Proceedings of
the United States
National Museum

SMITHSONIAN INSTITUTION • WASHINGTON, D.C.

Volume 119 1967 Number 3549

THE FREEZE-DRY PRESERVATION OF BIOLOGICAL SPECIMENS

By Rolland O. Hower [1]

Office of Exhibits

Introduction

Visitors today to the United States National Museum see specimens of small animals that accurately represent the form and color they possessed in life. This improved representation is the direct result of the new method of specimen preservation that has been brought about by the process of freeze-drying. By this process, the specimen retains most of its original characteristics without further need for preservation.

This new technique is based upon sublimation of frozen fluids. One of the earliest papers detailing the phenomenon of sublimation was delivered to the Royal Society of London in 1813 by William Hyde Wollaston, a physicist. Wollaston's commentary (1813) on water passing from the frozen to the gaseous state, apparently by-passing the liquid phase, discussed what was already a well-known phenomenon. It was not until the 1890's, however, that the removal

[1] As far as is known, Mr. Hower is the first to apply the freeze-dry process to museum work. Developing more sophisticated apparatus and establishing time schedules for the freezing of specimens, he is now investigating freeze-dry problems of color retention, fat stabilization, and the freezing of larger specimens.

of fluid from tissue by sublimation at low temperatures had been accomplished by Dr. Richard Altman of Leipzig (Glick, 1949, p. 4).

Only in recent years, with the advent of modern refrigeration and vacuum techniques and equipment that is widely available, has the process become practical for commercial or professional purposes. The process of freeze-drying, now greatly refined, is used currently for food processing and for the preservation of pharmaceuticals, human bone, tissue, and blood plasma.

The freeze-drying techniques utilized at the Smithsonian Institution for the preservation of biological specimens began in the late 1950's (Hower, 1962). There is now a well-established freeze-dry program to preserve many types of natural history specimens for exhibit. Current exhibits include freeze-dried rodents, reptiles, crustaceans, insects, and fishes.

Reflecting on events of past years that led to work on the freeze-dry process, I wish to express my gratitude to Dr. H. T. Meryman of the Biophysics Division of the Navy Medical Research Institute, who pioneered many of the techniques and has made himself always available for consultation.

Special thanks also are extended to James C. Nyce, who contributed invaluable aid in the preparation of this paper, to Mrs. Constance Minkin, for her writing contributions, and to John Hackmaster, who prepared the illustrations.

Principles of the Freeze-dry Concept

Freeze-drying consists bascially of dehydrating tissue while it is frozen. Whereas some tissue dried from a nonfrozen state becomes shrunken and consequently distorted in the process, tissue dried from a frozen state virtually retains its original appearance; thus, the process is not only valuable in preserving the quality of food but also in maintaining the appearance of museum specimens. An additional advantage is that the dehydrated tissue is not subject to decay.

Compared to other preservation methods, the shrinkage and distortion of freeze-dried museum specimens is minimal, but there is some distortion in certain types of animals, particularly some fishes. In most cases, however, the distortion is negligible or correctable. Furry or feathery specimens and those with exoskeletons appear completely natural when freeze-dried, provided they have been properly prepared. Such preparation includes the positioning of the specimen before dehydration, the actual dehydration, installation of artificial eyes, and the painting of mucous membranes or exoskeletons (mucous membranes become white after dehydration and crustaceans lose their coloration).

SUBLIMATION.—If biological tissue is first frozen to give it mechanical rigidity and water is then removed by sublimation, most tissue can be dehydrated without apparent physical change.

Consider this process in terms of plain frozen water: ice. Each ice crystal is a geometrically sound structure, made up of myriads of water molecules that are retained in their positions by the gravitational field of the surrounding molecules. Within the restricting confines of this lattice, each molecule moves randomly, and there is a possibility that one of the motions of a surface molecule will be great enough to propel it out of the confines of the lattice. The greater the crystal mass, the greater the probability of such escapes. As temperature is increased and molecular motion becomes accelerated, the probability of escape becomes greater; thus, the average number of water molecules that will escape from a given mass at a given temperature can be statistically predicted. Ice within a biological specimen behaves in nearly an identical manner.

Sublimation begins at the outer surface of a specimen and continues at the boundary between the frozen and the dried tissue. This boundary recedes toward the center of the specimen as drying proceeds. As water molecules continue to escape from ice crystals on the boundary, they move about at high velocity, colliding constantly with other molecules and with the structure of the dried tissue surrounding them. (As they are buffeted from collision to collision, they are virtually independent of external forces.) There is a heavy concentration of water-vapor molecules at the sublimation boundary, due to the great number of molecules escaping; consequently, there are more collisions, which ricochet molecules along the line of least resistance toward the outer shell of the specimen. The force of the molecules' collisions following their escape from the ice crystals on the sublimation boundary supports their drive through the dried tissue of the specimen and into the atmosphere beyond its outer shell. For ice to sublime efficiently, the vapor pressure within the specimen chamber must be lower than the vapor pressure of ice within the specimen itself.

VAPOR PRESSURE.—If the temperature of a vacuum chamber containing ice is maintained at $-10°$ C., evacuated with a vacuum pump, and then valved off so that no external air can enter, molecules will begin to escape from the ice crystals within the chamber (fig. 1). Some of these molecules will ricochet about, colliding with one another and with the sides of the chamber, while others will relocate themselves upon other ice crystals.

As the concentration of the vapor formed by these free molecules reaches a specific point (which is dependent upon the temperatures of their atmosphere), the rate at which molecules return to the ice will

become equal to the rate at which they depart, and the water vapor is then said to be in a state of equilibrium (fig. 1). The vapor pressure at which this equilibrium occurs is referred to as equilibrium vapor pressure. With the temperature of the chamber maintained at −10° C., the pressure indicated on its vacuum gauge will be 1.950 mm. Hg. This is the equilibrium vapor pressure (consequently the vapor pressure) of water at −10° C.

Water-vapor molecules may be removed from the area immediately surrounding the ice in a vacuum chamber, thereby upsetting the equilibrium and permitting more molecules to escape and allowing fewer to return. This principle applies to biological specimens as well as to ice.

The most effective method of continuously removing water-vapor molecules from a specimen chamber is to create a lower vapor pressure elsewhere. This can best be done by establishing a colder surface (condenser) nearby. Water vapor will diffuse to a colder surface and recondense to form new ice crystals.

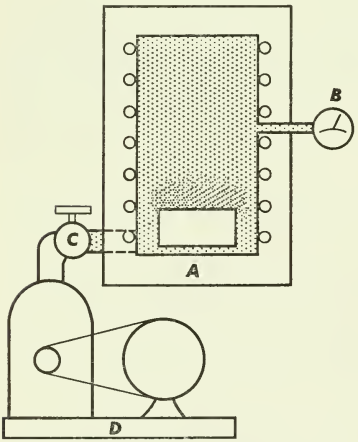


FIGURE 1.—Water-vapor equilibrium (*A*=refrigerated chamber; *B*=vacuum gauge; *C*=cut-off valve; *D*=vacuum pump).

A refrigerated condenser serving as an efficient water pump is employed for this purpose. As previously stated, when the temperature of a specimen chamber is −10° C., the vapor pressure of ice within it is 1.950 mm. Hg. If the temperature of the nearby condensing surface is −40° C., the vapor pressure is 96 micron Hg., creating a vapor-pressure differential of 1.854 mm. Hg. Water molecules will collect on the cold condenser surface. With this

much vapor-pressure difference, the only limitation to the effectiveness of a condenser is the surface area (fig. 2).

PRESSURE REDUCTION.—When vapor transfer occurs at atmospheric pressure, water molecules are impeded by collisions with air molecules. At atmospheric pressure, the mean free path (the average distance a vapor molecule can travel before colliding with another molecule) is approximately .005 microns (5×10^{-6} mm.). The mean free path of a water-vapor molecule with relation to pressure is as follows:

pressure	*mean free path*
10 mm. Hg.	0.0034 mm.
1 mm. Hg.	0.034 mm.
100 μ Hg.	0.34 mm.
10 μ Hg.	3.4 mm.
1 μ Hg.	34.0 mm.

The transfer of water molecules from an ice crystal to a condenser can be accelerated by increasing the length of their mean free paths; this can be done by reducing the pressure in the chamber with a

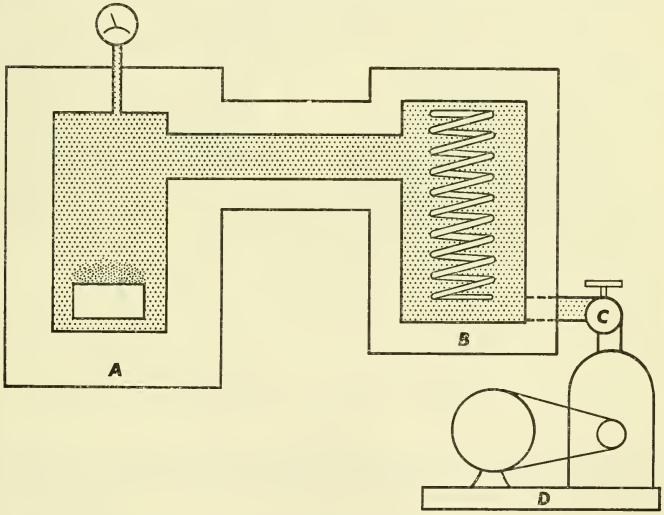


FIGURE 2.—Water-vapor differential (*A*=specimen chamber; *B*=condenser chamber; *C*=cut-off valve; *D*=vacuum pump.

vacuum pump. Not all vapors, however, are condensable; therefore, a pump must be used to remove these vapors so that chamber pressure will remain low enough to permit efficient removal of the water to its condenser.

Instrumentation of the Freeze-dry System

The basic equipment necessary to establish a freeze-dry apparatus consists of a specimen chamber, a condenser chamber, and a vacuum pump. The Smithsonian system also incorporates refrigeration control components that are very desirable for maintaining the dependability of the system.

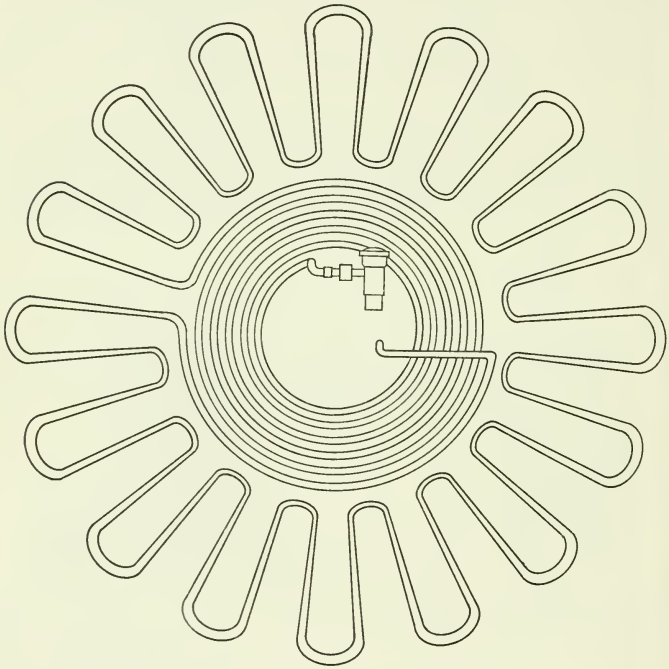


FIGURE 3.—Continuous copper tubing (⅝ in.) connected at alternate ends by return bends within the specimen chamber.

SPECIMEN CHAMBER.—The basic requirements for the specimen chamber are that (1) it must be vacuum tight; (2) it must be refrigerated; and (3) it must have a large access opening.

The ultimate vacuum within the chamber can be as low as 10 microns (1×10^{-2} mm. Hg.) of pressure, producing a differential between internal and external pressure of approximately 15 pounds

per square inch. Sturdy construction is required if a chamber is to withstand such force.

At the Smithsonian, the structural requirements for the chambers were met by the selection of a 60-gallon paint-spray pressure tank for the specimen chamber and a 30-gallon tank for the condenser. These tanks required modification but proved both satisfactory and economical.

The Smithsonian specimen chamber was mounted horizontally on a base shaped to the contour of its walls, with allowance for the thickness of an insulated outer plastic shell. The door on the Smithsonian chamber is two feet in diameter and withstands a total external pressure of 6780 pounds. The chamber itself, two feet in diameter and 36 inches long, withstands a total external pressure of a little more than 20 tons.

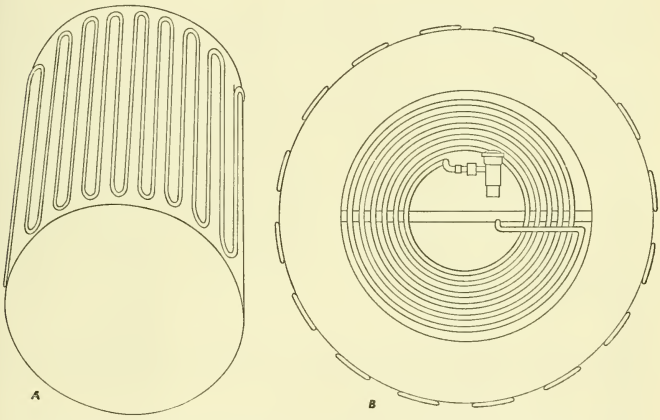


FIGURE 4.—Detachable refrigeration assembly: *A*, outside view; *B*, inside view.

The vapor line, a piece of steel tubing with an inside diameter of 3½ inches, was welded into a 3¾-inch hole midway down the side of the chamber.

The inside of the chamber was lined with ⅝-inch continuous copper refrigeration tubing, filling one end and running the length of the walls, where it was joined at alternate ends with 2-inch return bends. The tubing was soldered to the steel wall of the chamber, and a fillet of metallic plastic compound was added to improve heat conduction between wall and tubing (fig. 3).

Due to the difference in the coefficients of expansion of the two materials at operating temperatures, however, fracturing of the steel-to-copper attachments developed. To overcome this difficulty, the refrigeration line was soldered to the outer surface of a copper sleeve, negating the need for attachment between the copper tubing and the steel wall (fig. 4). This innovation not only solved the fracturing problem but also established the refrigeration line as a detachable unit within the system, allowing easy removal from the chamber for maintenance and repair.

Since the refrigeration line entered and left the chamber through two small openings in the rear of the tank, the openings around the tubing were sealed with soft solder. When installation of the refrigeration line was completed, the chamber was tested thoroughly for vacuum leaks.

Refrigeration of the specimen chamber.—Within an evacuated system, heat is transferred principally by radiation. Radiation travels in a line-of-sight path between a specimen and surrounding surfaces. There is no heat produced within the specimen chamber; therefore, the only appreciable load on the refrigeration system is ambient heat that leaks in through the insulation. This load can be calculated in BTU's per hour from the information given (p. 17).

Any refrigeration compressor (q.v.) serving the specimen chamber should be capable of producing controlled temperatures at the chamber walls of −15° to −30° C. (after heat leak has been taken into account).

When selecting a refrigeration compressor, it is desirable to double the calculated heat load (primarily heat leak) to allow a sufficient margin for error (table 1).

Table 1.—*Refrigeration compressor capacity in BTU's per hour* (based on a 90° F. ambient temperature; F–12 (freon-12)=Dichlorodifluoromethane; F–22 (freon-22)=Chlorotrifluoromethane)

Horsepower	Refrigerant	5° F	0° F.	−10° F.	−25° F.	−40° F.
0.25	F–12	1900	1700	1300	750	350
0.33	F–12	2275	2050	1550	950	445
0.50	F–12	3500	3175	2450	1550	1000
0.75	F–12	5100	4600	3550	2250	1400
1	F–12	7200	6500	5100	3350	2300
1	F–22		5590	4350	2890	1920

Condensing chamber.—The physical requirements for the condensing chamber are similar to those of the specimen chamber.

At the Smithsonian, a length of copper tubing, 3½ inches in diameter (later replaced with steel), was brazed into an opening, 3¾ inches in diameter, near the top of the 30-gallon tank. The opposite end of the tubing was connected to the specimen-chamber vapor line. At the base of the tank was an opening into which a 1½-inch steel pipe was welded, connecting it to the vacuum pump.

Inside the condensing chamber, a large complex of coils within coils was fashioned from 200 feet of copper refrigeration line ⅝ inches in diameter. The expansion valve was mounted inside the chamber and the refrigeration line was brought into and out of the chamber through openings in the side of the chamber, which were sealed with soft solder as in the specimen chamber (see fig. 4).

REFRIGERATION OF THE CONDENSER.—The force underlying the motion of water vapor from the ice, through the specimen chamber to the refrigerated condenser, is the vapor-pressure difference. This force is produced by the difference in temperature between the ice crystals within the specimen and the refrigerated condenser. Table 2

Table 2.—*Relationship between temperature* (° C) *and vapor pressure* (mm. Hg.)

° C	Vp	° C	Vp	° C	Vp	° C	Vp
0	4. 579	−16	1. 132	−34	. 1873	−66	. 00349
−1	4. 217	−17	1. 031	−36	. 1507	−68	. 00261
−2	3. 880	−18	0. 939	−38	. 1209	−70	. 00194
−3	3. 568	−19	0. 854	−40	. 0966	−72	. 00143
−4	3. 280	−20	0. 776	−42	. 0768	−74	. 00105
−5	3. 013	−21	0. 705	−44	. 0609	−76	. 00077
−6	2. 765	−22	0. 640	−46	. 0481	−78	. 00056
−7	2. 537	−23	0. 580	−48	. 0378	−80	. 00040
−8	2. 326	−24	0. 526	−50	. 0295	−82	. 00029
−9	2. 131	−25	0. 476	−52	. 0230	−84	. 00020
−10	1. 950	−26	0. 430	−54	. 0178	−86	. 00014
−11	1. 785	−27	0. 389	−56	. 0138	−88	. 00010
−12	1. 632	−28	0. 351	−58	. 0106	−90	. 00007
−13	1. 490	−29	0. 317	−60	. 0080	−94	. 00003
−14	1. 361	−30	. 2859	−62	. 0061	−98	. 000015
−15	1. 241	−32	. 2318	−64	. 0046		

demonstrates the fixed relationship between temperature and the vapor pressure of water. The temperature difference between the specimen and the refrigerated condenser need not be extreme in order to produce a substantial difference in water-vapor pressure; however, it should be noted that lowering the condenser temperature produced less gain logarithmically in vapor pressure difference.

219-947—66—2

It is clear, therefore, that any effort to produce extremely low condenser temperature is not economical since the cost per BTU of refrigeration increases rapidly as temperature is reduced, but the vapor-pressure differential increases only moderately. A temperature of −40° C. is satisfactory and is within the range of conventional freon-12 or freon-22 refrigeration units.

Another factor to be considered in designing a condenser is that the rate of water loss from the specimen will be very low and, thus, the heat load on the condenser's refrigeration unit will be slight.

The heat leak through the insulation of the condenser chamber should be calculated exactly, as for the specimen chamber.

Temperature Control: Temperature may be controlled by a simple mercury control switch with a 2- or 3-degree low-temperature differential. The control employed in the Smithsonian system is a sealed mercury switch, triggered by a vapor-pressure-actuated Bourbon tube. Exterior adjustments with visible settings over a calibrated dial are employed.

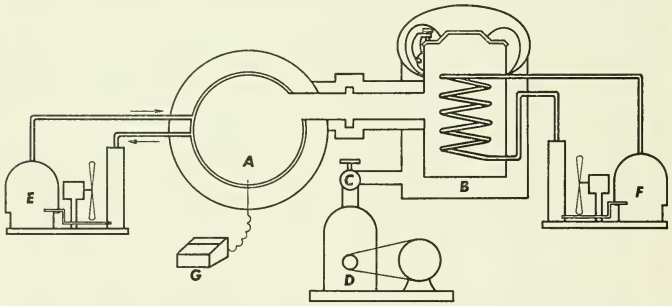


FIGURE 5.—Schematic of Smithsonian freeze-dry apparatus (*A*=specimen chamber; *B*=condensing chamber; *C*=recompression valve; *D*=vacuum pump; *E*, *F*=refrigeration compressors; *G*=vacuum gauge).

VACUUM PUMP.—The vacuum pump, very important to the freeze-dry system, should be selected with care. There are many suitable pumps available.

When a refrigerated condenser is employed as a vapor trap, the only function of the vacuum pump is to reduce air pressure mechanically within the chamber and evacuate the noncondensable gases released from the specimens during the drying process.

The capacity of a pump is described in terms of displacement. Displacement, expressed in liters per second or cubic feet per minute, is a measure of a pump's capability to produce a vacuum in a given time, as well as to keep the system evacuated as gases are released

from volatile materials within the system. Pump displacement or pumping speed must be calculated according to the volume of the system and pressure desired. The time required to pump the system down to the ultimate pressure (about 10 to 100 microns Hg.) should be established. The pump-down chart (fig. 6) may be used to calculate

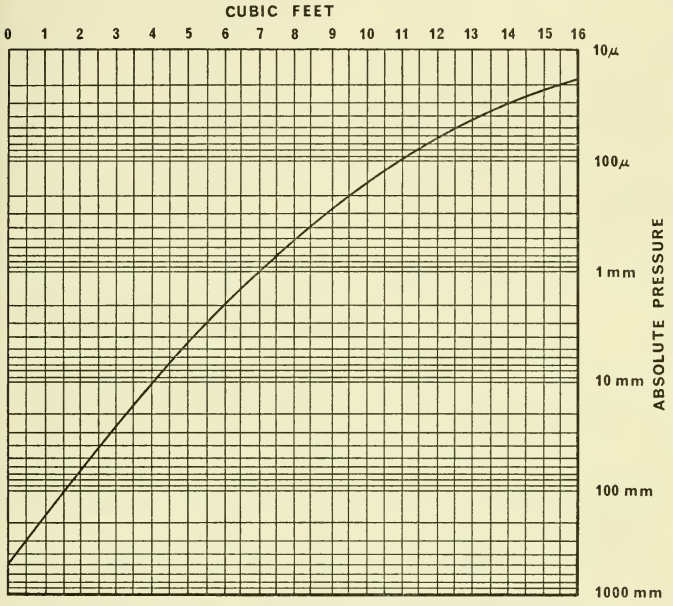


FIGURE 6.—Pump-down factor chart.

displacement requirements. By way of illustration, the following two examples are cited:

(1) To determine the required pumping displacement to evacuate a system with a total volume of 15 cubic feet to a pressure of 100 microns in 5 minutes, reference to the pump-down chart shows that at 100 microns the factor is 10.9. Multiply 10.9 by the volume (15 cu. ft.) to obtain the total number of cubic feet to be pumped (163.5). To determine the required pump displacement, divide this total by the number of minutes (5) allowed for the evacuation.

$$10.9 \times 15 = 163.5$$

$$\frac{163.5}{5} = 32.7 \text{ cu. ft./min. or } 55{,}563.6 \text{ liters/sec.}$$

(2) To determine how long it will take a pump with a displacement of 27 cubic feet per minute to evacuate a system with a volume of 160 cubic feet to a pressure of 200 microns (at 200 microns the factor is 9.50), calculate as follows:

$$9.50 \times 160 = 152$$

$$\frac{152}{27} = 56.29 \text{ min.}$$

Under certain conditions the refrigerated condenser can be eliminated and water vapor can be removed directly through the vacuum pump, provided the pump is capable of removing the vapor at a rate equal to its release from the specimen. To determine whether the condenser can be omitted, the approximate quantity of water vapor being released each minute by the specimens within the chamber must be computed.

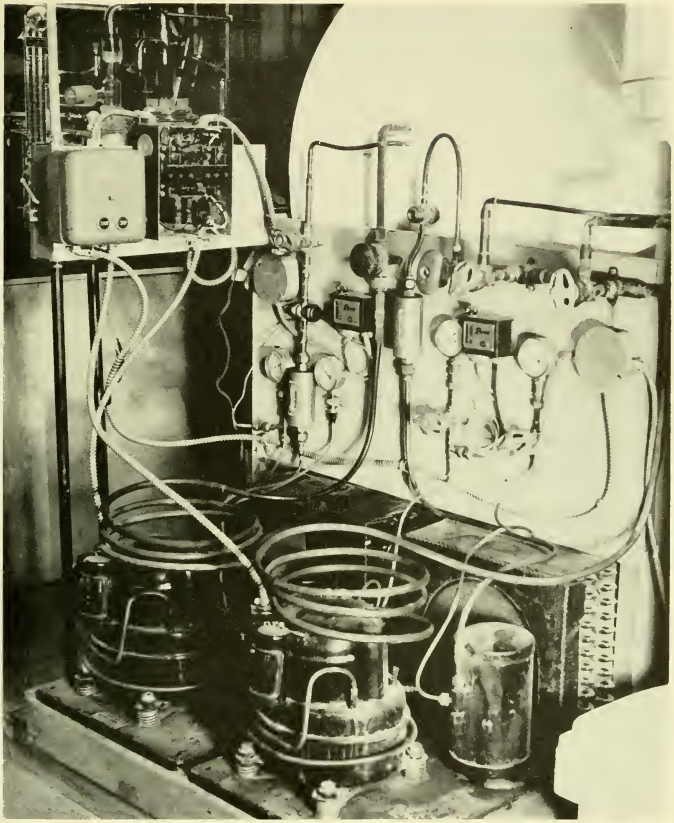
If, for example, a specimen chamber contains three squirrels, two flickers, a toad, and a garter snake (at various drying stages and all of average weight), the average daily release of water vapor will be approximately 12 grams.

If 150 microns of pressure were maintained within the chamber, it would be necessary to pump 200 cubic feet of water vapor for each gram of water removed from the system, or 2400 cubic feet per day (1.66 cu. ft./min.). If the vacuum pump cannot handle this volume of vapor, the pressure within the specimen chamber will slowly rise; and, as it rises, the volume occupied by a unit weight of water vapor decreases. At a pressure of 300 microns, two grams of water will occupy the same volume as would one gram at half that pressure. It is obvious, therefore, that overloading a chamber or providing an inadqeuate vacuum pump will cause an increase in operating pressure in the specimen chamber. To avoid this, a refrigerated condenser should be used.

Since the differential between the water-vapor pressure within the chamber and the vapor pressure of the ice within the specimen provides the driving force for the movement of water vapor through the system, an increase in pressure within the chamber causes a decrease in efficiency.

An increase in chamber pressure from 150 to 300 microns with a specimen temperature of $-10°$ C. would result in a vapor pressure differential of from 1.80 to 1.65 mm. Hg., or a reduction of a little more than 8 percent.

While this example demonstrates that increasing water-vapor load is not as deleterious as expected, it nevertheless establishes a relationship between water-vapor load and vacuum-pump capacity and



Refrigerating apparatus for the specimen and condenser chambers (Smithsonian photo).

Interior of the specimen chamber; dehydration is almost complete (Smithsonian photo).

Author checking the telethermometer of the apparatus (Smithsonian photo).

A specimen that has been freeze-dried (Smithsonian photo).

indicates that a vacuum-pump limitation can limit the number of specimens that can be produced in a reasonable span of time.

A 2-stage gas-ballast vacuum pump is recommended for all freeze-dry use. Gas ballast on the pump enables it to remove condensable vapors such as water vapor from a vacuum chamber with a minimum of internal condensation.

In the gas ballast, a valve provides a means for controlling the entry of air into the exhaust stage of the pump's operation. The ballast of air acts as a transporting medium carrying diluted vapors through the exhaust part to the atmosphere.

Such pumps are generally rated with ultimate pressure ranges from 1 to 10 microns; however, pumping speed usually begins to decrease at about 100 microns, which is considered a satisfactory pressure for freeze-drying.

Oil should be changed frequently in any pump used in a freeze-dry system. Even though the pump is gas-ballasted, a certain amount of water vapor will contaminate the oil and damage the pump.

TABLE 3.—*Vacuum table*

Terminology	Pressure Range
Ultrahigh vacuum	pressures below 1×10^{-6} mm. Hg. or .001 microns Hg.
High vacuum	$1\times10^{-3}-1\times10^{-6}$ mm. Hg.
Fine vacuum	1 mm. Hg. 1×10^{-3} or 1 micron Hg.
Rough vacuum	760 mm. Hg. to 1 mm. Hg.
1 mm. Hg.	1000 microns .00132 atmosphere .01934 p.s.i. .0393 in. Hg.
1 micron	.001 mm. Hg. 1×10^{-3} mm. Hg.
Atmospheric pressure	760 mm. Hg. 14.7 p.s.i. 29.921 in. Hg.

The possibility of future expansion of the freeze-dry system should also be considered when selecting a vacuum pump. Under certain conditions the addition of a second chamber may require doubled pumping capacity (see tables 3–5).

VACUUM-LINE DIMENSIONS.—The diameter of vacuum lines affects the efficiency of a freeze-dry system since a pump is effective only if the vacuum lines are large enough to handle the vapor transfer.

Conductance (the volume of flow of vapor through a line) is a well-investigated function, and there are a variety of ways to calcu-

TABLE 4.—*Metric conversion table*

1 in.	002.5400 cm.	025.4000 mm.
1 ft.	000.3048 m.	000.0304 cm.
1 in.^{2}	006.4520 cm.^{2}	645.2000 mm.^{2}
1 ft.^{2}	929.0000 cm.^{2}	
1 in.^{3}	016.3870 cm.^{3}	016.3870 ml.^{3}
1 ft.^{3}	000.0283 m.^{3}	000.2830 cm.
		.283 ml.
10 mm.	1 cm.	0.3937 in.
010 cm.	1 dm.	3.9370 in.
100 mm.^{2}	1 cm.^{2}	
100 cm.^{2}	1 dm.^{2}	
010 ml.	1 cl.	0.3380 fl. oz.
010 cl.	1 dl.	6.1025 cu. in.
010 dl.	1 l.	1.0567 fl. qt.
010 l.	2.64 gal.	

TABLE 5.—*Conversion multipliers* (e.g., liters per second times the factor equals cubic feet per minute)

From	To	Factor
liters per second	cubic feet per minute	02.1200
liters per minute	cubic feet per minute	00.0353
liters per second	liters per minute	60.0000
cubic centimeter per second	liters per second	00.0010
cubic feet per minute	liters per minute	28.3200

late the vacuum-line dimensions required to permit adequate conductance.

The flow of fluids (or vapors) within a vacuum system is usually either molecular flow or viscous flow. Molecular flow occurs at pressures at which the molecule's mean free path is greater than the average diameter of the tubing through which it flows.

Since the mean free path of a molecule does not approach the diameter of any large vacuum line at above 1-micron pressure, we are concerned only with viscous flow.

It should be noted that restriction of tubing to the viscous flow of vapors is significant only when the volumes to be moved are very large or the pressure differential is small. The law that expresses the viscous flow of fluids through a tube was first deduced by Jean L. M. Poiseuille, the French physicist (Daniels et al, 1949, p. 71). This law establishes relationships between the coefficient of viscosity (viscosity=poises/dyne sec./cm.^{2}), the volume of the fluid flowing through

the whole section of the tube in unit time (seconds, as applied here), the pressure differential at each end of the tube, the radius, and the length of the tube.

V=Volume (cm.^{3}/sec.)
p=Pressure (dynes/cm.^{2})
r =Radius (in cm.)
L=Length (in cm.)

$$V=\frac{pr^4}{8L}$$

The formula states that the volume of vapor transferred through a tube in a specific period of time is proportional to the fourth power of the tube radius. This means that a small increase in tube diameter will produce a considerable increase in vapor conductance.

To utilize the formula, it is necessary to know the coefficient of viscosity, which for air is approximately 1.7×10^{-4} poises at a pressure of 1 dyne/cm.^{2}=7.5×10^{-4} mm. Hg. 1.

For example, to calculate the conductance of vapor through a tube that is 193 cm. long, 7.62 cm. in diameter, and with a pressure differential between the specimen and the condensing surface of 2000 microns, the following values are:

V=cm.^{3}/sec.
p=2666.7 dynes/cm.^{2} (2000μ pressure differential)
r=3.81 cm.
L=193 cm.
v=1.7×10^{-4} poises

$$V=\frac{(3.14)(2666.7)(3.81)^4}{(8)(193)(.017)}=\frac{1737861.723}{26.248}$$

V=66209 cm.^{3}/sec.

Testing for leaks.—The overall system must be tested for vacuum leaks. Leaks in a vacuum system can be detected easily with a Peroni pressure gauge used in conjunction with acetone. The vapor pressure of acetone is so high that, when joints or surfaces are brushed with it, any leak will cause a marked increase of pressure within the system. The gauge head should be installed at a point near the vacuum pump and then watched for pressure increases after the system has been evacuated and tested.

Another test is to inject freon under slight pressure into the system and locate the leaks with a refrigeration leak detector. When the system is initially pumped down, the pressure in the system may be higher than anticipated due to gases being given off by zinc coating or other materials of high vapor pressure that may be inside the system. The pressure can be effectively reduced by allowing air to reenter the system and by pumping it down repeatedly until these vapors have been evacuated.

Repairs should be made while the system is evacuated because the vacuum helps a sealant penetrate a leak. Unless the pressure is unusually high when the pump is operating, it may be assumed that a leak is small and can be sealed with glyptol varnish or lacquer applied by brush. The instant that either material reaches the vacuum through a pinhole leak, it dries and plugs the leak. If the pressure remains high, the leak is large and should be plugged with soft solder.

Refrigeration compressors.—The major consideration in refrigeration is that constant temperature be maintained.

The hermetically sealed refrigeration unit is composed of a motor and compressor shaft of 1-piece construction. The motor (cooled by the flow of refrigerant gas) and compressor assembly are within a gas-tight housing that is welded shut. This method of construction eliminates the need for certain parts (pulley, belt, compressor flywheel, and compressor seal) found in an open unit and, of course, avoids the servicing and replacing of those parts.

One objection to this type of unit is that, under freeze-dry operating conditions, there is some danger of the motor's burning out because of the very small amount of refrigerant being circulated.

The Smithsonian's compressors, however, have not overheated despite continuous operation for several years in a room with an average temperature of about 90° F. If overheating should occur, it could be remedied by installing a water-cooled condenser in the discharge side of the compressor.

A second type, the air-cooled compressor, usually operates with a belt and pulley; the motor is in the open, where it is cooled by air circulating around it.

Operating characteristics of *both types* of refrigeration compressors are, otherwise, essentially the same.

The selection of a fractional-horsepower refrigeration compressor should be based upon calculation of heat load on thermal insulation (see p. 17 and also table 1).

Expansion valves.—For maximum vaporization of the refrigerant, it is important to select thermostatic expansion valves of correct capacity. It is equally important that the valves be installed at the proper locations, since both factors can influence the success or failure of the entire system.

The thermostatic expansion valve selected for the condensing chamber should be a type designed to control temperatures below −40° C.

Although the thermostatic expansion valves in either chamber may be mounted in any position, they should be installed as near the

evaporator inlet as possible. The valves in the Smithsonian system are mounted inside the chambers, where maximum efficiency is gained.

Bulb Location: For satisfactory expansion-valve control, good thermal contact between the bulb and the suction line is essential. The bulb, which controls the expansion valve, should be fastened securely with two metal straps to a clean section of the suction line inside the chamber. The bulb should be located near the midpoint of the line around the coil. It should not be near the bottom of the line because a refrigerant-and-oil mixture is usually present there, which would result in incorrect control of the expansion valve.

Filter and Drier: These should be installed in the liquid line ahead of the thermostatic expansion valve.

Sight Glass: Further protection is easily and inexpensively provided with a sight glass through which the refrigerant level can be determined by the presence or absence of bubbles in the liquid line. Bubbles indicate that the refrigerant level is low.

THERMAL INSULATION.—Insulation material is required to substantially reduce heat leak, which loads the refrigeration unit. This material may be glass wool, with a K factor of 0.29, rock wool, with a K factor of 0.26, compressed cork, with a K factor of 0.30, or other similar material. The Smithsonian freeze-dry apparatus is insulated with a foam-in-place plastic (polyurithane). Foam-in-place plastics range in density from 2 to 10 pounds per cubic foot; they offer K factors of from 0.02 to 0.24. These plastics can be used without great difficulty and can be made to conform to any contour. Such properties make plastic foam an ideal material to use as insulation.

Calculation for the determination of thermal impedence and heat load is as follows:

K=insulation factor (BTU/hr./sq. ft.)

S=surface area of insulation (sq. ft.)

A=Ambient temperature (difference between the inside and outside of chambers in °F)

IT=thickness of insulation (in.)

$$\frac{(K)\ (S)\ 2A}{IT} = \text{BTU/per hr.}$$

TEMPERATURE READING AND RECORDING.—Temperatures throughout the drying process should be carefully watched and accurately recorded. The record is indispensable for establishing drying times and for determining ideal temperatures for various types of specimen material. An automatic temperature-recording device is valuable for indicating the temperature variations as the refrigeration compressors are cycling, and it may also call attention to problems that would otherwise go unnoticed, such as a faulty temperature-control switch.

The system at the Smithsonian incorporates a telethermometer that utilizes a thermistor probe as a sensing element and provides temperature readings directly from a temperature scale. The telethermometer's range is from −40° to 150° C. Both surface and air probes are used and the probe leads are connected through a multiple-selector switch to permit convenient temperature reading at several places.

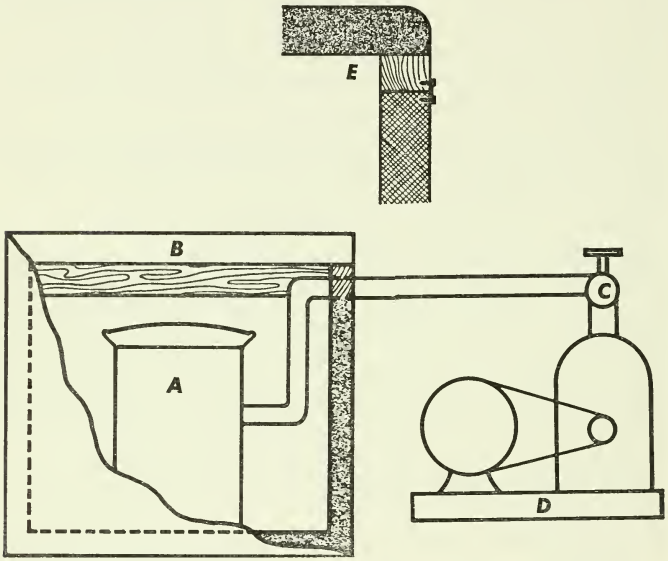


FIGURE 7.—Simple freeze-dry apparatus (*A*=specimen chamber; *B*=commercial deep-freeze; *C*=recompression valve; *D*=vacuum pump; *E*=wood-spacer insert).

RECORDER.—Any versatile general-purpose D.C. voltage and current recorder may be used to record temperatures continuously. One with a low-to-high-level range (200 microvolts to 500 volts and 200 millimicroamperes to 100 microamperes) is suitable for freeze-dry purposes. It is important that the recorder be compatible with the temperature device in scale range and voltage output.

Construction of a Freeze-dry System

The simplest apparatus suitable for freeze-drying consists of a commercial deep-freeze containing a small chamber connected to a gas-ballasted vacuum pump (fig. 7).

The main requirement for the deep-freeze chest is that it maintain a constant temperature of −15° to −20° C. The only alteration needed is removal of its hinges and latch and the insertion of a wood spacer between the chest and its lid to permit passage of a vapor line between them. The vapor line must pass *through* the wood spacer.

The hinges and latch should be relocated for the raised top; the thickness of the wood spacer is determined by the diameter of the vapor line that will pass through it.

A small specimen chamber may be made from a 5-gallon paint-spray pressure tank roughly 18 inches in diameter and 21 inches high. A hole must be cut in the side of the chamber for a vacuum line, which can be brazed into place.

The major consideration in selecting a gas-ballasted vacuum pump is the volume occupied by the water vapor at the temperature and pressure being used. Assuming that the chamber temperture is −20° C., the vapor pressure is 0.8 mm. Hg. At a vapor pressure of 0.8 mm., one gram of water occupies a volume of 1200 litters. If the pressure gradient is approximately 0.6 mm., we find one gram of water will occupy approximately 1400 liters. A gas-ballasted vacuum pump with a 25-liter-per-minute capacity will take approximately one hour to pump one gram of water. A pump with a capacity of 79 liters per minute will remove approximately one gram of water in 18 minutes.

If the vapor line is 150 cm. (5 ft.) long, the diameter is calculated to be approximately 2.5 cm. or 1 inch. Tubing 1¼ inches in diameter will allow a sufficient operating margin when using the larger capacity pump.

The chamber must not be loaded beyond pumping capacity. In a system of this size, it is suggested that a single specimen be placed in the chamber and a second one added two or three days later, followed by a third specimen on the completion of the first, and so on, in this pattern. Observations of drying times during experimental drying cycles will determine the capacity of each individual system. The drying cycle can be interrupted, provided specimens are not permitted to thaw.

The apparatus described above is limited by two basic characteristics of its construction: the vapor-handling capacity and the size of the specimen chamber (fig. 8). As stated, the most efficient device for removing water vapor from the specimen chamber is a refrigerated condenser. If an external refrigerated condenser of sufficient size is used, the only limitation to the size of the specimen chamber is the size of the deep-freeze unit that holds the specimen chamber.

A refrigerated condenser can be constructed within a paint-spray pressure tank similar to the one used for the specimen chamber. The condensing surface may be a coil of ⅝-inch copper refrigeration tubing

with an expansion valve mounted at the top or inlet side of the coil. The coil should have as many turns as space permits. A coil-within-a-coil arrangement is most desirable (fig. 9).

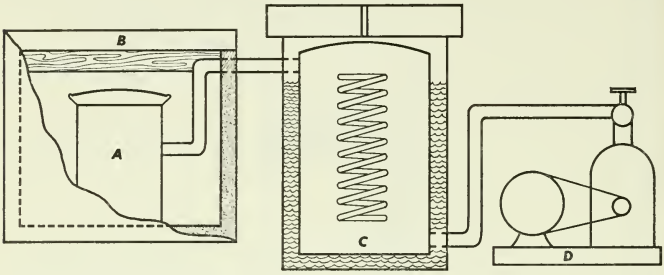


FIGURE 8.—Simple freeze-dry apparatus utilizing a condenser surface (*A*=specimen chamber; *B*=commercial deep freeze; *C*=condenser chamber; *D*=vacuum pump).

A vacuum-tight drain should be installed at the bottom of the condensing chamber to conveniently remove the condensation from the chamber during defrosting. The condensing chamber should be encased in an insulated box with openings in appropriate positions for vapor lines and refrigeration tubing.

An important requirement in any freeze-dry system is a valve (see fig. 5) that will permit atmospheric air to enter the system in order

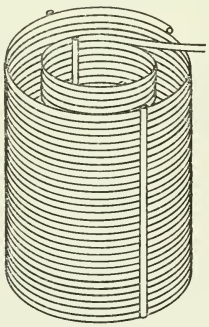


FIGURE 9.—Condensing coil.

that the chamber can be opened. This valve should be so located as to insure that the condensing chamber is situated between the valve and the specimen chamber, thereby assuring condensation of atmospheric moisture on the condensing coil rather than on the specimen. The valve must be vacuum tight when closed and need not be large; a ¼-inch intake is sufficient to shut down the system. It is recom-

TABLE 6.—*Freezing mixtures* (PS=primary substance and its proportion; SS=secondary substance and its proportion; TS=tertiary substance and its proportion; A=temperature (° C throughout) of substance before mixture; B=temperature of mixture; C=total reduced temperature; D=temperature when all snow is melted (when snow is used); E=heat absorbed (calories when A is grams); *=lowest temperature obtained. Table modified from "Smithsonian Physical Tables," 1959, 9th rev. ed., by W. E. Forsythe.

PS		SS	TS	A	B	C	D	E
$NaC_2H_3O_2$ (cr)	85	H_2O 100		10.7	4.7	15.4		
NH_4Cl	30	" "		13.3	−5.1	18.4		
$NaNO_3$	75	" "		13.2	−5.3	18.5		
NaS_2O_3 (cr)	110	" "		10.7	−8.0	18.7		
KI	140	" "		10.8	−11.7	22.5		
$CaCl_2$ (cr)	250	" "		10.8	−12.4	23.2		
NH_4NO_3	60	" "		13.6	−13.6	27.2		
$(NH_4)_2SO_4$	25	" 50	NH_4ND_3 25			26.0		
NH_4Cl	25	" "	" "			22.0		
$CaCl_2$	25	" "	" "			20.0		
KNO_3	25	" "	NH_4Cl 25			20.0		
$NaSO_4$	25	" "	" "			19.0		
$NaNO_3$	25	" "	" "			17.0		
K_2SO_4	10	snow 100		−1	−1.9	.9		
$NaCO_3$ (cr)	20	" "		−1	−2.0	1.0		
KNO_3	13	" "		−1	−2.85	1.85		
$CaCl_2$	30	" "		−1	−10.9	9.9		
NH_4Cl	25	" "		−1	−15.4	14.4		
NH_4NO_3	45	" "		−1	−16.75	15.75		
$NaNO_3$	50	" "		−1	−17.75	16.75		
NaCl	33	" "		−1	−21.3	20.3		
$H_2SO_4+H_2O$	1	" 1.097		−1	−37.0	36.0	−37.0	.0
(66.1% H_2SO)	1	" 1.26		−1	−36.0	35.0	−30.2	17.0
"	1	" 1.38		−1	−35.0	34.0	−25.0	27.0
"	1	" 2.52		−1	−30.0	29.0	−12.4	133.0
"	1	" 4.32		−1	−25.0	24.0	−7.0	273.0
"	1	" 7.92		−1	−20.0	19.0	−3.1	553.0
"	1	" 13.08		−1	−16.0	15.0	−2.1	967.0
$CaCl_2+6H_2O$	1	" .35		0			.0	52.1
"	1	" .49		0			−19.7	49.5
"	1	" .61		0			−39.0	40.3
"	1	" .70		0			−54.9*	30.0
"	1	" .81		0			−40.3	46.8
"	1	" 1.83		0			−21.5	88.5
"	1	" 2.46		0			−9.0	192.3
"	1	" 4.92		0			−4.0	392.3
Alcohol at 4° C	77	" 73		0	−30.0			
"		CO_2 solid			−72.0			
Chloroform		" "			−77.0			
Ether		" "			−77.0			
Liquid SO_2		" "			−82.0			
NH_4NO_3	1	H_2O .76		20	5.0			
"	1	" .94		20	−4.0			
"	1	" "		10	−4.0			
"	1	" "		5	−4.0			
"	1	snow "		0	−4.0			
NH_4NO_3	1	H_2O 1.20		10	−14.0			
"	1	snow "		0	−14.0			
"	1	H_2O 1.31		10	−17.5*			
"	1	snow "		0	−17.5*			
"	1	H_2O 3.61		10	−8.0			
"	1	snow "		0	−8.0			

mended that this valve be opened while the pump is still running to prevent atmospheric air from forcing the pump to run backward or forcing oil from the pump into the condensing chamber.

Figure 8 illustrates assembly of the modified apparatus using a refrigerated condenser.

Preparation of Specimens

The freeze-dry process will not improve a poor specimen. If a specimen is emaciated, slightly deteriorated, or otherwise inferior when it enters the chamber, it will be in no better condition when it leaves. Sagged tissue, however, can often be reshaped with subcutaneous injections of water, and sagged body cavities can be restored with cotton before freezing.

The art and skill of the taxidermist will help decide the success or failure of the freeze-dry technique. Many of the same tools and methods of conventional taxidermy can be employed advantageously in the freeze-dry process. Wires, supports, and other tricks of the trade are useful. Also rapid freezing with liquid nitrogen or freezing mixtures will hold a specimen in position.

Initial freezing of the specimen.—Of all compounds in animal tissue, water is the most abundant. For the average, it constitutes 70 percent of the animal's total weight. Water is found in cellular and vascular spaces, and, in small quanties, in protein and carbohydrates. Of the total liquid content, 20 percent is usually in extracellular fluid; approximately 25 percent of this extracellular fluid is plasma, and the remainder is interstitial fluid, mostly water. About 76 percent of muscle tissue is water. The Rowntree data (see Harrow, 1951) relating to the biochemistry of man is a good general guide to the distribution of tissue water in most mammals.

Water in tissue is found almost always in combination with naturally occurring salts; for this reason, freezing should be brought about in the shortest possible time. Slow freezing invariably leads to the formation of eutectics through the concentration of salts. As freezing water separates itself from a solution by the process of ion diffusion, the salts become more highly concentrated than they were in the original solution.

Eutectics have lower freezing points and lower vapor pressures than water; thus, their formation during the process slows down drying. Due to unfrozen saturated fluid in the tissue and resulting surface tension, shrinkage occurs. Rapid freezing, which reduces the formation of eutectics, can be accomplished by using the freezing mixtures listed in table 6, liquid nitrogen, or a freezing chest with a

temperature of less than −25° C. Rapid freezing also creates smaller ice crystals, causing less tissue distortion during the process.

Techniques for faster drying.—The greatest amount of water is removed early in the drying cycle when a specimen's dried shell is thinnest and offers the least impedence to the escape of water vapor. The rate of weight loss due to the escape of vapor approaches zero with the completion of drying. If a specimen chamber is loaded with a great number of fresh specimens, the water-vapor atmosphere will be very great during the first few days, possibly exceeding the capacity of the system. If, however, specimens are introduced at spaced intervals, the same number of specimens can probably be processed without exceeding the system's vapor-removing capacity, and a constant level of water-vapor removal may be approached.

By drilling holes in the back or bottom portions of specimens, their drying surfaces are increased and the area of their epidermal layers is decreased, allowing water vapor to escape more rapidly. Skinning one side of a specimen serves a similar function in that it removes epidermal tissue that would otherwise act as a vapor barrier.

Evisceration of a specimen also reduces its water content considerably; however, the removal of water-laden organs requires incision, removal of the viscera, filling the body cavity with cotton or similar material, all of which requires time and involves some of the less desirable features of taxidermy.

A record should be maintained of each specimen's weight since observing the weight reduction (by removing the specimen from the chamber and weighing it) is the best method for determining when a specimen is completely dry. A specimen should be left in the chamber for one or two days after the loss of weight ceases to be apparent. This is especially true for rodents such as mice or rats with a scaly epidermis on their tails. The tails dry slowly and it is therefore advisable to perforate their undersides and keep the specimens in the chamber beyond their apparent drying times.

Conclusion

As technology advances, it becomes apparent that freeze-dry will be an ever-growing field. Plans are already underway for increasing the Smithsonian facilities. Theoretically, there is no limitation to the ultimate size or numbers of specimens that at one time could be processed in this manner. It is hoped that this paper will help to encourage the growth and exchange of further ideas on the freeze-drying process.

References

Braddick, H. J. J.
1954. The physics of experimental method. New York: John Wiley and Sons.

Daniels, F.; Mathews, J. H.; Williams, J. W., et al
1949. Experimental physical chemistry, 4th ed. New York: McGraw-Hill Book Co.

Glick, David.
1949. Techniques of histo- and cytochemistry.

Harrow, B.
1951. Textbook of biochemistry. Philadelphia and London: W. B. Saunders Co.

Hower, R. O.
1962. Freeze-drying biological specimens. Smithsonian Inst. Inf. Leafl., no. 324.
1964. Freeze-drying biological specimens. Mus. News Techn. Suppl., vol. 1, no. 1.

Meryman, H. T.
1960. The preparation of biological museum specimens by freeze-drying. Curator, vol. 3, no. 1.
1961. Biological museum specimens. Naval Medical Research Institute, Bethesda, Md.
1961. The preparation of bilogical museum specimens by freeze-drying, 2: Instrumentation. Curator, vol. 4, no. 2.

Strong, J.
1953. Procedures in experimental physics. New York: Prentice-Hall.

Wollaston, William Hyde
1813. On a method of freezing at a distance. Roy. Soc. Phil. Trans., pp. 71–74.

U.S. GOVERNMENT PRINTING OFFICE: 1967