet, 1962).

The apparatus (Fig. 2) consists of: (1) a specimen chamber SC, which contains a wire basket suspended from a spiral tungsten spring, (2) a condenser C, to which is attached a stopcock S, and from which the specimen chamber may be removed, (3) a trap T which prevents the passage of oil vapors to the condenser. The apparatus is equipped with air inlets and vacuum gauges; provisions are made, in particular, for connecting the thermocouple placed within a freeze-drying specimen to a recorder.

The glass vessel which served as the drying chamber of the freeze-drying apparatus was generally precooled in an alcohol bath at -10°. Then 25 grams of the frozen tissue were transferred to the wire basket contained in that vessel and, after a copper-constantan junction had been placed as nearly as possible at the center of one of the pieces of tissue selected as typical, the vessel was attached to the freeze-drying apparatus and the latter put in operation. Checks were made periodically, during the latter stages of each run, of the degree of dehydration; for that purpose, we closed the stopcock S and noted the equilibrium pressure indicated by gauge G1. An empirical relationship between the pressure at equilibrium and the water remaining in the sample, established for each specimen-chamber temperature, permitted the operator to discontinue freeze-drying when the residual moisture reached a level of about 2%. The freeze-dried material was then stored in a desiccator previously emptied of desiccant.

The frozen tissues were freeze-dried under three different sets of conditions: (1) The freeze-drying chamber was maintained in a bath

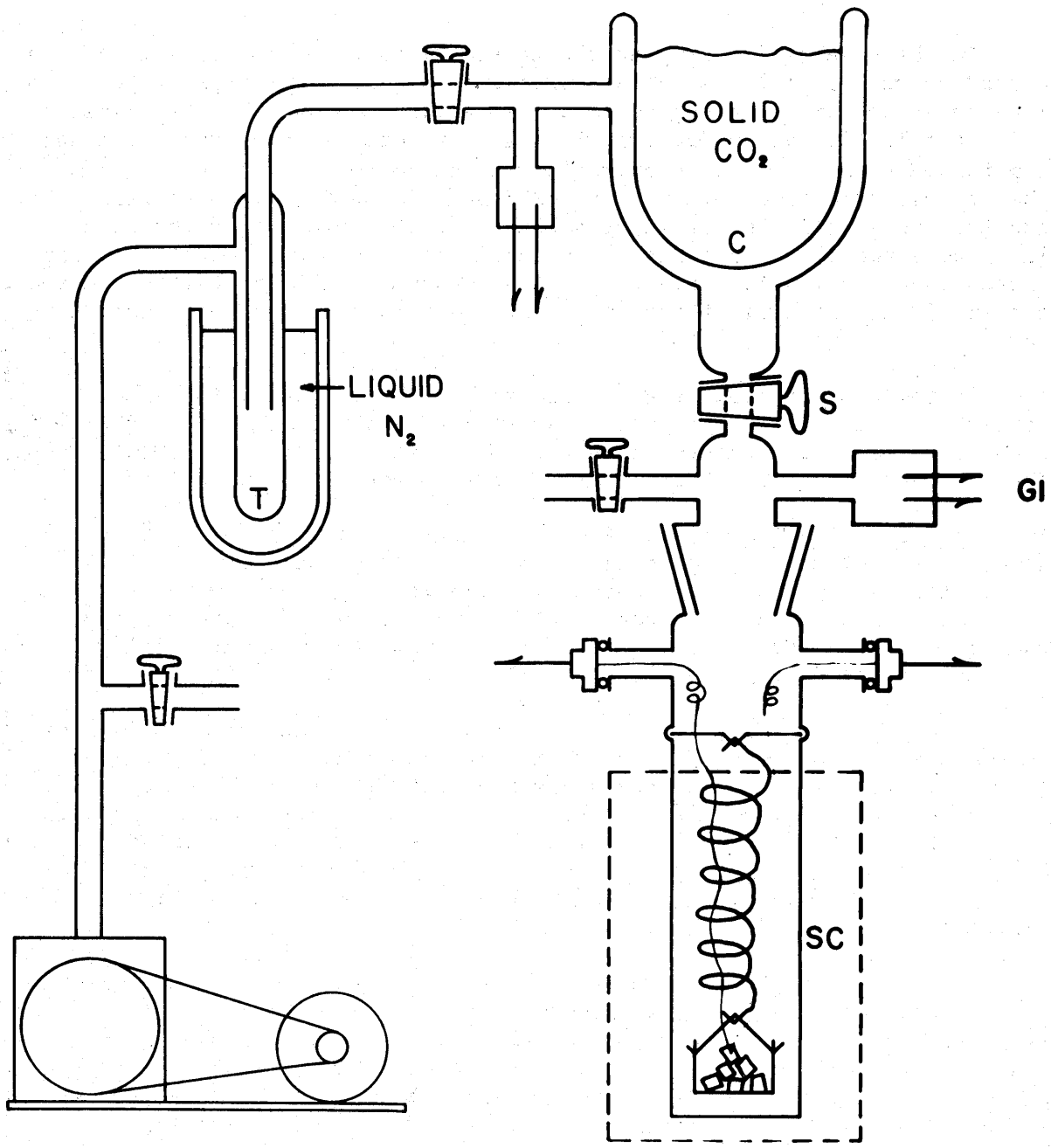


Fig. 2. Diagram of the apparatus used in freeze-drying experiments.

at -10° during the entire freeze-drying operation; (2) the vacuum pump was started while the drying chamber was at -10°, then the cooling bath was immediately removed and radiant heat applied. The source of radiant heat, a 250-watt G. E. infra-red lamp, placed 18 inches to one side of the specimen chamber (a pair of mirrors was set up on the other side) caused the specimen temperature to rise, ultimately, to 50°; (3) Freeze-drying was completed at -10°, as in (1), then the sample, still under vacuum in the specimen chamber, was warmed to 80° in an oil bath and kept at that temperature for 24 hours.

To study the effects of changes in the temperature of the specimen, at various stages of dehydration during the freeze-drying process, we recorded: (a) the temperature within the specimen by means of the thermocouple inserted in it, (b) the loss in weight, that is, the degree of dehydration, by measuring the contraction of the tungsten spring to which was suspended the wire-gauze basket containing the specimen, (c) the vapor pressure by means of the Pirani gauge placed between specimen and condenser. These three values are represented in Fig. 3 in the cases of slowly frozen material, freeze-dried at -10° (Fig. 3,A) and freeze-dried at +50° (Fig. 3,B).

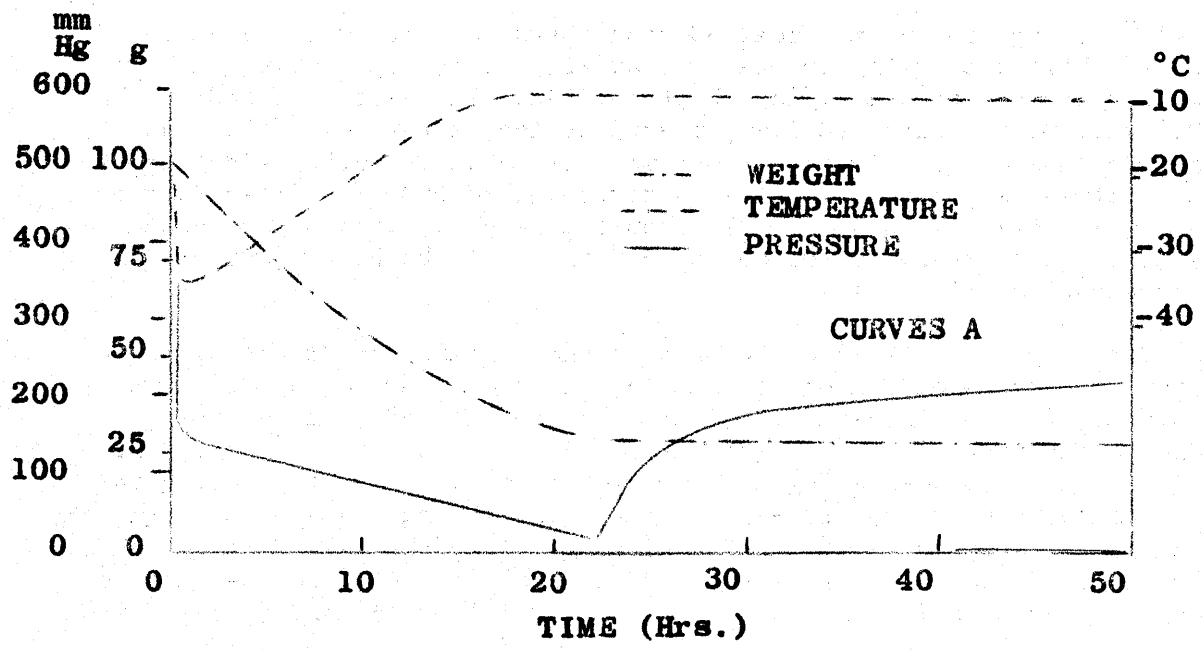
The procedures just outlined for freezing and freeze-drying were used throughout the work unless indicated otherwise. Certain particular details of the procedures will be given, when pertinent, in the description of the other operations (Parts 1 to 5).

PART I

PROTEIN EXTRACTABILITY OF VARIOUSLY TREATED MUSCLE TISSUE

A. Methods

Protein Extraction. Our original plan was to extract the proteins of the muscle by the Cori-Mommaerts method (Mommaerts, 1958), which uses 0.5% NaCl, 0.4M KCl and 0.6M KCl as extractants. But Helander (1957) pointed out that the potassium chloride solution does not extract all the contractile proteins of muscle and, in a thorough study of the solvent properties of the various alkali and alkaline earth halides, he showed that a 1.1M solution of potassium iodide in 0.1M potassium phosphate, at pH 7.4, extracts approximately 20% more contractile protein than does 0.6M potassium chloride. Questions could be raised over the likelihood that potassium iodide exert a degrading action on the actin or the myosin. Tonomura, Sekiya and Imamura (1962) gave a partial answer to some of these questions by showing that potassium iodide causes no irreversible effect on the alpha-helix content of myosin.



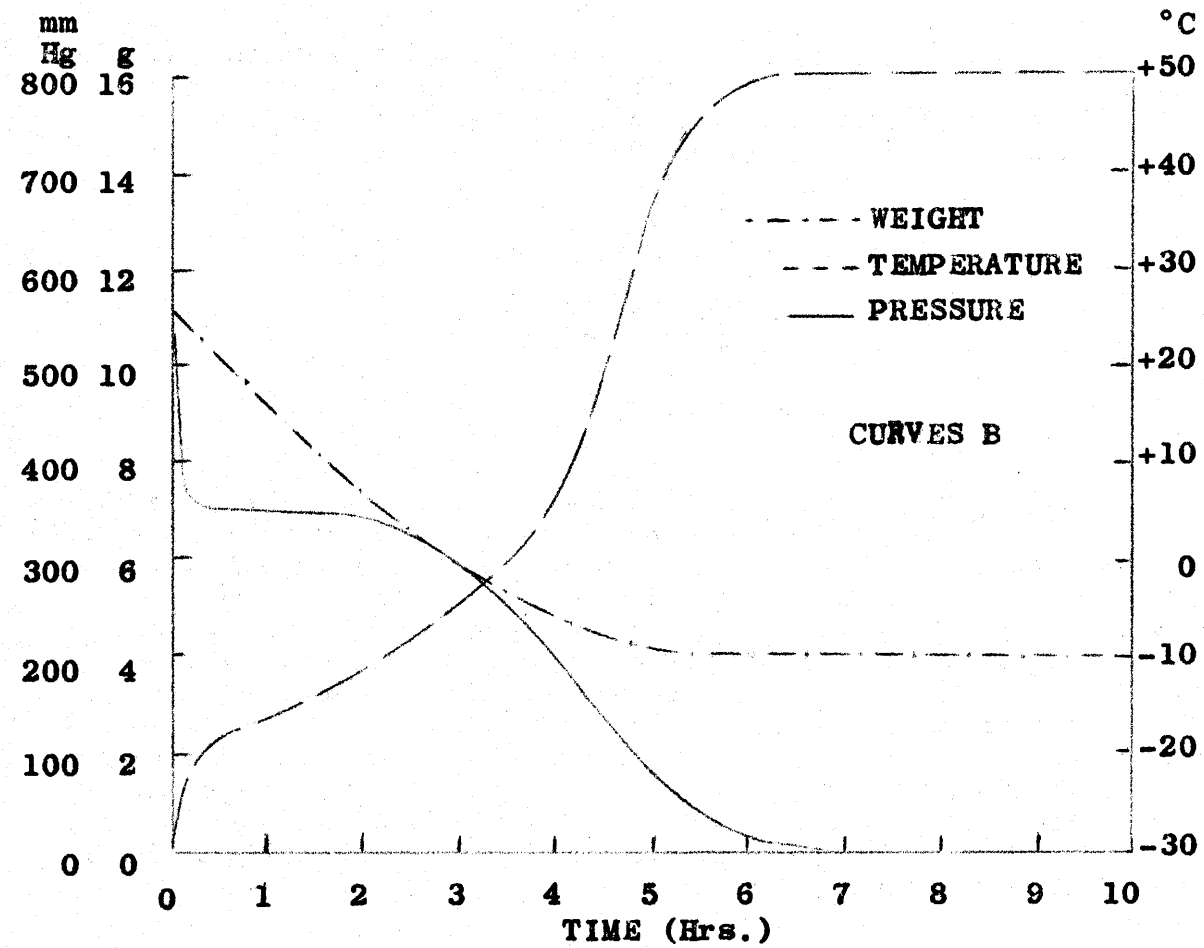


Fig. 3. Sets of curves illustrating the progress of freeze-drying in typical experiments conducted (a) at a specimen-chamber temperature of -10°C. (Curves A) and (b) with a specimen chamber warmed by radiation from an infra-red lamp (Curves B).

We therefore resorted to Helander's method which with slight modifications suggested by Dr. E. J. Briskey of the University of Wisconsin, is, briefly, as follows: (I) Frozen muscle tissue is disintegrated in 0.03M potassium phosphate buffer at pH 7.4, at 2°C., in a Waring Blendor. (II) The suspension is stirred at 2° for three hours and submitted to centrifugation. (III) Droplets of fat are removed from the supernatant by filtration on a large filter paper. (IV) The residue is extracted with 1.1M potassium iodide at pH 7.4, at 2°, for three hours and centrifuged (note that 1.1M KI is 0.1M with respect to potassium phosphate). (V) The protein content of each extract and the nonprotein nitrogen content of the low-salt-soluble extract are estimated by the biuret reaction (nonprotein nitrogen is estimated after precipitation of proteins with 20% trichloroacetic acid).

The supernatant from (II) contains the low salt soluble proteins; the supernatant from (IV) contains actin, myosin and tropomyosin; the residue from (IV) consists of stroma proteins, together with any proteins that may have been rendered insoluble by the various treatments.

(Note: In order to provide materials for the determination of the adsorption isotherms, we obtained samples in a salt-free condition from each fraction of several of the first extractions. Subsequently, we tried to determine these isotherms but had to abandon the attempt as will be explained in Part II.)

The successive steps in the procedure are represented graphically in Table 2.

B. Results

The results of 21 series of extractions from muscle tissue frozen at one or the other of the three rates mentioned, and vacuum-sublimed under one or the other of the three sets of conditions described, are given in Tables 3(A) and 3(B).

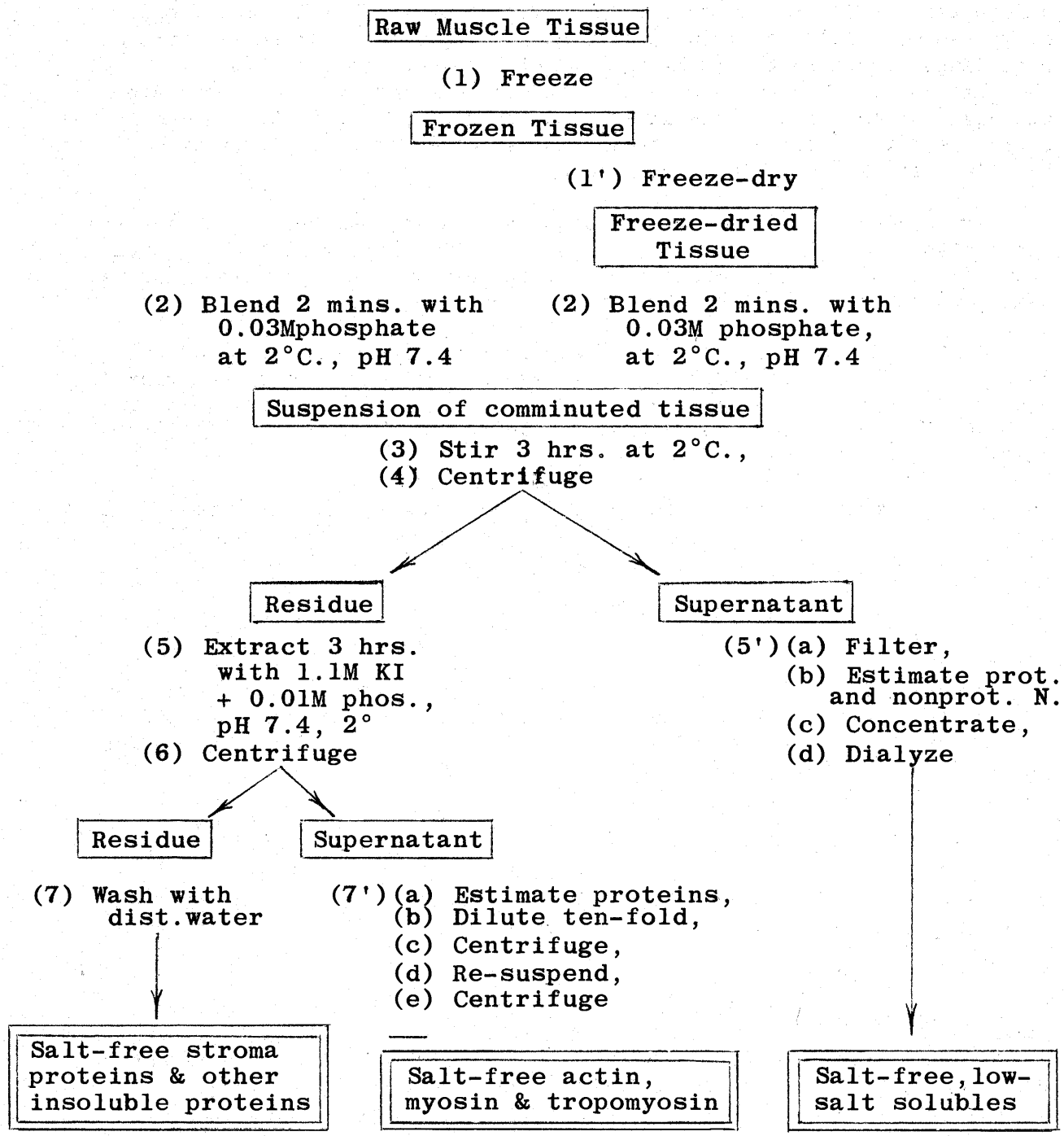
The most outstanding feature in Table 3(A) is that the values obtained for the two extracted fractions and for the insoluble residue, when the freeze-dried material was exposed to +80° for 24 hours (Experiments 6, 7, 12, 19, 20 and 21) differ greatly from those obtained in all other conditions. This treatment evidently exerts a very drastic effect.

Then there are two exceptional figures: (1) a high-salt-soluble fraction of 17.1% in Experiment No. 9, (2) a high value of 4.5% for the insoluble residue in Experiment No. 11. The first of these is apparently due to an error in measurement; in the second case, a rather large quantity of connective tissue was observed in the freeze-dried sample after fractionation.

Table 2

Outline of Extraction Procedure

(The steps in the procedure are numbered; the stages of the material are enclosed in a frame)



As for the values reported in each of the four columns: low-salt-soluble, n.p.n., high-salt-soluble, and insoluble residue, they do not differ enough in the specimens frozen at different rates, in those freeze-dried under different conditions (Table 3A), and in the specimens frozen at different rates and not freeze-dried (Table 3B) to indicate a significant effect attributable to any of these factors.

The differences reported from one run to another in experiments of the same category are probably due to variations in the composition of the material, such as, in the fat and connective tissue contents and in the water content.

Conclusions: (A) The data reported thus concur in showing no definite differences in extractablity (1) when the samples were frozen rapidly, or at an intermediate rate, or slowly; (2) when they were not freeze-dried, or were freeze-dried at -10°, or were freeze-dried in conditions in which the dry tissue reached $+50^{\circ}$; (3) when they were freeze-dried to residual moisture contents of 2%, or to moisture contents of less than 1% (no matter whether this limit had been reached by freeze-drying at -10° or after the application of radiant heat which resulted in a rise of the temperature to $+50^{\circ}$).

(B) Greatly decreased high-salt extractabilities resulted from exposure of samples to $+80^{\circ}$, following completion of freeze-drying at -10°, regardless of the initial mode of freezing (rapid, intermediate or slow). (One should note also that low-salt-soluble fractions are decreased much less than high-salt-soluble ones.) — Further, exposure to $+80^{\circ}$ resulted in about the same decrease in extractability for tissues freeze-dried at 2% residual moisture and for tissues freeze-dried to less than 0.2%, these moisture levels being equivalent, roughly, to the presence and the absence of a water monolayer on the protein molecules.

Appendix 1

Effect of Long-Storage of Freeze-dried Tissue on Protein Extractability

We extracted muscle tissue which had been freeze-dried in 1960 by the U. S. Army Food and Container Institute and kept in sealed cans for three years. The entire muscle, the longissimus dorsi, had been exposed to an air-blast at -20°C.; its internal temperature had remained between 0° and -10° for about 2 hours (cf. Luyet, 1962, p. 196). Table 4 gives the results obtained with that material, with fresh muscle extracted after recent freeze-drying and with controls not freeze-dried.

Table 3(A)

Protein Extractability of Tissue Frozen at the Three Standard Rates and Freeze-Dried at the Three Standard Temperatures

Experiment No.	Freeze-Drying Temp.[1] (°C.)	Residual Water (%)	Low-Salt-Soluble Fraction (%)	Non-Prot. Nitr. (%)	High-Salt-Soluble Fraction (%)	Insol. Residue (%)
		(a) Tissue Frozen Rapidly				
1	-10	0	7.8	1.5	12.2	1.0
2	-10	1.7	5.7	0.9	12.8	0.9
3	-10	1.8	6.6	1.8	10.8	0.9
4	-10	2.6	6.5	0.6	10.8	1.5
5	+50	1.4	6.6	1.3	11.6	1.4
6	+80	0	4.2	0.5	3.5	10.1
7	+80	4.0	6.1	2.0	4.6	9.8
		(b) Tissue Frozen at Intermediate Rate				
8	-10	-	5.4	-	11.3	0.8
9	-10	-	6.5	-	17.1	2.1
10	-10	2.0	8.0	1.0	13.2	2.4
11	+50	0	8.1	-	11.6	4.5
12	+80	2.0	7.0	0.7	3.6	14.5
		(c) Tissue Frozen Slowly				
13	-10	-	7.2	-	12.1	1.1
14	-10	1.3	7.0	0.4	11.7	1.6
15	-10	2.2	6.5	0.4	11.9	2.0
16	-10	2.6	6.7	0.3	12.8	1.2
17	+50	0.6	6.6	0.7	11.6	2.5
18	+50	0.8	5.9	1.1	12.1	2.3
19	+80	0	4.3	0.4	3.7	9.8
20	+80	0	4.1	1.2	4.2	13.8
21	+80	2.0	4.1	1.6	4.0	8.0
		Averages and Extremes[2]				
1 to 5, 8 to 11, 13 to 18, 6,7, 12,19, 20,21	-10 and +50	Max.:	8.1	1.8	13.2	2.5
		Av.:	6.7	0.9	11.9	1.5
		Min.:	5.4	0.3	10.8	0.8
	+80	Max.:	7.0	2.0	4.6	14.5
		Av.:	5.0	1.1	3.9	11.0
		Min.:	4.1	0.4	3.5	8.0

[1] See text for details of procedure in cases of tissue marked "freeze-dried at +50 and +80°."

[2] The data for tissues freeze-dried at -10° and +50° are averaged together, as they show no evident difference.

Table 3(B)

Protein Extractability of Tissue Frozen at the Three Standard Rates but not Freeze-Dried (Controls for Material Reported in Table 3(A)

Experiment No.[1]	Low-Salt-Soluble Fraction (%)	Non-Prot. Nitr. (%)	High-Salt-Soluble Fraction (%)	Insol. Residue (%)
	(a) Tissue Frozen Rapidly			
1C	7.4	2.5	13.5	0.5
2C	6.4	0.2	11.4	2.1
3C	7.4	2.5	13.5	0.5
4C	5.6	0.4	11.2	1.4
5C	6.3	0.6	11.3	2.1
6C	7.4	2.5	13.5	0.5
7C	7.4	2.5	13.5	0.5
	(b) Tissue Frozen at Intermediate Rate			
8C	5.0	-	11.6	0.5
9C	7.7	-	12.0	2.7
10C	6.7	0.9	13.0	1.0
11C	7.8	0.6	13.6	0.5
12C	6.7	0.9	13.0	1.0
	(c) Tissue Frozen Slowly			
13C	4.6	0.6	12.1	0.9
14C	6.3	0.3	11.5	1.1
15C	6.8	0.4	11.3	2.6
16C	6.3	0.3	11.5	1.1
17C	6.3	0.6	11.3	2.1
18C	6.3	0.6	11.3	2.1
19C	6.3	0.3	11.5	1.1
20C	6.3	0.3	11.5	1.1
21C	6.3	0.3	11.5	1.1
	Averages and Extremes			
1C Max.:	7.8	2.5	13.6	2.7
to Av.:	6.4	0.7	12.0	1.4
21C Min.:	4.6	0.2	11.2	0.5

[1] The experiments reported in this table being the controls for those reported in Table 3(A) receive the same numbers with the addition of symbol C (for Control).

Table 4

Protein Extractability of Tissue Recently Freeze-dried and of Tissue Stored for Three Years

	Control Frozen not Freeze-Dried	Tissue Recently Fr.-Dried After Slow Freezing (Av. of 2 runs)	Tissue Fr.-Dried After Slow Freezing, Stored for 3 years
Low-Salt Sol. Fraction (%):	7.1	6.3	4.8
Contractile Proteins (%):	10.5	11.9	5.2
Stroma Proteins (%):	0.72	2.4	4.7
Totals:	18.3	20.6	14.7

Thus, muscle tissue stored for 3 years gave a noticeably lower yield of low-salt-soluble and contractile proteins and a much higher proportion of stroma proteins than tissue recently freeze-dried.

Appendix 2

Protein Extractability in Prerigor and Postrigor Muscle

Tissue extracted in the prerigor state gave practically the same results as that extracted in the postrigor state. The figures for the three fractions and the totals are given in Table 5.

Table 5

Protein Extractability of Prerigor and Postrigor Muscle

	Prerigor Muscle	Postrigor Muscle
Low-Salt-Sol. Fraction (%):	8.3	7.1
Contractile Proteins (%):	10.8	10.5
Stroma Proteins (%):	0.75	0.72
Totals:	19.9	18.3

Appendix 3

Effect of Freeze-Drying at -30° and at Room Temperature on Protein Extractability

In addition to the three modes of freeze-drying reported in the main body of Part I (-10°, and at temperatures rising to +50° and to +80°), some freeze-drying experiments were made in which the temperature was maintained at -30° throughout the operation (a 20-gram batch of tissue was found to freeze-dry in about 3 days in a specimen-chamber maintained at -30°), and other experiments in which the specimen-chamber, maintained at -10° until the start of freeze-drying, was allowed thereafter to warm to room temperature.

As in the case of freeze-drying in the three standard conditions, completion of the process was checked each time by the "vapor pressure-rise-to-equilibrium" method. The freeze-dried materials were then submitted to the extraction procedure already described. The results are presented in Tables 6(A) and 6(B) and the freeze-drying curves in Fig. 4.

The data show that, in general, neither the freeze-drying at -30° nor the freeze-drying at room temperature leads to any significant reduction in protein extractability. The slightly higher figures for insoluble residue, noted in Runs 2, 4 and 11 are most probably due to a failure to exclude small quantities of connective tissue from the test materials. While it is possible that freeze-drying at -30° leads to an increase in insoluble residue, it seems unlikely that dehydration at this low temperature could cause changes (such as, denaturation) not observed after freeze-drying at -10° (see Table 3A).

Appendix 4

Protein Extractability of Tissues Dried Without Freezing

The high extractabilities obtained with freeze-dried samples prompted us to try some drying procedures more likely to lower extractability. Samples were dried, either at +22° or at +2°, in a vacuum desiccator adapted to permit use of thermocouples and the measurement of pressure. In one case, after dehydration was completed, the temperature was raised to 80° and maintained there for 24 hours. The following three desiccants were used: (1) Linde "molecular sieve," which was chosen for its excellent dehydrating properties when we wanted a high degree of desiccation; (2) 55% sulphuric acid, which, at 20°, exerts a partial vapor pressure slightly greater than that of ice at 0°, was selected when a less drastic action was desired and the danger of freezing by evaporative cooling was to be avoided; (3) saturated potassium fluoride solution, which exerts about the same water vapor pressure as 55% sulphuric acid, and was substituted for the latter when we suspected that SO_2 released from the sulphuric acid might have led to losses in tissue protein extractability.

Questions arose too about the possibility that traces of hydrofluoric acid might have led to some protein denaturation. We shall, thus, concentrate our attention on the results obtained with molecular sieve, although we give in Table 7, the results obtained with the three desiccants.

A comparison of the data presented in the first five lines of this table with the data presented previously on frozen and on freeze-dried tissue does not reveal any evident effect of drying without freezing on the extractability of the proteins.

Dehydration by molecular sieve followed by heating at 80°, at very low moisture contents, leads to an almost complete loss of extractability. Since desiccation with sieve reduces only slightly the solubility, the damaging action of the heat is clearly evident, and the effect is similar to that reported above on freeze-dried tissue.

Table 6(A)

Protein Extractability of Tissue Frozen at the Three Standard Rates and Freeze-Dried at -30° and at Room Temperature

Experiment No.	Freeze-Drying Temp. (°C.)	Residual Water (%)	Low-Salt-Soluble Fraction (%)	Non-Prot. Nitr. (%)	High-Salt-Soluble Fraction (%)	Insol. Residue (%)
		(a) Tissue Frozen Rapidly				
1	-30	2.8	5.1	-	10.1	1.5
2	-30	3.2	6.1	0.1	10.8	3.6
3	Rm. Temp.	-	6.3	-	13.2	2.3
4	Rm. Temp.	0.7	5.4	-	11.4	4.1
5	Rm. Temp.	3.3	7.8	-	13.2	1.4
		(b) Tissue Frozen at Intermediate Rate				
6	-30	2.5	5.6	0.7	15.5	2.3
7	Rm. Temp.	-	6.4	-	11.8	0.8
8	Rm. Temp.	-	7.5	0.5	11.2	1.8
9	Rm. Temp.	1.4	6.9	-	11.5	1.3
10	Rm. Temp.	1.9	7.0	0.5	11.2	1.9
		(c) Tissue Frozen Slowly				
11	-30	3.3	6.2	0.6	11.0	3.9
		Averages and Extremes				
1,2,		Max.:	6.2	0.7	15.5	3.6
6,11	-30	Av.:	5.8	0.5	11.9	2.8
		Min.:	5.1	0.1	10.1	1.5
3,4,5,		Max.:	7.8	0.5	13.2	4.1
7,8,9,	Rm. Temp.	Av.:	6.8	0.5	11.9	1.9
10		Min.:	5.4	0.5	11.2	0.8

Table 6(B)

Protein Extractability of Tissue Frozen at the Three Standard Rates but not Freeze-Dried (Controls for Material Reported in Table 6(A)

Experiment No.		Low-Salt-Soluble Fraction (%)	Non-Prot. Nitr. (%)	High-Salt-Soluble Fraction (%)	Insol-Residue (%)
	(a) Tissue Frozen Rapidly				
1C		4.0	-	6.5	1.5
2C		6.4	0.5	10.8	0.6
3C		7.5	-	11.3	0.8
4C		7.5	-	11.3	0.8
5C		7.5	-	11.3	0.8
	(b) Tissue Frozen at Intermediate Rate				
6C		6.7	0.9	13.0	1.0
7C		5.0	-	11.6	0.5
8C		7.8	0.6	13.6	0.5
9C		7.5	-	11.3	0.8
	(c) Tissue Frozen Slowly				
10C		6.3	0.3	11.5	1.1
11C		5.6	0.4	11.2	1.4
	Averages and Extremes				
1C,2C, 6C,11C	Max.:	6.7	0.9	13.0	1.5
	Av.:	5.7	0.6	10.4	1.1
	Min.:	4.0	0.4	6.5	0.6
3C,4C,5C, 7C,8C,9C, 10C	Max.:	7.8	0.6	13.6	1.1
	Av.:	6.7	0.5	12.1	0.7
	Min.:	5.0	0.3	11.3	0.5

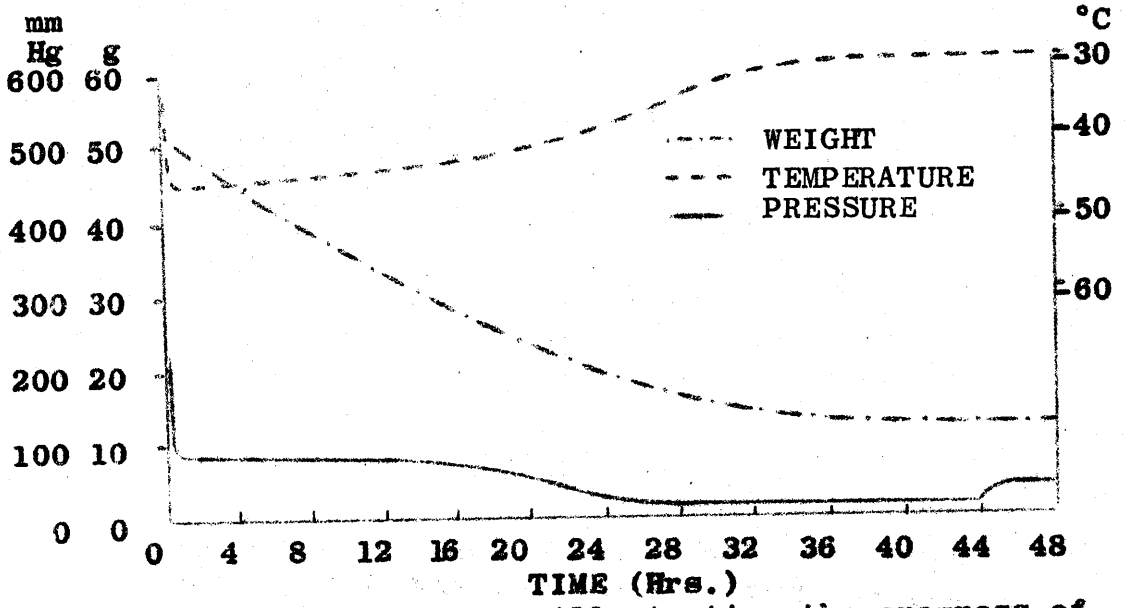


Fig. 4. Set of curves illustrating the progress of freeze-drying in a typical experiment conducted at a specimen-chamber temperature of -30°C.

Table 7

Protein Extractability of Tissues Dried Without Freezing

Experiment No.	Drying Temp. (°C.)	Type of Desiccant	Residual Water (%)	Low-Salt-Soluble Fraction (%)	Non-Prot. Nitr. (%)	High-Salt-Soluble Fraction (%)	Insol. Residue (%)
		(A) Tissue Dried					
1	+2	Mol. Sieve	-	5.9	0.2	10.3	2.7
2	+22	Mol. Sieve	-	6.4	0.9	12.0	1.1
3	+2	Mol. Sieve	2.0	5.8	1.3	10.4	2.2
4	+22	Mol. Sieve	2.0	5.2	0.5	8.7	3.1
5	+22	Mol. Sieve	1.3	6.7	1.8	10.1	0.5
6	+22	55% H_2SO_4	5.4	4.8	1.3	5.6	9.0
7	+22	55% H_2SO_4	6.3	5.8	1.3	8.8	5.6
8	+22	Satd. KF	5.5	6.6	0.9	7.1	11.8
9	+22 & +80	Mol. Sieve	-	1.8	0.3	1.8	18.4
		Averages and Extremes					
1		Max.:		6.7	1.8	12.0	3.1
to		Av.:		6.0	0.9	10.3	1.9
5		Min.:		5.2	0.2	8.7	0.5
		(B) Tissue Not Dried (Controls for Nos. 1 to 9)					
1C	-	-	-	6.4	0.5	10.8	0.6
2C	-	-	-	6.4	0.5	10.8	0.6
3C	-	-	-	5.6	0.4	11.2	1.4
4C	-	-	-	6.4	0.5	10.8	0.6
5C	-	-	-	7.4	2.5	13.5	0.5
6C	-	-	-	6.3	0.6	11.3	2.1
7C	-	-	-	5.4	2.0	10.7	6.1
8C	-	-	-	6.1	0.4	10.0	2.8
9C	-	-	-	6.1	0.4	10.0	2.8
		Averages and Extremes					
1C		Max.:		7.4	2.5	13.5	1.4
to		Av.:		6.5	1.1	11.8	0.8
5C		Min.:		5.6	0.4	10.8	0.5

General Conclusions of Part I

1. Tables 3(A) and 3(B), 6(A) and 6(B) show that the freeze-drying process, i.e., the act of removing, by sublimation, the ice formed from water in the tissue, does not affect the extractability of any of the proteins present. This conclusion holds for widely

differing freeze-drying conditions, with the possible exception of freeze-drying in a specimen chamber maintained at -30 . (The effects of the last-mentioned treatment deserve further investigation.)

2. Furthermore, sufficiently gentle but more or less complete drying from the nonfrozen state has very little or, perhaps, no effect on extractability. It, therefore, appears that dehydration may be accomplished at temperatures in the range 0° to $+20^{\circ}$, with corresponding concentration of proteins and salts, without the occurrence of reactions leading to "denaturation" of either the low-salt-soluble or the structural proteins.

3. Again, Tables 3(A) and 3(B), 6(A) and 6(B) show that the secondary drying stage in the freeze-drying operation, can be carried beyond the 2% residual moisture level without affecting extractability. Thus there does not seem to be any "vital water" layer, the removal of which causes damage to the system.

4. Heat applied to the tissue in the dried state - whether the tissue be freeze-dried, or merely dried, and whether the water content be 2% or high, or 0.2% or lower - leads to reactions which cause reductions in extractability, particularly in extractability of the actin, myosin and tropomyosin.

PART II

WATER BINDING OF VARIOUSLY TREATED MUSCLE TISSUE AS STUDIED BY ADSORPTION ISOTHERMS

(A) Method and Apparatus

In principle, a piece of freeze-dried tissue of known dry weight is placed in an atmosphere of given relative humidity until it is in vapor pressure equilibrium with that atmosphere; then its weight is measured again. A plot of the increasing weight of the piece in terms of the increasing water vapor pressure will then give information on the water-binding properties of the tissue.

To facilitate such determinations we set up the apparatus represented in Fig. 5. The specimen A (a piece of freeze-dried tissue), weighing less than 5 mg., is suspended at the lower end of a quartz fiber B which hangs on the arm C of a torsion microbalance. The specimen is exposed to the atmosphere D in a glass vessel of about 500 ml. capacity which contains a mixture E of water and sulphuric acid that controls the relative humidity. Measured quantities of water are added from a burette F to provide stepwise increases in water vapor pressure. The temperature is controlled by a thermostat in the bath G. A double stirrer H agitates the air and the solution in the vessel.

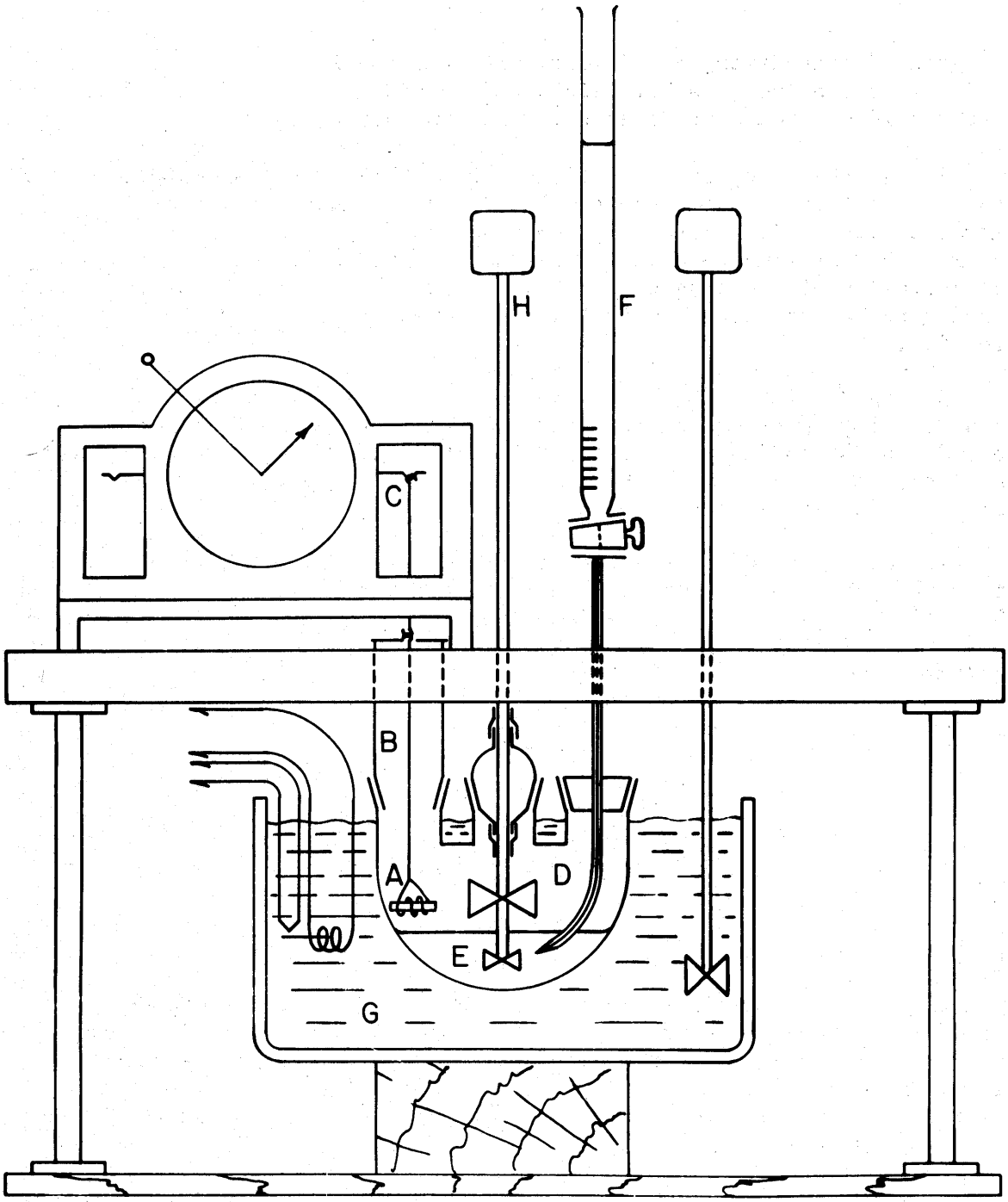


Fig. 5. Apparatus for the determination of water binding by the method of vapor pressure equilibrium.

Our plans called for two series of determinations of adsorption isotherms, one on whole pieces of tissue freeze-dried which had been subjected to one of the nine standard treatments (combinations of freezing rates and freeze-drying conditions), the other on their extracted fractions. The results of our experiments will be presented in two corresponding sections.

I. Adsorption Isotherms of Whole Pieces of Freeze-Dried Tissue. A plotting of the results of 16 final determinations of the isotherms of all the categories of tissues studied revealed that the curves were classifiable into 3 groups according to the rate of freezing. The averages of the 3 groups are represented in Fig. 6.

Marked differences appeared also between the isotherms of the tissue heated to +80° after having been freeze-dried and those of tissue not so treated. We represent in Fig. 7 the isotherms obtained with material frozen at one of the three standard rates and exposed to 80° after having been freeze-dried; they are to be compared with the isotherms of Fig. 6 which were obtained with material frozen at one of the three standard rates and not exposed to +80° after having been freeze-dried.

An analysis of the data leads to the following conclusions:

(1) The three freezing rates yielded different water-binding properties in determinations made in the range of relative humidities (RH) extending from 0 to somewhere between 10 and 20% (cf. Curves R, I and S of Fig. 6, for rapid, intermediate and slow freezing, respectively). The high freezing velocity gave the greatest readsorption of water; the low velocity, the smallest. - In the range of RH extending from 20 to 30%, the vertical separations between the three average isotherms are the greatest. - Above 30% RH, these separations are gradually smaller until, around 85% RH, the three curves intersect. - Above 90% RH, curves A and C seem to cross each other, C becoming the highest and A the lowest.

(2) Freeze-drying at -10° or at +50°, after freezing at any of the three rates, had no definite influence on the readsorption of water.

(3) Exposure of the freeze-dried tissue for 24 hours to +80° lowered the isotherms, very drastically after slow freezing, but less so after freezing at intermediate or high rate (compare curves A, B and C in Figs. 6 and 7).

Analysis by the method of Brunauer, Emmett and Teller (B.E.T.) of the isotherms leads to values for the area of the adsorbed water monolayer which are almost independent of the initial freezing rate

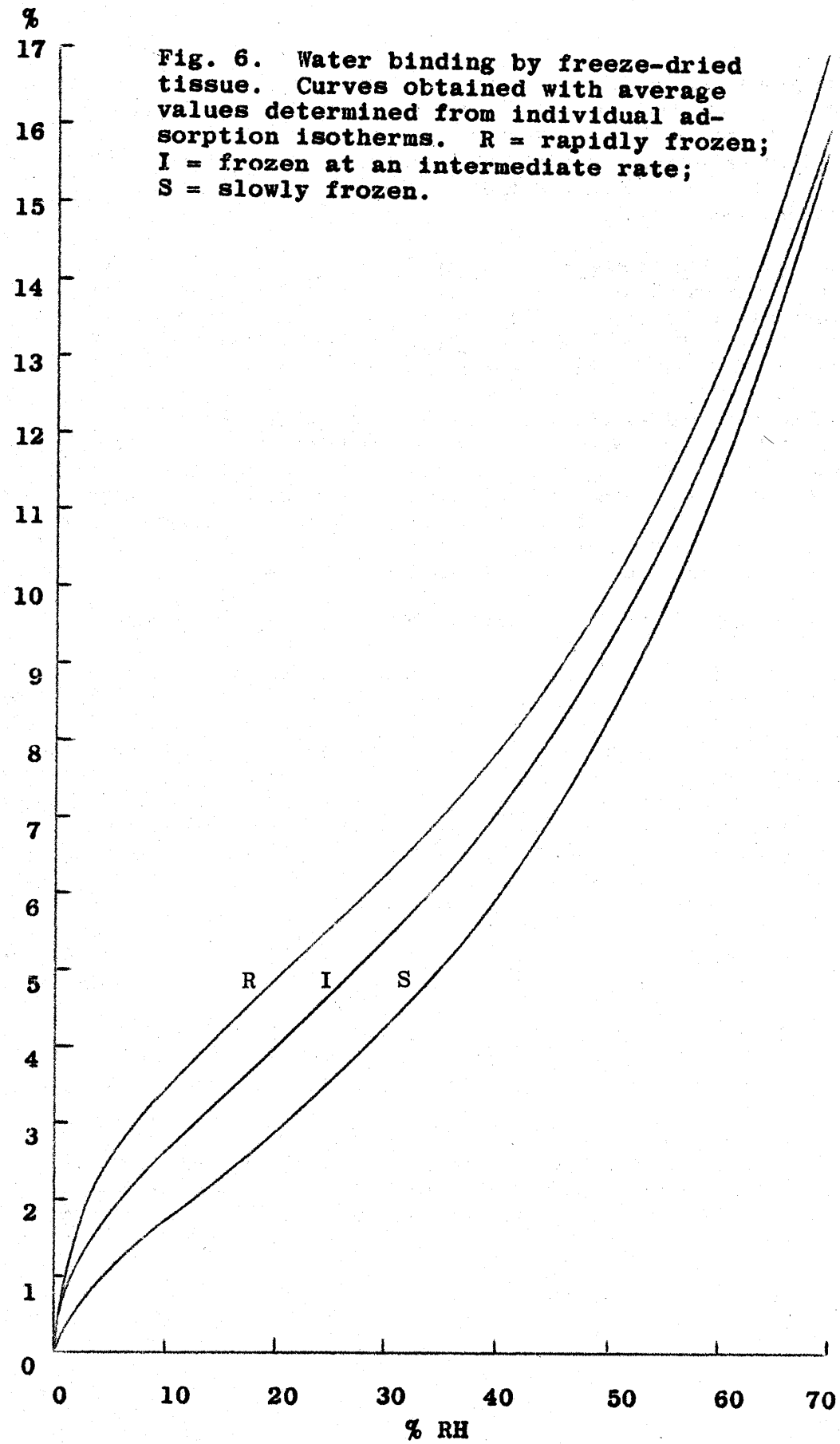


Fig. 6. Water binding by freeze-dried tissue. Curves obtained with average values determined from individual adsorption isotherms. R = rapidly frozen; I = frozen at an intermediate rate; S = slowly frozen.

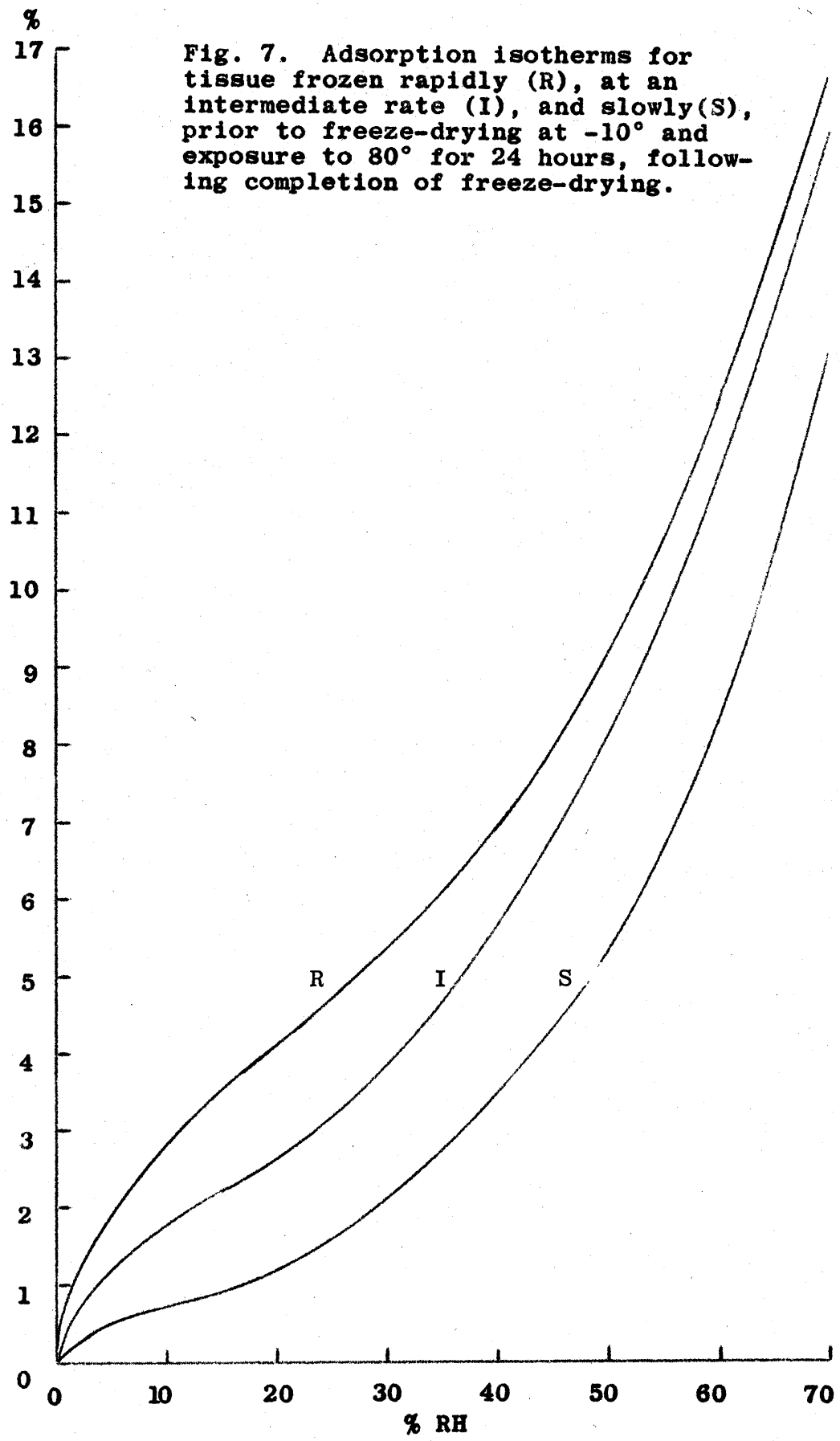


Fig. 7. Adsorption isotherms for tissue frozen rapidly (R), at an intermediate rate (I), and slowly(S), prior to freeze-drying at -10° and exposure to 80° for 24 hours, following completion of freeze-drying.

(0.188, 0.183 and 0.180 m^2/mg. for rapid, intermediate and slow freezing rates, respectively) but indicates that the monolayer is bound more strongly, the higher the initial freezing rate.

B.E.T. analysis of the isotherms of tissue heated to 80° after freeze-drying shows that the heating has reduced the area of the surface available for the formation of a water monolayer. The reductions in area were of the order of 15%, 40% and 75% for the tissues initially frozen at high, intermediate and low rates, respectively; we may thus conclude that the higher the initial freezing rate, the less the susceptibility to reduction in monolayer area by heating to 80°.

These observations raise interesting questions. For instance, it is curious that, at 20% RH, the samples yielding curve A have almost twice as high a water-binding ability (5%, w/w) as the samples giving curve C (2.8%, w/w), whereas, in the range 80 to 85% RH, all samples show the same water-binding ability (28.5%, w/w at 85% RH) and above 85% the trend may be reversed. Ability to "bind" or "hold" water between 90% and 100% RH is, of course, of considerable significance.

But both the establishment of the facts and their interpretation need further investigation.

Adsorption Isotherms of Protein Fractions. We made numerous trial experiments to find suitable methods for determining adsorption isotherms of low- and high-salt-soluble proteins and of insoluble residues, but found it impossible to obtain significant data. "Descending isotherms," i.e., isotherms requiring equilibration at successively lower relative humidities, of material which is initially in the wet state, appeared to be determined by the rate of dehydration in the range 100 to 80% RH, and steady values for water content at lower relative humidities were apparently not established thereafter.

We then tried to determine "ascending isotherms" both for the soluble and insoluble fractions and, again, could not make equilibrium measurements of the adsorption of water, except at very low relative humidities. Interpretation of these "ascending isotherms" was further complicated by the fact that all fractions, whether from freeze-dried or from control tissue samples, were subjected to complete dehydration prior to determination of the isotherms.

The difficulties encountered in these attempts were in sharp contrast to the ease with which reproducible "ascending isotherms" were obtained from whole freeze-dried tissue. Since the same techniques were employed in all adsorption experiments, it would seem that the difficulties encountered were due to some physical properties of the fractionated materials and possibly to chemical reactions made possible by these physical properties.

PART III

WATER-HOLDING CAPACITY OF VARIOUSLY TREATED MUSCLE TISSUE AS STUDIED BY THE PRESSURE METHOD

As was already stated, we used the pressure method of Grau and Hamm (1957) and Wierbicki and Deatherage (1958) in four categories of muscle tissue, namely: (a) in tissues in the fresh state (control, not frozen); (b) after freezing and thawing (control, not freeze-dried); (c) after freezing at each of the three standard rates, freeze-drying at one of the three standard temperatures, and rehydrating; (d) after treatment by heat (cooking) the tissue previously treated as in (c). The method - one will recall - consists in determining the water-holding capacity of the tissue by measuring the proportion of water which cannot be expressed by the application of a given mechanical pressure.

(A) Methods

The methods used in this project which have not yet been described include: (1) pressing, (2) rehydrating the freeze-dried material and (3) cooking.

Pressing Procedure. Samples of muscle tissue weighing about 300 mg. were placed on No. 1 (9 cm.) Whatman filter paper preconditioned according to Wierbicki and Deatherage's method, and pressed for 1 minute at 500 p.s.i. between plexiglass plates mounted in a hydraulic press.

Water-holding capacity was determined by subtraction of the quantity of water expressed from the total quantity of water present before pressing. This difference, i.e., the water not removed by pressing, may then be expressed as a percentage of the total water present initially as a percentage, w/w, of the total solid content of the tissue before pressing. (For more details we refer the reader to the original papers of the authors cited.)

Rehydration Procedure. Rehydration of freeze-dried tissue was accomplished with the apparatus and in the manner described by Luyet et al. (1961). Cubes of tissue were weighed in the freeze-dried state, covered on five sides with aluminum foil (to help prevent the loss of water-soluble proteins), and packed, exposed face uppermost, in the bottom of the rehydrating chamber of the apparatus. A weighted wire gauze disc, placed over the cubes, served to prevent them from floating on contact with water. The chamber was then evacuated and water was admitted, in vacuo, until the pieces were totally submerged. Under these conditions, rehydration is rapid and the tissue is thoroughly impregnated. The cubes were then removed from the rehydration chamber and, after having been freed from the foil, were tumbled over wet filter paper in such a way that the excess surface moisture was removed from the six sides of the cubes.

Table 8

Water-Holding Capacity of Muscle Tissue: (1) Freeze-Dried and Rehydrated, (2) Freeze-Dried, Rehydrated and Cooked, (3) Controls: (a) Not Frozen, (b) Frozen and Thawed, (c) Not Frozen, Cooked

Mode of Freezing	Freeze-Drying Temp. (°C.)	(1) Tissue Freeze-Dried and Rehydrated: Water Held (% of Total Water)	(1) Tissue Freeze-Dried and Rehydrated: Water Held (% of Dry Wt.)	(2) Tissue Freeze-Dried, Rehydrated and Cooked: Water Held (% of Total Water)	(2) Tissue Freeze-Dried, Rehydrated and Cooked: Water Held (% of Dry Wt.)
Rapid	-10	50.9, 53.1, 53.3	65.2,64.2	24.9	41.3
	+50	37.2	85.8	14.7	27.3
	+80	34.4, 41.1	91.5,78.0	5.4	9.7
At Interm. Rate	-10	51.8	120.1	19.4	28.1
	+50	62.4	64.9	17.8	23.8
	+80	17.8	23.8	7.1	13.0
Slow	-10	48.8, 49.3	116.8	17.5	24.9
	+50	49.8, 55.3, 66.8	117.4,94.5	22.5	33.9
	+80	37.1, 37.8	80.3	13.4	19.9

(3) Controls
Water Held (% of Total Water)
(a) Not Frozen: 58.7, 59.9, 61.8, 64.4, 67.3
(b) Frozen and Thawed: 42.6, 46.0, 55.4, 63.6, 64.4
(c) Not Frozen, Cooked: 53.5, 84.2

Heating (Cooking Procedure). Pieces of tissue, having a wet weight of about 300 mg., either fresh or rehydrated after having been freeze-dried, were wrapped individually in aluminum foil (or were already wrapped for rehydration). The wrapped pieces, one of which contained a thermocouple, were further enclosed together in a single, watertight foil envelope, placed in a boiling water bath for a few seconds (until the thermocouple indicated an internal temperature of 70°) and transferred to a second water bath at 70° for 15 minutes.

(B) Results

The results are reported in Table 8, in which the amount of water held by the tissue submitted to pressure is expressed in percent of the dry weight and also in terms of the total water present before pressing.

General Observations and Comments. (1) The first important feature in Table 8 is the enormous variations obtained in experiments made under similar conditions, but on different pieces of tissue; for example, in two controls, not frozen, cooked (last line). On several occasions, we observed that a tissue firmer to the touch had a higher water-holding capacity.

(2) Despite these great variations, one may distinguish two main trends: (a) Tissue cooked after having been rehydrated holds much less water than non-cooked tissue. (b) Tissue heated to 80° after having been freeze-dried, loses much of its water-holding capacity in that treatment. (c) Tissue freeze-dried at +50° had about the same water-holding capacity as tissue freeze-dried at -10°.

PART IV

REHYDRATION RATE OF VARIOUSLY TREATED, FREEZE-DRIED MUSCLE TISSUE

The problem of rehydration rate is closely related to that of the obstacles to rehydration, in particular the entrapping of air bubbles. Accordingly, Part IV of this report will consist of two sections, one dealing with the rehydration rate proper, the other with entrapping of air.

(A) Methods

Rate of Rehydration. Of several methods that we tried for measuring the rate of rehydration of freeze-dried muscle tissue, we chose the "drop method" which recommends itself for simplicity and reliability. It consists in allowing a drop of water to fall on the piece of tissue from a given distance, and in measuring the time required for the drop to be absorbed.

The details of the procedure are as follows: A drop of distilled water, delivered from a one-millimeter (I.D.) capillary tube, of which the lower end is placed 5 mm. above the piece of tissue, falls on the latter, and the time elapsed until the originally smooth, unbroken, wet surface of the tissue ceases to reflect light uniformly is recorded.

We soon realized the importance of two particular factors in that technique: (1) the orientation of the muscle fibers with reference to the plane of the surface studied, (2) the previous history of that surface, namely, whether it originated from a section made before freezing or after freeze-drying. We then decided (1) to determine the rate of rehydration on two surfaces, one parallel, the other perpendicular to the direction of the fibers, and (2) to use only pieces cut before freezing in one or the other of the two desirable orientations.

Amount of Entrapped Air. Pieces of freeze-dried tissue, held with their fibers orientated in a vertical or horizontal direction, were immersed abruptly in the rehydrating fluid, distilled water. These pieces were cubes, approximately one centimeter on a side, except in the case of rapid freezing, in which they were layers one to two millimeters thick. In that case, each layer was rehydrated

separately and enough layers were then assembled to provide approximately the same quantity of material as with the cubes. All samples were held in the rehydrating fluid for three minutes.

To determine the quantity of air entrapped in the course of rehydration, we squeezed out, under water, with the aid of a single piston device, the air remaining in pieces of tissue after the three-minute immersion, collected it, and measured its volume in an eudiometer.

The apparatus - which has been described in a paper by Luyet et al. (1961) to which we refer the reader for details - consists of (1) a compressing chamber, (2) a eudiometer, (3) a system of connecting tubes, vessels and stopcocks for transferring the air from the compressing chamber to the eudiometer.

(B) Results

Rate of Rehydration. The results are given in Table 9.

In all cases the samples warmed to +80° required a longer time to absorb the drop of water. This is apparently due, to large extent, to the melting, or softening of the fats that tend to close the channels left by the sublimation of the ice. Such a view is confirmed by the fact that often the surface has a glossy appearance, as if fats have spread over it.

Table 9

Rates of Rehydration as Determined by the "Drop Method"

Mode of Freezing	Temp. of Fr.-Drying (°C.)	Surface Perpend. to Fibers Av. Time (sec)	Ex-tremes (sec)	Nr. of Determ-inations	Surface Parallel to Fibers Av. Time (sec)	Ex-tremes (sec)	Nr. of Determ-inations
	-10	4	1-6	9	44	3-107	11
Rapid	+50	2	1-4	10	38	15-90	10
	+80	12	7-17	5	40	22-100	9
At	-10	1.5	1-4	11	4	2-16	15
Interm.	+50	4	1-9	10	10	2-26	18
Rate	+80	27	10-49	10	78	38-138	6
	-10	2	1-5	12	2	1-3	13
Slow	+50	2	1-3	10	2	1-4	10
	+80	20	7-46	12	35	11-70	8

The drop was absorbed faster on the surfaces perpendicular to the fibers than on the surfaces parallel to them; apparently the channels left after the sublimation of the ice, which are known to be predominantly parallel to the fibers, act as capillary tubes.

Generally, tissues frozen at higher cooling rates had lower absorption rates. Since we know that higher cooling rates result in the formation of smaller ice crystals and finer channels which, after freeze-drying, would exhibit stronger capillary action, we are led to consider the intervention of another inhibiting factor, which may be the counter effect of the air entrapped in such small channels.

Amount of Entrapped Air. The volumes of air entrapped during rehydration, and remaining after three minutes, are shown in Table 10 which gives the results of two series of experiments with fibers placed vertically and two with the fibers placed horizontally. (In the case of the thin slices used for rapid freezing, such orientation being impossible, we limited ourselves to three series.)

The following trends are apparent in Table 10: (1) Less air was entrapped in the slowly frozen than in the more rapidly frozen samples; (2) less air was entrapped when the fibers were orientated vertically during rehydration than when they were orientated horizontally; and (3) samples freeze-dried at higher temperatures had more entrapped air than those freeze-dried at low temperatures.

Table 10

Amounts of Air Entrapped During Rehydration

Mode of Freezing	Fr.-Drying Temp. (°C.)	Entrapped Air: Fibers Placed Horizontally (ml/g/dry wt.)	Entrapped Air: Fibers Placed Vertically (ml/g/dry wt.)
	-10	.25, .30, .33	
Rapid	+50	.24, .27, .30	
	+80	.25, .43, .61	
At	-10	.78, 1.28	.59, .68
Interm.	+50	.84, .92	.70, .72
Rate	+80	1.55, 1.82	1.57, 1.15
	-10	.36, .50	.29, .31
Slow	+50	.44, .46	.22, .34
	+80	1.07, 1.09	.58, .53

Note: So many factors and complex relationships, on which we have little information, would be involved in the interpretation of these trends that further speculation would hardly be justified. Thus a factor which is, no doubt, important is the dissolution of the entrapped air by the invading rehydration fluid, dissolution which, itself, depends on the surface areas involved, that is, on the size of the ice crystals, and the latter, in turn, depends on the freezing-rate.

PART V

TESTS FOR TENDERNESS

We used, in this work, two methods of determining tenderness: sensory evaluation and mechanical testing. - The types of tissue tested included: (1) freeze-dried, rehydrated and cooked tissue of the nine standard categories, (2) fresh tissue (not frozen), cooked.

(A) Methods

Preparatory Treatments. A piece of stock tissue was divided into four parts which were then used to provide rapidly frozen slices, cubes frozen at an intermediate rate, larger pieces frozen slowly, and control material. Portions of each of the three types of frozen material were then freeze-dried in one or other of the three standard conditions, thus yielding the nine different types of freeze-dried product from the same batch of tissue.

Rehydration was accomplished by the method described in Part III, after minimal storage periods in the freeze-dried condition (one to two days), and cooking was performed in the manner also described in Part III.

Sensory Determination of Tenderness. The test was carried out with the aid of a taste panel composed of seven members of the Foundation's staff. The panel members were requested to compare the tenderness of each of the nine processed samples with that of the control (nonfrozen, cooked) and to make semi-quantitative estimates of the extents to which tenderness was greater or less than that of the control. Estimates from the various members of the panel were then averaged and expressed in figures on an arbitrary scale from 1 to 5, 5 being the most tender.

Mechanical Testing of Tenderness. For this purpose, we designed and constructed a simple apparatus which consisted of an aluminum bar, 20 cm. long, pivoted at one end to a heavy base and containing a stainless steel razor blade, with its single cutting edge parallel to the length of the bar, at the other end. The moving end of the bar rested on a split specimen-table, about 2 x 2 cm., such that the razor blade fitted into a split between the two 1 x 2 cm. halves of the table but did not touch them. For the test, we raised the arm, placed the samples, one at a time, across the slit in the table, and released the arm from a fixed, predetermined position. The razor blade cut into the sample; the depth and the length of the cut were measured and multiplied to yield the area of the cut surface. A series of 5 to 7 cuts were made parallel to the fibers in the sample, and a similar number of cuts at right angles to the fibers in each 1 cm. cube of tissue examined.

The tenderness of the sample for a given orientation of the fibers was then defined as the average area of the cut, produced in the manner described, for that orientation. Quite consistent results were obtained first in several preliminary tests on controls, and then in the case of freeze-dried, rehydrated and cooked samples.

(B) Results

The experimental findings recorded with the two testing methods are presented in two sections of Table 11. The information obtained from the tasting panel is expressed in figures in the scale of tenderness (Score: 1 to 5) and in verbal comments by the members of the panel (tender or tough, very or medium (v. or m.) tender or tough; the information from the mechanical test is expressed in "area of cut surface" (in $cm.^2$) and in a corresponding figure for relative tenderness (again, Score: 1 to 5).

Table 11

Tenderness of Freeze-Dried, Rehydrated and Cooked Meat

Freeze-Drying Temp. (°C.)	Sensory Evaluation		Mechanical Test: Perpend. to Fibers		Mechanical Test: Parallel to Fibers	
	Score	Comments:	Score	Cut Surf. Area (cm^2)	Score	Cut Surf. Area (cm^2)
		Tissue Rapidly Frozen				
-10	4.0	m.tender	4	0.41	3	0.45
+50	5.0	tender	3	0.38	6	0.68
+80	2.0	tough	-	-	-	-
		Tissue Frozen at Intermediate Rate				
-10	3.6	m.tough	3	0.35	3	0.43
+50	3.3	tough	1	0.18	3	0.45
+80	1.3	v.tough	2	0.31	2	0.31
		Tissue Slowly Frozen				
-10	4.0	m.tender	4	0.43	4	0.53
+50	5.0	tender	4	0.43	4	0.49
+80	0.8	v.tough	2	0.28	5	0.62
		Control (not Frozen), Cooked				
-	5.0	tender	5	0.49	5	0.62

Conclusions: 1. Freeze-drying leads to slight or moderate loss of tenderness, the loss being (a) least detectable in slowly frozen tissue and greater in tissue frozen at intermediate and high rates, and (b) independent of the Freeze-drying temperature (-10 or +50°),

2. The application of heat at 80° for 24 hours, after freeze-drying at -10°, causes extreme toughness in cooked tissue, save when the toughness is measured parallel to fibers in tissue slowly frozen before being freeze-dried. In this latter case, heating at 80° has apparently loosened the fiber bundles.

3. Results of sensory and mechanical testing are in good agreement if one compares results of sensory tests with an average of results from mechanical tests made with the blade of the testing instrument perpendicular and parallel to the direction of the fibers.

General Evaluation

1. (a) The sarcoplasmic proteins seem to be highly resistant to damage by freeze-drying. Though we could not find a way to obtain a meaningful isotherm from this fraction extracted from freeze-dried tissue, we observed that the extracted fraction was entirely soluble after a second freeze-drying in the presence of the extracting salts.

(b) There was considerable evidence that the structural proteins are susceptible to damage by freeze-drying and to heat applied to the whole freeze-dried tissue. Salt-free quantities of structural proteins readily lost their ability to re-dissolve in salt solutions and exhibited lower adsorption isotherms after repeated dehydration, whether by freeze-drying or by air-drying.

(c) The contribution of the stroma to the properties of the meat and the effects of freeze-drying on this fraction are less easily assessed. In fact, we have been unable to find any evidence for a relationship between changes in tenderness and changes in properties of the stroma fraction.

2. As for the water binding properties of the freeze-dehydrated meat, we would only note the relationships expressed in Figs. 6 and 7. That is, water binding by the freeze-dried tissue in greater when the initial freezing rate has been higher, and is reduced by the application of heat after freeze-drying, the reduction being greater when the initial freezing rate has been lower.

3. No direct relationship was observed between water binding ability and water holding capacity after rehydration. A relationship existed, however, by coincidence, between these two characteristics in the case of tissues heated to 80°, in as much as tissue heated to 80° after freeze-drying exhibits lower water-binding ability and, after rehydration, exhibits lower water holding capacity in comparison with similar freeze-dried tissues not exposed to heat.

4. There is a relationship between water-binding ability and rehydration rate (and extent of rehydration) in as much as water-binding ability is greater when the initial freezing rate has been higher (see Part II), and rehydration rate (and extent) are greater when the initial freezing rate has been lower (see Part IV). Thus there is an inverse relationship between fixed bound hydration water and rehydration rate and extent.

The following values for variables susceptible to control in freeze-drying are suggested:

(a) Low freezing rates - these permit good rehydration characteristics and relatively high tenderness scores after cooking. Furthermore, the low water binding ability after freeze-drying of slowly frozen tissue does not seem to be of practical significance. We would even suggest that further trials be made in which tissues are frozen very slowly prior to freeze-drying (time at the freezing plateau of a day or more).

(b) Moderate specimen chamber temperatures - there does not seem to be an advantage to low ($-10^{\circ}C.$) or extra low (-30°) temperatures in freeze-drying of meat. Moderate application of infra-red radiation has led to as acceptable a product as freeze-drying at -10°.

We suggest, however, that many more experiments be made on the lines we have taken to determine the effects of specimen chamber temperature. - Freeze-drying is achieved more rapidly (though sometimes not more economically) with greater temperature gradients; but higher temperatures on the specimen lead to loss of protein extractability and incidence of toughness after rehydration and cooking. - A study of the kinetics of the development of insolubility, in tissue freeze-dried to various moisture contents, and subsequently exposed to 50°, 60°, 70°, etc., for various times, should lead to an estimate of the maximum safe specimen chamber temperature for a given batch of tissue.

6. In view of the limited correlation between the measured tenderness of tissue, the water-binding ability and the water-holding capacity, we suggest that further attention be devoted to a search for a measurable characteristic which correlates well with tenderness after cooking.

Since the pressure of 500 p.s.i. was chosen by Wierbicki and Deatherage from a consideration of the properties of fresh tissue, and since it is likely that the relationship between pressure applied and fluid expressed is different for tissue freeze-dried and rehydrated, we suggest that attention be focused on a study of this very relationship, as it is affected by freeze-drying.

SUMMARY

The purpose of this contract was to study the water sorption characteristics of raw beef muscle which had been freeze-dried by different methods. The sorptive properties were examined on the basis of the adsorption isotherm, water holding capacity, extractability, solubility, rehydratability and tenderness. When the results are considered on an "overall texture" basis, optimum rehydration and tenderness characteristics after cooking were obtained in meat which was frozen at a slow rate and dehydrated at a final 50°C temperature in the dehydration chamber. Higher temperatures immediately after the final stage of dehydration were found to have the most detrimental or toughening effect on the texture of meat. On the other hand, no advantage existed when low (-10°C) or extra low (-30°C) chamber temperatures were used.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*

American Foundation for Biological Research
Madison, Wisconsin

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

STUDY OF THE WATER BINDING PROPERTIES OF FREEZE-DEHYDRATED MEAT IN RELATION TO PROTEIN COMPOSITION AND PROCESSING TREATMENT

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Final Report 21 Feb 63 - 20 Feb 64

5. AUTHOR(S) *(Last name, first name, initial)*

Luyet, B. J. and MacKenzie, A. P.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1967	35	12

8a. CONTRACT OR GRANT NO.
DA19-129-AMC-63(N)

b. PROJECT NO.
7X84-06-003

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

67-90-FL FL-57

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12. SPONSORING MILITARY ACTIVITY

U. S. Army Natick Laboratories
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13. ABSTRACT

(1) Determination of the low- and high-salt-soluble protein fractions, and of the insoluble fraction in beef muscle frozen at 3 rates and freeze-dried at 3 final temperatures. (2) Establishment of the adsorption isotherms of the freeze-dried muscle tissues. (3) Measurement, by the mechanical pressure method of the water-holding capacity of the freeze-dried, cooked and noncooked tissues. (4) Evaluation of the rate and extent of rehydration of freeze-dried tissues subjected to various pretreatments. (5) Tests of tenderness of meat cooked after being freeze-dried.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Freeze-drying	8				6	
Freezing	8				6	
Heating	8				6	
Rehydration	8				7	
Beef	9		9		9	
Muscle tissues	9		9		9	
Evaluation			8			
Water-binding properties			8,9			
Water-holding capacity			8,9			
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Pressure			10			
Acceptability					7	
Proteins					7	
Tenderness					7	

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