A CALORIMETRIC AND HISTOLOGICAL METHOD FOR STUDYING FREEZING RATES IN TURKEYS

Thesis for the Degree of Ph. D.

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Joseph Herman MacNeil

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This is to certify that the

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A CALORIMETRIC AND HISTOLOGICAL METHOD

FOR STUDYING FREEZING RATES IN TURKEYS

presented by

Joseph Herman MacNeil

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L. E. Dawson
Major professor

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A CALORIMETRIC AND HISTOLOGICAL METHOD FOR STUDYING FREEZING RATES IN TURKEYS

By

Joseph Herman MacNeil

AN ABSTRACT OF A THESIS

Submitted to
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ABSTRACT

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A number of turkey processors have installed equipment to freeze their turkeys rapidly in a liquid medium instead of the more common method of using air blast freezers. Current procedures involve both liquid freezing and air blast freezing. By measuring the amount of heat removed from a turkey while immersed in a liquid it is possible to relate other processing elements to liquid freezing.

A calorimeter was designed and constructed to study heat removal rates in turkeys immersed in a flowing liquid. The accuracy of the apparatus was determined by comparing the observable heat removal from four propylene glycol models, with the calculated theoretical amount of heat removed from each during a known temperature change. It was found that an average of 96.5 per cent of the calculated heat removed from each of the four models was measured by this apparatus.

Each of four unpackaged turkeys was frozen for one hour in the calorimeter through which refrigerated propylene glycol was flowing at the rate of .268 cubic feet per minute. After freezing for one hour, it was found that the turkeys still contained from 54 to 61 per cent of the total amount of heat to be removed in order to lower the temperature of the bird to 0° F. When four other birds were packaged in evacuated Cryovac bags and frozen for one hour in the calorimeter, this percentage was increased to from 75 to 85 per cent. When the

liquid flow rate was adjusted from .268 cubic feet per minute to .159 cubic feet per minute, the residual heat increased from the above range of 54 to 61 per cent to a range of 61 to 75 per cent for the four turkeys frozen.

In order to observe by microscopic examination the effects of freezing rate and ice crystal formation, it is necessary to have a method of tissue fixation which will preserve the tissue elements as they were in the frozen state. A standard method, two different freeze drying methods, and a frozen fixation method were investigated. It was found that the method of frozen fixation was the most useful method in this study and could be adapted to small laboratories not having elaborate histological facilities, and at the same time gave significant results. This method could be used to study the effects of rate of freezing on tissue structure and could be used satisfactorily in conjunction with heat removal studies.

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INTRODUCTION

Turkey production in the United States in 1959 amounted to eighty-two million birds which averaged 16.9 pounds each on a live weight basis. More than one and one-fourth billion pounds of turkey meat was processed and an estimated 90 per cent of this meat was frozen before being merchandised.

Until recently turkeys were frozen in insulated rooms in which air was circulated by fans or in freezing tunnels through which air as low as -50° F. was passed at high velocities. Several turkey processing plants in Michigan were visited and these plants did not have sufficient freezing capacity to rapidly freeze the volume of turkeys handled. Many of these turkeys frozen at a slow rate lacked the light color of well finished and properly processed frozen birds.

A number of turkey processors have recently installed equipment to freeze their turkeys rapidly in a liquid medium producing a light-colored turkey. The incorporation of this freezing method required an expansion in the processing area for unboxed birds, but has resulted in more effective utilization of the available freezing and storage rooms. Turkeys, partially frozen in a liquid medium, are box packed and held for further freezing and storage in insulated rooms maintained at sub-freezing temperatures.

Some turkey processors utilize calcium chloride brine and others use a solution of propylene glycol as the heat-transfer medium. Several methods are used to partially immerse the bird and to apply the low temperature liquid over the exposed areas of the birds. Since

the application of this liquid method of freezing turkeys is relatively new, questions have arisen regarding its effectiveness and efficiency. Immersion freezing times and temperatures which will result in a desirable color and appearance of turkeys which can be maintained throughout marketing channels have been derived successfully; however, there are very little data pertaining to the amount of heat removed during immersion freezing in relation to the total amount of heat present. Experimental procedures providing such essential information can effectively be used in studying heat removal rates under varying processing conditions, along with providing information of the effect of freezing rate on quality and maintenance of quality in frozen turkeys. Mathematical determinations of freezing rates have been carried out successfully using models. The difficulty involved in measuring heat removal from an irregular-shaped object such as a turkey led to the design and development of a calorimeter capable of holding a whole turkey. This calorimeter was constructed to allow a refrigerated liquid to be pumped through the calorimeter chamber which contained a turkey. By observing the heat energy increase in the liquid after passing over the turkey, the amount of heat removed from the turkey in terms of British thermal units (B.t.u.) was computed.

In order to observe by microscopic examination the effects of freezing rate and ice crystal formation, it is necessary to have a method of tissue fixation which will preserve the tissue elements as they were in the frozen state. Several methods cited in the literature were not adaptable to the present laboratory scheme. A method of frozen fixation was elaborated and tested for its ability to accurately represent tissue structure as a result of freezing. With an expansion in immersion freezing, investigations leading to detailed

information, not only with regard to heat removal rates during short time immersion but also on the effects of freezing rate on the meat tissue, must be expanded.

With these points in mind, the following objectives were outlined with the aim of investigating the feasibility of using a calorimeter to study heat removal rates from turkeys immersed in a liquid and to be able to examine by histological methods the effects of freezing.

(1) To design and build a calorimeter to be used in determining the rate of heat removal from turkeys during freezing in a liquid medium.

(2) To develop procedures to be used in the precise measurement of freezing rates using a calorimeter.

(3) To demonstrate the uses of such apparatus in evaluating packaging and flow rates on freezing rates of turkey.

(4) To develop a simple method of examining the histological changes which occur in muscle tissue during freezing.

(5) To compare histological methods and demonstrate their uses in observing the effect of freezing rate microscopically.

This research study is divided into two parts: Part I deals with the development of the calorimetric apparatus and procedure along with a demonstration of its uses; Part II deals with the evaluation of several methods of examining frozen turkey muscle tissue.

LITERATURE REVIEW

Artificial refrigeration was first used to freeze food products commercially in the United States about 1865 (Tressler and Evers, 1947). At that time meats and fish were frozen in crude rooms insulated with sawdust and cooled by ice and salt mixtures. The principles of freezing by direct immersion and spraying with liquid have been known for some time. A brine spray freezing process was used in the poultry industry several years ago; however, present practices of freezing turkeys by immersion in liquids maintained at low temperatures is relatively new.

Measurement of Heat Loss

A search of the literature did not reveal a description of a calorimeter which could be used to measure heat removal from turkeys. However, other types of calorimeters have been used in connection with food freezing for some time.

Short and Bartlett (1944) constructed and tested four different calorimeters for use in determining the specific heat of various food products. Mannheim et al. (1955) constructed a Dewar flask calorimeter to use in the determination of enthalpy changes involved in freezing and thawing of several food products. These calorimeters, however, were not suitable for measuring the amount or rate of heat loss from whole turkeys.

Short and Staph (1951) reported that the energy which must be removed from a particular foodstuff, in order to lower its temperature from that at which it is received to that at which it is stored, equals the sum of the sensible energy removed from each constituent and the

latent heat of fusion of the water and the fats. The fats may not undergo a freezing change within the normal freezing storage temperature range; hence only the heat of fusion of the water which is frozen should be considered. Staph (1951) suggested that food be thought of as a simple mixture of a dry material and pure water. This mixture would have some of the properties of a solution or compound in which the fusion point decreases as the solution becomes more concentrated.

Ede (1955) stated that it is difficult to predict, by calculation, the true temperature distribution in a product due to the involvement of latent heat. Furthermore, a change in physical properties occurs during freezing which is usually too large to be ignored. He points out, however, that the calculation of temperature distribution can be simplified in most cases, with a negligible reduction in accuracy, by regarding the temperature distribution in the outer frozen material as if it was of the steady state type. The rate of extraction of heat at the freezing boundary can then be estimated. This rate of heat removal depends on the rate at which the freezing boundary advances which, in turn, is due to the magnitude of latent heat.

Long (1955) described the effects of initial temperature on the subsequent freezing patterns for fish. He reported that no simple explanation of initial temperature effect has yet been found except in qualitative terms. The magnitude of these effects appears to be due to a combination of circumstances. He pointed out that when the initial temperature of the fish was at the initial freezing point, the center of the product remained in the freezing zone much longer than it did when the initial temperature was higher. He explained that the difference was due to the difference between the heat transfer across low conductivity unfrozen material and high conductivity frozen

material.

"When food is uniformly frozen, the final point of solidification will normally be located at the geometric center of the product; however, when the food is irregular in shape, this point may be elongated and located midway between the two centers or at several separate points", (Finnegan, 1941). Irregularities in the freezing method can change the point of final solidification and may substantially increase or retard the rate of freezing. Since turkeys are irregular in shape, the geometric center is difficult to locate and the mean temperature of the mass, at any specified time, would remain unknown.

Esselen et al. (1954) studied freezing rates in several classes of poultry by using a time-temperature relationship. Thermocouples were placed in the thick portion of the breast and thigh of the various classes studied and the endpoint was taken as approximately 15°F. Freezing rates were reported as the time necessary to lower the temperature to 15°F. and then comparing the observed freezing times of each of the classes studied. Klose and Pool (1956) reported freezing rates for chilled poultry as the time required for the temperature just under the skin to reach 20°F. Lentz and van den Berg (1957) in studying immersion freezing of poultry used procedures similar to Esselen et al. ; however, in addition, they placed thermocouples at the surface between bag and skin, under the skin, one-fourth inch, one-half inch, and also two locations of one inch and one and one-half inch deep in the breast muscle. Except where the effect of high initial temperature was studied, all birds were frozen from an internal temperature of 32-35°F. and freezing times were reported as the time for the temperature to fall from this level to 25°F. Van den Berg and Lentz (1957) found that the large variability

in freezing times of comparable birds necessitated a large number of replicates to ensure reasonable precise results. To overcome this problem of replicate numbers they constructed gelatin models of definite dimensions. Thermocouples were used to measure the temperature distribution throughout the model and by using a mathematical formula the time required to freeze the model to any radius could be computed. A temperature of 25°F. was considered to be the end point.

Freezing Time for Poultry

Stiles (1922) stated that the factors affecting the cooling rate of a product can be classified into two categories: (1) internal factors depending on the nature of the cooled substance; and (2) external factors depending on the properties of the external medium. Internal factors are thermal conductivity, specific heat, density, latent heat, specific surface and nature of the surface of the cooled body; and external factors are temperature, conductivity, specific heat, density and degree of agitation of the external medium.

Van den Berg and Lentz (1957) using gelatin models with thermal properties similar to those of poultry found that the time required to freeze a model varied widely with kind of liquid and agitation rate. Freezing times in each of five liquids tested (methanol, calcium chloride, ethylene glycol, propylene glycol, glycerol) varied by a factor of 1.5 to 2.0 with agitation rate, while the maximum variation between liquids at a given agitation rate was slightly greater than a factor of two. Freezing times as well as the effect of agitation of freezing time depended primarily on viscosity of the liquid medium and were maximum in glycerol and minimum in calcium chloride. Spraying the liquid on the models was as effective in reducing freezing times as

the highest rate of agitation used. Parsons et al. (1957) froze turkeys to a depth of one-half inch in a brine solution maintained at temperatures of 20, 15, 10, 5, 0° F. and in a glycol solution at 5° F. intervals from 20° F. to -20° F. Differences in viscosity of the solutions were related to temperature. A turkey immersed in the least viscous salt solution resulted in an average freezing time of 1 hour and 59 minutes, while a glycol solution resulted in an average freezing time of 3 hours and 53 minutes when both were frozen at +20° F. At 0° F. the average freezing time in brine was 13 minutes. To obtain the same freezing rate using a glycol solution, it was necessary to lower the glycol solution to -20° F.

Esselen et al. (1954) reported a time range for immersion of from 20 to 30 minutes per pound to completely freeze poultry. For a quick dip in brine at -20° F. followed by holding in cold storage recommended freezing times were 6, 35, and 45 minutes for broilers, 12-pound turkeys, and 22 to 27 pounds ready-to-cook turkeys, respectively. Lentz and Van den Berg (1957) reported results similar to this; however, they suggest that these times should be increased to 40 minutes for optimum results.

Influence of Packaging Material on Freezing Rates

Winter (1947) reported that, with other factors equal, there is a direct relationship between the quality of wrapping material and the length of time that frozen meat, poultry, and fish will retain their original flavor and color during storage at 0° F. He based his conclusions regarding packaging materials on the theory that the best packaging materials appear to be those which are most effective in excluding oxygen. When packaging materials are used on turkeys

immersed in a liquid, two additional considerations are necessary (Mitchell et al. 1958). The film must be completely water-tight to prevent the freezing solution from contacting the product and the package must be "skin tight" to avoid "skin blotches" which form under air pockets. Parsons et al.(1957) summarized the factors involved in selecting a packaging material. He reported that the packaging material must be moisture-proof, have strong mechanical properties, and be capable of holding a partial vacuum. The removal of air from within the package to permit complete contact between the packaging material and the surface of the products is essential. Close contact between packaging materials and product contributes directly to a decrease in mechanical breakage and subsequently, a decrease in contamination of the poultry. The effect of packaging on freezing times of turkeys immersed in a liquid was reported by Esselen et al. (1954). In general it took about twice as long to freeze packaged poultry as it did to freeze unwrapped birds. It took only 5 hours to freeze packaged 12-pound turkeys in brine at -20° F. This is only about one-twentieth of the 60 hours required to freeze a similar sized turkey in some air freezing operations. Van den Berg and Lentz (1958) indicated that the packaging film used, vinylidene-chloride (Cryovac), did not significantly affect either freezing time or appearance after freezing.

Efficiency of Immersion Freezing

Brownlow (1959) reported the use of a tank which had a capacity to partially freeze approximately 9,000 pounds of turkey per hour with a liquid temperature of 0°F. maintained with a $7\frac{1}{2}$ by $7\frac{1}{2}$ single stage compressor. He indicated that it would take twice the above compressor capacity to do

a less than comparable job as far as color is concerned using conventional freezing methods.

Davis (1954) reported that immersion freezing of poultry is 12 times faster than some of the other cold air methods which follows chilling in slush ice; however, he recommended that the immersion process, at present, be used only for ready-to-cook poultry packaged in moisture-proof and vapor-proof bags prior to chilling or freezing.

Effects of Immersion on Color

A preference by consumers for light-colored turkeys, capons, or chickens was reported by Pflug (1957). MacNeil et al. (1958) reported the consumer acceptance of turkey parts frozen at various freezing temperatures. They showed that a Detroit Consumer Preference Panel preferred turkey quarters frozen in a liquid over quarters frozen at 0° F., -20° F., and -40° F. in an air blast freezer. After storage periods of over four months this preference declined.

Since consumers select poultry on the basis of appearance, the factor of color may be very important for industry consideration. Apparently, although much research had been done on freezing procedures, poultry is still subjected to freezing in a haphazard fashion. Baker (1953) reported that almost half of the frozen turkeys in New York state became discolored during the freezing operation. The effect of freezing rate on color of meat was discussed by Ramsbottom et al. (1949). They reported that steaks frozen by contact with dry ice (-110° F.) were much lighter in color than were steaks frozen at -20° F., while steaks frozen at 0° F. were intermediate in color and approached the color of fresh steaks. Baker (1953) froze turkeys at -20° F., 0° F., and 5° F., and reported results similar to those obtained

by Ramsbottom et al. with meat. He showed that the turkeys frozen at -20° F. were the most pleasing in appearance, while those frozen at 5° F. were very dark in color. After thawing, the original color of all birds was restored. By freezing the birds in a reversed order, that is, the birds originally frozen at 5° F. were frozen at -20° F., similar results were obtained in that the birds frozen at -20° F. were the most pleasing in color.

Similar results on color of frozen birds were obtained by Esselen et al. (1955). They also reported that subscalded poultry (138 - 140° F.) was particularly susceptible to discoloration when frozen.

Baker (1955) reported that the discoloration involved in slow freezing took place in the flesh rather than the skin. He froze the skinless birds and skin separately at two temperatures, -20° F. in air blast and 20° F. in still air. After freezing at -20° F. the flesh and skin were both white, while after freezing at 20° F. the flesh was dark while the skin was light. Results of studies conducted by Klose and Pool (1956) were in general agreement with those reported by Baker on the need and importance for fast freezing; however, they differed in respect to the processes involved in the color change. Klose and Pool stated that appreciable and easily detectable optical changes take place in the skin in addition to those in the surface layer of the flesh.

While temperature is the main factor influencing rate of freezing and color of resulting product, Baker (1953) and Lentz and Van den Berg (1957) reported that the movement of the surrounding atmosphere was also very important, as well. Van den Berg and Lentz (1958)

showed that lightness and uniformity of appearance in air blast increased markedly when air velocity at -20° F. was increased from 0 to 700 feet per minute. Baker (1953) indicated that a warm layer of air surrounds the bird in still air and acts as insulation.

Effect of Freezing Rate on Ice Crystal Formation

The preservation of food by freezing is based upon the fact that a decrease in temperature retards the action of destructive agents, such as microorganisms, and reduces enzymatic and chemical reactions. Bartlett (1944) reported that no direct relationship existed between the efficacy of a given temperature for preserving food and the quantity of ice which is present.

Birdseye (1946) reported that both plant and animal tissues are composed of a multitude of individual cells, in and around which is a complex liquid containing numerous minerals, salts, vitamins, proteins, and other substances. As the temperature of this liquid is brought below 32° F., there are a number of physical and chemical changes which take place in it. One of the most important changes is the formation of ice crystals.

The size of the crystals formed, in the temperature range of plus 32° F. to plus 25° F., is determined to a large extent by the speed with which the product goes from plus 32° F. to plus 25° F. The faster the rate of freeze, the smaller the ice crystals and the slower the rate of freeze, the larger the ice crystals. The temperature range mentioned above is referred to by Pennington (1941) as the "zone of maximum crystallization." Koonz and Ramsbottom (1939) found that rate of freezing affects the size, number and location of ice formations. Nearly instantaneous freezing produced minute,

evenly distributed ice columns within the fibers. With a somewhat slower rate of freezing, the ice columns within the fibers were larger in diameter and fewer in number. The importance of the size of the ice crystals was emphasized by Birdseye (1946). He stated that large ice crystals, as a result of slow freezing, could result in physical damage to the cell (cell rupture) or a physio-chemical change which he termed salt dehydration. Sair and Cook (1938) in studies with fish and beef, found that when these two products had been frozen slowly in air, they exude a certain amount of fluid or drip when thawing indicating physical damage to the cell and consequent loss of liquids.

DuBois, Tressler, and Fenton (1942) reported that it was difficult to note, by casual examination, the difference between chickens frozen rapidly and those frozen more slowly. However, they could be differentiated by a microscopic appearance of cross sections of the muscle tissue.

Willis, Lowe, and Stewart (1948) showed that birds frozen at 10° F. showed a considerable amount of desiccation and darkening while birds frozen at -10° F. were in much better condition, but even at this temperature they did begin to darken after nine months of storage.

Studies on Tissue Structure after Freezing

Rapatz and Luyet (1959) prepared photomicrographs of ice crystal formation in muscle tissue. They reported that the main difference between physical systems such as solutions and tissues is that the latter have a structure of which the component parts (membranes, filaments, microsomes) act as obstacles to the formation and the propagation of the ice. One of the predominant features in the mode of propagation of the ice within muscle fibers is the preference of the

developing ice phase for the longitudinal direction of the fiber. They also indicated that satisfactory tissue sections for microscopic observation were obtained by performing all manipulations (tissue preparation and photographing) in a +2°C. cold room.

Cook et al. (1926) reported that increasing the period of freezing increased the changes in beef as determined by the quantity of drip, net nitrogen loss on thawing and by the microscopic appearance in the frozen condition. When the period of freezing was longer, the ice crystals were observed, while still in the frozen state, to be smaller in numbers but of large size and there were more bands of compressed dehydrated fibers and fewer undistorted fibers than in the rapidly frozen sample.

Koonz and Ramsbottom (1939), using a freeze drying method similar to the Altman-Gersh method as cited by Hoerr (1936) studied the structure of frozen poultry. They reported results similar to Cook et al. and presented photomicrographs which showed that when a whole bird was frozen at -40°F. in air the water was frozen within the fibers and accumulated approximately in the center of the fibers with the fiber protoplasm being distributed at the periphery of the large ice columns. By lowering the freezing temperature to -55°F. they found that the ice columns assumed a peripheral arrangement.

Hiner et al. (1945) froze the longissimus dorsi muscle of beef at 18, 0, -10, -40, and -114°F. in air without forced circulation, to investigate histological changes that occur with different rates of freezing. Freezing at 18°F. resulted in the formation of large interfibrillar ice areas which pushed the fiber together into groups. No intrafibrillar ice crystals were observed. However, intrafibrillar freezing and some fiber-wall damage were visible at 0°F. This latter

process became more extensive as temperature was further lowered.

Two different methods of preparing slides were used by Hiner and his co-workers. One method carried out at 18° F. consisted of cutting the frozen tissue on a sliding microtome, placing the section on a slide and photographing it immediately. The second technique consisted of cutting the tissue and unrolling it carefully on a cold slide, thawing by warming the slide with the finger and then placing the slide in a series of alcohol baths starting at 70 per cent. After fixing, the sections were stained with hematoxylin and eosin.

Studies on tenderness conducted by Hiner and Hankins (1946) showed that freezing beef at temperatures of -10° F. or lower resulted in the splitting or shredding of the individual muscle fibers. Slow freezing at the relatively high temperatures of 18° F. caused only a distortion of the fibers with little or no visible injury to them. They explained differences in drip loss on thawing by the fact that the formation of ice crystals within the fibers resulted in fiber splitting at the lower temperatures, which, in turn, enables the water to be reabsorbed upon thawing. In cases where freezing was slow the ice crystals formed outside the fibers, leaving the fiber walls intact but pulling the fiber moisture out. On thawing this frozen water seeped out.

PART I: Calorimetric Method for Study of Freezing Rates in Turkeys

PROCEDURE

Description of Apparatus

The equipment available for preliminary immersion freezing studies consisted of a rectangular steel tank insulated with six-inch expanded polystyrene board form insulation (Styrofoam).[1] The interior of the tank measured 48 inches in length, 30 inches in width, and 36 inches in depth. The tank contents were cooled by coils on the bottom of the tank connected to a direct expansion ammonia system. Directly below the refrigeration coils were rows of perforated pipe which permitted air to be bubbled through the cooling medium to assure good temperature distribution. The temperature of the tank contents was regulated by adjusting the back pressure regulator valve.

This agitation system failed to function properly after several hours of operation. The air supply used for agitation contained enough moisture to produce a gradual accumulation of ice in the perforated pipes until air bubbling ceased. A circulating pump was then installed which pumped the liquid solution over the coils and returned it to the tank at the opposite end.

In preliminary trials freezing curves were determined for turkeys which had been immersed in a liquid for different lengths of time, followed by complete freezing in an air blast freezer. The lack of control of the variables involved in immersion freezing led to the

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[1]The metal tank used was constructed by the Agricultural Engineering Department.

construction of an insulated calorimeter.

Figure 1 is a diagram showing a side view of the apparatus used to measure the amount of heat transferred from the products studied to the liquid medium. The temperature controller was used to regulate the temperature of the liquid coolant. The circulating pump mixed the tank contents to maintain uniform temperature throughout. The calorimeter pump was used to pump the constant temperature liquid through the calorimeter. A by-pass valve was used to regulate the flow of liquid coolant through the calorimeter, while a second valve, termed the flowmeter valve, located in the pipe leading from the calorimeter to the flowmeter, was used for minor adjustments in the flow rate. A rotameter (flowmeter) was used to measure the volume of liquid which passed through the calorimeter. The thermometers are shown in their proper position for measuring the temperatures of the liquid entering and leaving the calorimeter.

Figure 2 is a diagram showing a top view of the same apparatus shown in Figure 1.

A diagram of the calorimeter is shown in Figure 3. This calorimeter was constructed of two-inch expanded polystyrene board form insulation (Styrofoam) covered on both sides with a one-eighth inch thickness of plywood coated with a layer of fiberglass. The joints were sealed with epoxy resin. The liquid entered the calorimeter through a plastic pipe which had two slots inside the calorimeter. Styrofoam forms were used to decrease the area inside the calorimeter and to deflect the liquid flow around the product in the calorimeter.

Mercury in glass thermometers calibrated in degrees Fahrenheit were used to measure liquid temperatures. The thermometers were graduated in two-tenth degree intervals and temperatures could be

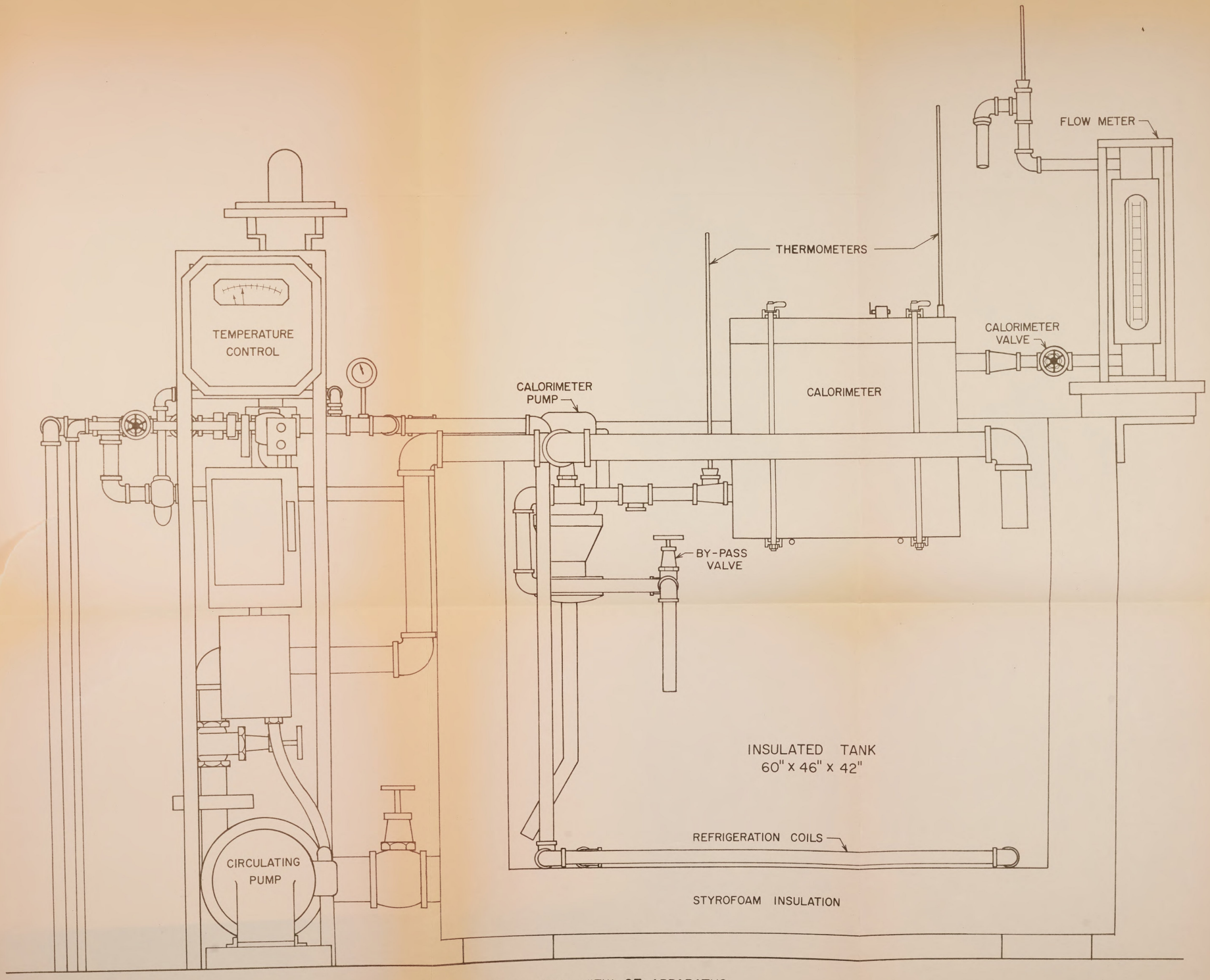

FIG. 1 - SIDE VIEW OF APPARATUS

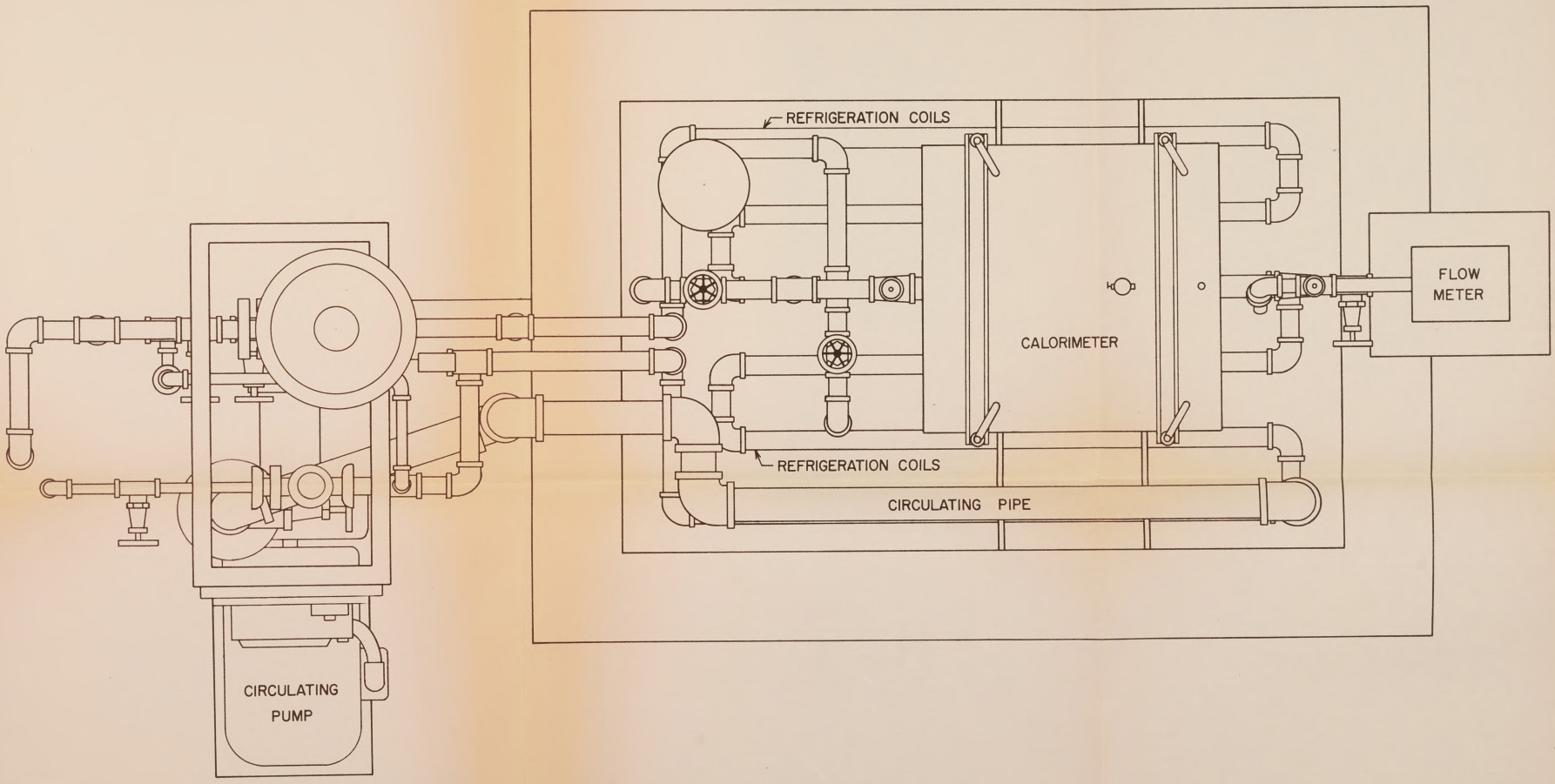

FIG. 2 - TOP VIEW OF APPARATUS

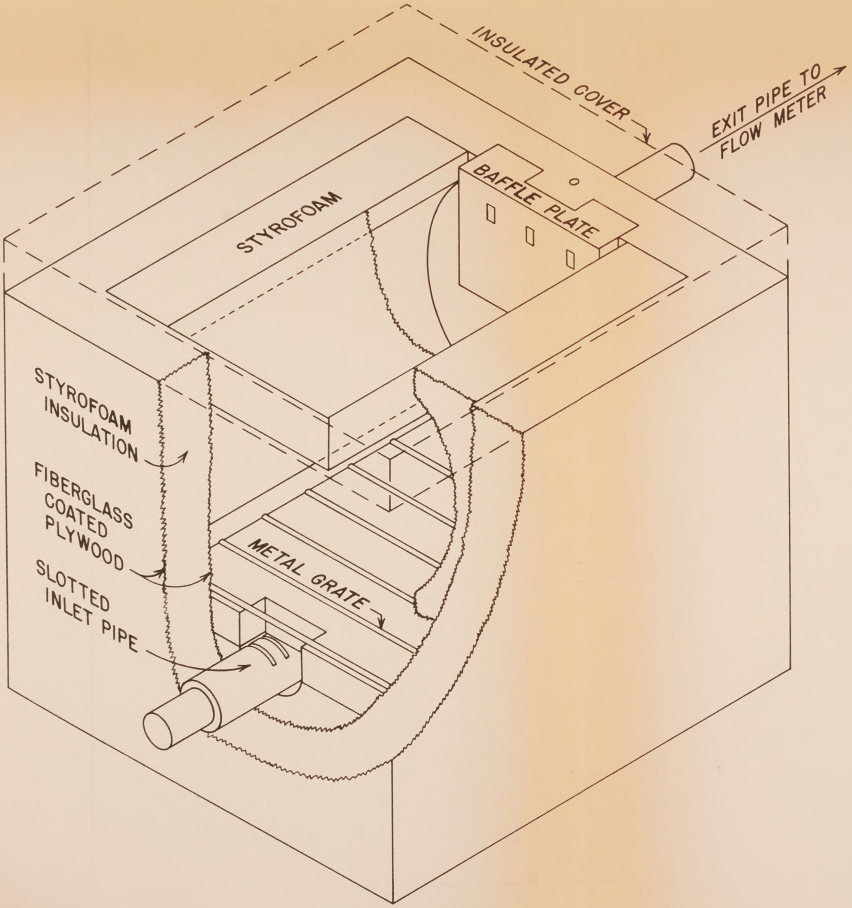

FIG. 3 – CALORIMETER

accurately estimated to the nearest one-tenth of a degree.

One of the major problems that still existed was one of accurate temperature recording. During the course of several freezing trials it was found that the temperature fluctuations of the liquid emerging from the calorimeter were so large and occurred with such frequency that any attempt at accuracy was impossible. A theory was proposed to account for this interesting phenomenon and steps were then taken to correct it.

The liquid used in these trials increased in viscosity as the temperature was lowered and at a temperature of 2°F. this liquid could be compared to a thick sugar syrup. As this liquid passed over the product in the calorimeter, heat was removed from the product. Due to the viscosity of the liquid, it was theorized that the heat was not distributed uniformly throughout but rather passed into the exit pipe in the form of thin ribbons of heat. It was further theorized that when one of these ribbons came in contact with the thermometer the temperature increased. When it veered away from the thermometer, the temperature dropped.

To overcome temperature fluctuations, a metal box (baffle plate) was placed over the exit pipe and this box was so designed that by the time the liquid reached the thermometer it was considered to be mixed and have a constant temperature. The installation of the baffle plate did reduce serious temperature fluctuations making temperature recording more accurate.

A photograph of the apparatus ready for use is shown in Figure 4. The potentiometer is shown with the thermocouple leads inserted into the product inside the calorimeter. Figure 5 is a photograph of the interior of the tank without the liquid coolant and calorimeter.

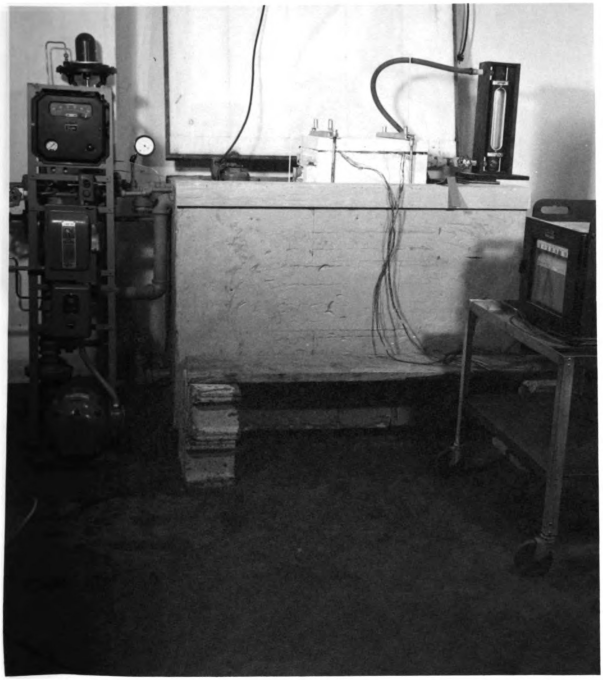

Fig. 4. Photograph of tank and apparatus developed to study freezing rates of turkeys.

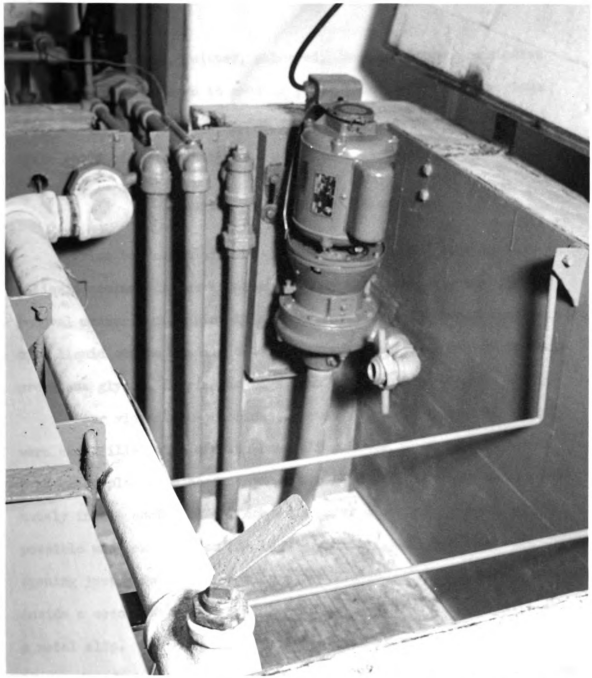

Fig. 5. Photograph of the inside of the steel tank without the liquid or calorimeter and shows placement of the calorimeter pump, refrigerant pipes and large circulating pipe.

An electric clock with a second hand was used as the timing device.

Conventional, twisted, soldered, 24-gauge copper constantan thermocouples were used to measure product temperatures at various times and locations. A 16-point recording potentiometer having a cycle speed of four minutes was used for most trials.

Accuracy Evaluation of Calorimeter

Prior to determining the rate of heat removal from turkeys in a liquid coolant it was necessary to determine the accuracy of heat removal measurements using this apparatus. Since the mean temperature of a liquid can be readily obtained at any specific time, 60 per cent propylene glycol, in plastic bags, was used in this determination.

Four vinylidene-chloride (Cryovac) bags (12 inches by 18 inches) were each filled with one and one-half gallons of propylene glycol. A thermocouple was placed inside the bag so that it would be approximately in the center of the contents after closure. As much air as possible was removed from the bags by forcing the liquid through the opening just before sealing with a metal clip. This bag was placed inside a second Cryovac bag which was air evacuated and sealed with a metal clip. Each bag of propylene glycol weighed approximately 12 pounds after sealing. These bags were then held at room temperature until used. The bags will hereafter be called propylene glycol models.

To prevent any heat from being transferred from the calorimeter to the liquid during a trial, the calorimeter and its contents were cooled to the temperature of the liquid in the tank before each trial. This was accomplished by operating the calorimeter for several hours or until the temperature of the liquid entering was the same as the

temperature of the liquid leaving the calorimeter.

After the calorimeter was precooled, a propylene glycol model was removed from the holding room, weighed, and placed inside the calorimeter. The thermocouple lead was connected to the potentiometer at the same time to obtain an accurate initial temperature. The model was tied to the metal grate to prevent it from floating or otherwise changing its position.

After one-half hour had elapsed the calorimeter pump was stopped, the remaining liquid drained from the calorimeter and the propylene glycol model removed. The contents of the model were thoroughly mixed by shaking to obtain an average temperature which was recorded on the recording potentiometer.

Determination of Heat Loss from Turkeys

Preparation of Turkeys

Turkeys used in this experiment were obtained from the Michigan State University Poultry Science Department. Birds were slaughtered and then chilled for a minimum of 24 hours in a slush ice tank before use. To maintain a uniform initial temperature the turkeys were held in an agitated slush ice tank which was stored in a 35° F. cooling room (Figure 6).

When the thermocouples were used in unpackaged turkeys, they were placed in the bird at least ten hours prior to use; thus a uniform temperature was maintained for all birds. A sharp instrument was used to facilitate the thermocouple placement in the bird. The thermocouples were then threaded through the flesh into the body cavity and inserted back into the cavity wall at the point of greatest thickness of flesh. The body cavity opening was plugged with a fitted piece of

Fig. 6. Photograph of a chill tank containing slush ice and circulating liquid used to maintain constant temperatures of turkeys.

styrofoam. The bird was then trussed with string and placed in a slush ice tank. Figure 7 is a photograph of a trussed turkey with thermocouples inserted.

When thermocouples were used with packaged birds, it was difficult to evacuate and seal the bag since thermocouple leads protruded through the bag openings. A small hole was cut in the bottom of the bag through which the thermocouple leads were passed. The bag material was carefully folded around the lead wires and fastened with a metal clip. Plastic cement was used to seal this area. The thermocouples were inserted into the bird in the manner previously described. The turkey was then placed inside the bag which was then evacuated, sealed, and returned to the slush ice tank.

Heat Loss Determination

A previously prepared turkey was removed from the slush ice tank, weighed, and placed inside the precooled calorimeter. The turkey was tied securely in place with a rubber tube, and the calorimeter was readied for operation.

After the desired time had elapsed the calorimeter pump was stopped. After draining the calorimeter, turkeys to be used for subsequent freezing treatments were removed from the calorimeter and placed in the desired freezers.

Determination of Liquid Composition

Many of the properties of the propylene glycol solution change with a change in concentration, particularly specific heat and viscosity. The specific gravity was determined by using specific gravity bottles and hydrometer. No significant difference in specific gravity was

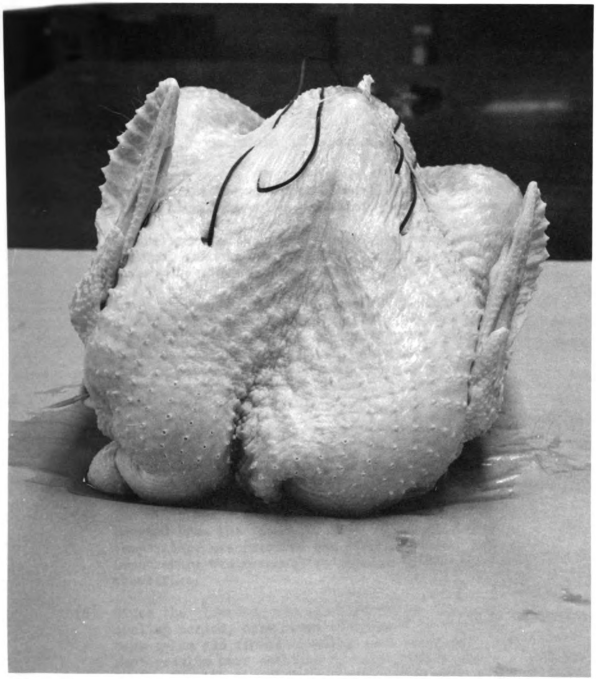

Fig. 7. Photograph of a turkey showing thermocouple lead wires in position for temperature measurements.

observed when determined using these two methods; therefore, the hydrometer was later used exclusively in checking liquid composition. By reference to tables (Dow, 1957) the specific heat[1] of the propylene glycol solution was obtained.

Operation of Calorimeter

(1) The calorimeter was operated until the temperature of the liquid entering and leaving were the same.

(2) The calorimeter pump was stopped, thermometers removed, calorimeter drained, and the clamp, cover, styrofoam forms, and baffle plate removed.

(3) The previously prepared product was immediately placed inside the calorimeter and tied down with a rubber tube to prevent floating. The baffle plate was placed in position. Styrofoam forms were replaced and the cover securely clamped. Thermometers were placed in their proper position.

(4) The calorimeter pump was started with the air vent open. After approximately two minutes the calorimeter was filled with liquid, and the air vent was closed.

(5) The timer was started when the liquid first emerged from the flowmeter exit pipe. The first temperature was recorded ten seconds later. The temperature was recorded at regular intervals thereafter.

(6) Since the flowmeter was adjusted during the precooling period, only minor adjustment to the by-pass valve and flowmeter valve were required. Best results were obtained by making minor adjustments with the flowmeter valve; larger adjustments with the by-pass valve.

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[1]Specific heat is the number of British thermal units needed to raise one pound of the substance through one Fahrenheit degree.

RESULTS AND DISCUSSION

When a body absorbs heat energy and does not undergo simultaneously some endothermic process such as a chemical or phase change, the heat appears as a rise in temperature of the body. The most common method of determining the amount of heat used or produced in a physical change utilizes measurement of temperature rise of a known amount of material of known specific heat. An insulated calorimeter chamber was constructed and operated so that there was practically no heat transfer from or to the inside of the calorimeter. Any temperature rise of a liquid passing through the calorimeter and over a warm product was assumed to be the result of heat lost by the product.

Many preliminary trials were conducted in which turkeys and propylene glycol models were used to determine the rate at which heat was transferred from a product to the liquid coolant. There were a number of factors which were found to cause variations in results, such as:

(a) position of the product in the calorimeter,

(b) problems related to the flow pattern around the product due to viscosity,

(c) the shape of the interior walls of the calorimeter.

The calorimeter was modified a number of times until results were repeatable and considered accurate. The following tests were then conducted to determine whether the apparatus was effectual in measuring heat removal rates under various experimental conditions.

Heat Removed from Propylene Glycol Models

The amount of heat removed from four propylene glycol models was determined using a calorimeter. These models consisted of approximately twelve pounds of propylene glycol packaged in Cryovac bags. The amount of heat loss was determined for periods of one-half hour in a liquid at 2° F. and flowing at a rate of .268 cubic feet per minute. To determine the accuracy of the calorimeter, it was necessary to calculate the theoretical amount of heat removed from the product during a known change in temperature, and to relate this heat loss to the heat loss measured by the calorimeter.

The theoretical heat loss was computed by inserting the values for the variable factors in the following formula (Hausman and Slack, 1948).

$$Q = mct$$

where Q = quantity of heat in B.t.u.

m = mass in pounds

c = specific heat B.t.u. per pound per degree Fahrenheit

t = temperature change in degree Fahrenheit

Method of Calculation

A propylene glycol model weighing ten pounds, having a specific heat of .78 and an initial temperature of 70° F. was immersed in a liquid and cooled to 10° F. The theoretical heat loss was:

$Q = mct$

= 10 lbs. x .78 (B.t.u.) per lb. per °F. x 60° F.

= 468 B.t.u.

The percentage of the theoretical heat removed as measured by the calorimeter for four propylene glycol models is shown in Figure 8. The method of calculation is the same as that previously described. Between 95.5 and 97.6 per cent of the heat removed was measured by this apparatus. The mass, in this case, was the weight of the volume of liquid which passed through the calorimeter during the trial period and this volume was determined by a previous standardization of the flowmeter. The specific heat was determined by referring to the "Dow Charts" which show the relationship between percentage composition of the liquid coolant by weight and specific heat. The temperature change was obtained by recording the temperature of the ingoing liquid and the temperature of the outgoing liquid and calculating differences. The differences were then averaged for the period.

It was found that in all these tests carried out with the propylene glycol models that there was a discrepancy between the amount of heat removed when determined theoretically and the amount of heat removed when computed from the calorimeter data. At the end of the cooling trial, the liquid remaining in the calorimeter was drained from the inlet pipe. This liquid contained heat energy which had been removed from the model. With present procedures, only a rough estimate could be made of this amount of heat. This could account for part or all of the 2.4 to 4.4 per cent of heat removed from the models but not measured by the calorimeter.

Determination of Heat Removal from Turkeys

Four unpackaged Beltsville White tom turkeys were prepared for freezing trials as described in the procedure. These were each carefully positioned and fastened in the calorimeter and the procedure

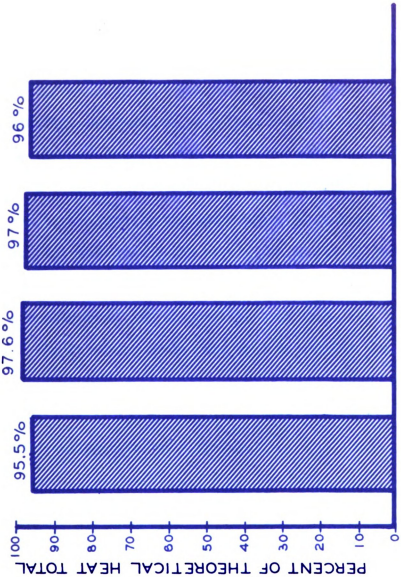

Fig. 8. Per cent of the total theoretical heat, as calculated from calorimeter data, which was removed from propylene glycol models held in the calorimeter for 30 minutes. (Liquid temperature, 20°F.; flow rate, .268 cubic feet per minute.)

for operating the calorimeter was then followed. Temperature of the liquid coolant entering and leaving the calorimeter was recorded at one-minute intervals. The average temperature difference was calculated for each ten-minute interval. The mass in this case was the weight of the liquid which passed through the calorimeter during a ten-minute period.

The specific heat was obtained from the Dow charts. These experimental observations were then inserted into the formula Q = mct giving the heat removed from the turkey as measured by the calorimeter.

A theoretical determination of the total B.t.u. which would be removed in lowering the temperature of each of four turkeys from its initial temperature was made. The change in physical properties of the turkeys in freezing made it necessary to use several steps in the computation of the theoretical heat loss.

The following method was used to compute the theoretical heat loss in lowering the temperature of a 14.38 pound turkey from 34° F. to 0° F. (Trial 1, Table I). A temperature of 27° F. was selected as the freezing point of a turkey.

[1]Specific heat before freezing .80 B.t.u. per lb. per °F.

[1]Specific heat after freezing .41 B.t.u. per lb. per °F.

[1]Latent heat of fusion 99.00 B.t.u. per lb.

Heat loss before freezing (34° F. - 27° F.) =
7° F. x .80 B.t.u. per lb. = 5.60 B.t.u. per lb.

Latent heat of fusion = 99.00 B.t.u. per lb.

Heat loss after freezing (27° F. - 0° F.) =
27° F. x .41 B.t.u. per lb. = 11.07 B.t.u. per lb.

Total per lb. 115.67 B.t.u. per lb.

Total = 115.67 B.t.u. per lb. x 14.38 lbs. = 1663 B.t.u.

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[1]Tressler and Evers (1957).

The heat removed from each of the four unpackaged turkeys is reported in Table I. After holding in the calorimeter for one hour with a liquid flow of .268 cubic feet per minute, 39 to 45.8 per cent of the total heat was removed. To show graphically the variation that existed between birds, the residual heat as a percentage of the total was plotted on semi-logarithmic paper. The rate of heat removal when plotted in this manner appeared to be approximately linear.

Figure 9 shows the residual heat in each of these turkeys after periods from 10 to 60 minutes in the calorimeter. The variation in percentage of residual heat after 60 minutes ranged from 54 to 61 per cent. This slight variation could be a result of differences in turkey weight, turkey shape, and turkey composition, as well as those factors previously mentioned.

Demonstration of Packaging Effects

The objective of this study was the development and use of this apparatus and procedure in detecting the effect of packaging on the rate of heat removal from a turkey. To test the ability of this apparatus in detecting these packaging effects, the following test was designed.

Four turkeys were packaged with Cryovac and properly placed in the calorimeter. Calorimeter operating procedures were then followed.

The results reported as a percentage of the theoretical heat to be removed to lower the temperature of the turkey from its initial temperature to 0° F. was computed and are given in Table II. In this case the per cent of total heat removed from the turkeys ranged from 14.8 per cent to 24.6 per cent in the four packaged turkeys. Larger

TABLE I

Percentage of Total Theoretical Heat Removed from Unpackaged Turkeys Held in Propylene Glycol for 60 Minutes at 2° F. with a Flow Rate of .268 Cubic Feet per Minute

Initial Bird Weight	Pounds				
	14.38	12.06	13.75	13.13	
Time Min.	Trial Number				Average
	1	2	3	4	
	Per cent of total heat removed				
10	16.5	14.7	12.2	16.0	14.9
20	7.0	6.8	6.5	6.9	6.8
30	6.8	5.5	5.5	5.6	5.9
40	6.2	5.3	5.4	4.5	5.4
50	5.4	4.9	5.0	4.1	4.9
60	3.9	4.0	4.4	4.1	4.1
Total	45.8	41.2	39.0	41.2	42.0

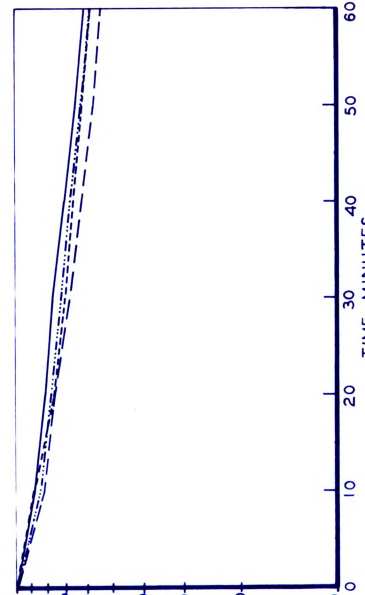

Fig. 9. Percentage of heat (B.T.U.) remaining in four unpackaged turkeys held for periods up to 60 minutes in propylene glycol at 29°F., and a flow rate of .268 cubic feet per minute.

TABLE II

Percentage of Total Theoretical Heat Removed from Cryovac Packaged Turkeys Held in Propylene Glycol for 60 Minutes at 2° F. with a Flow Rate of .268 Cubic Feet per Minute

Initial Bird Weight	Pounds				
	13.75	12.00	12.75	14.00	
Time Min.	Trial Number 1	2	3	4	Average
	Per cent of total heat removed				
10	11.8	9.3	4.7	5.1	7.7
20	5.1	2.9	3.2	2.5	3.4
30	3.3	1.9	2.2	2.4	2.5
40	1.7	1.2	1.1	3.0	1.8
50	1.9	0.8	1.8	3.4	2.0
60	0.8	0.7	1.8	3.2	1.6
Total	24.6	16.8	14.8	19.6	19.0

variations than those obtained could be expected if there were differences in the amount of air evacuated from the package or the effectiveness of the package seal. Figure 10 shows the residual heat remaining in the turkeys after periods from 10 to 60 minutes in the calorimeter. From 75 to 85 per cent of the total heat still remained after cooling in the calorimeter for 60 minutes. This was much higher than the 54 to 61 per cent residual heat reported in unpackaged turkeys.

Demonstration of Flow Rate Effects

Variations in heat transfer rate were observed as a result of differences in the flow rate of the liquid through the calorimeter. The liquid flow rate was decreased from .268 cubic feet per minute to .159 cubic feet per minute.

Four unpackaged turkeys were prepared as described in the procedure. After 60 minutes of cooling in the calorimeter, it was found that the heat loss reported as percentage of the total theoretical heat necessary to be removed to lower the turkeys to 0° F. ranged from 24.8 to 38.8 per cent as shown in Table III. This represented a decrease in heat removed when compared to that observed in the four turkeys cooled using the higher flow rate. However, the heat removed from turkeys at this flow rate (.159 cubic feet per minute) was greater than that observed with packaged turkeys cooled in a liquid at the high flow rate. Figure 11 shows the residual heat at 10-minute intervals up to 60 minutes in the four turkeys cooled using a flow rate of .159 cubic feet per minute. The amount of heat remaining in the turkey after one hour in the calorimeter ranged from 61 to 75 per cent.

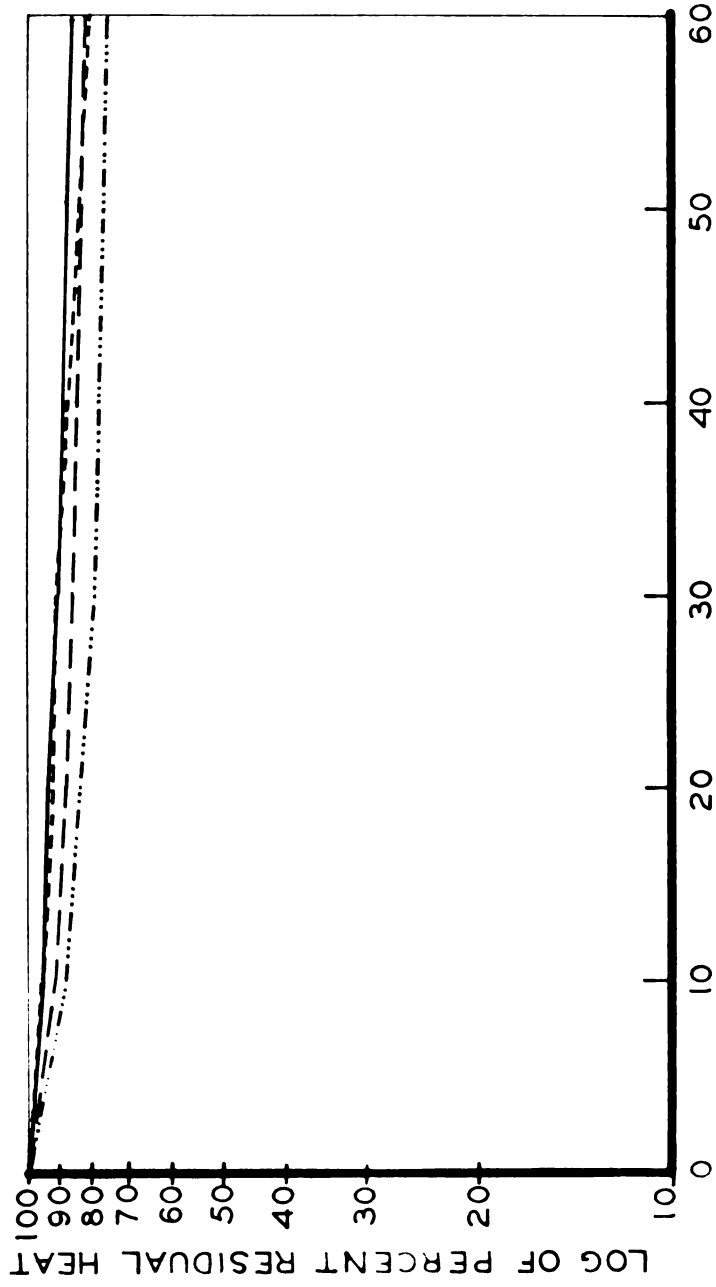

Fig. 10. Percentage of heat (B.T.U.) remaining in four packaged turkeys held for periods up to 60 minutes in propylene glycol at 2°F. with a flow rate of .268 cubic feet per minute.

TABLE III

Percentage of Total Theoretical Heat Removed from Unpackaged Turkeys Held in Propylene Glycol for 60 Minutes at 2° F. with a Flow Rate of .159 Cubic Feet per Minute

Initial Bird Weight	Pounds				
	14.13	14.50	13.13	14.75	
Time Min.	Trial Number				
	1	2	3	4	Average
	Per cent of total heat removed				
10	7.3	9.4	12.9	7.3	9.2
20	4.3	7.5	5.9	4.1	5.5
30	5.0	6.0	5.0	4.0	5.0
40	4.3	5.1	3.8	3.9	4.3
50	3.6	5.7	2.8	3.0	3.8
60	3.1	5.1	2.6	2.5	3.3
Total	27.6	38.8	33.0	24.8	31.1

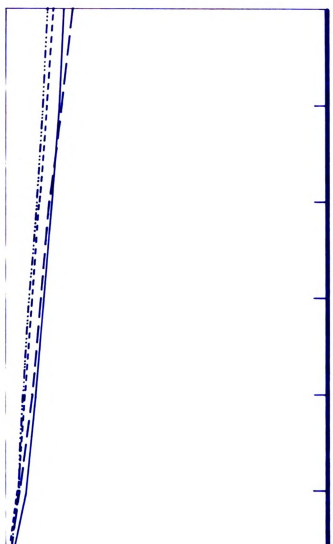

Fig. 11. Percentage of heat (B.T.U.) remaining in four unpackaged turkeys held for periods up to 60 minutes in propylene glycol at 20°F. with a flow rate of .159 cubic feet per minute.

The percentages of the total theoretical heat to be removed from each of the four turkeys, from each of the three experimental situations, were averaged and are presented graphically in Figure 12. It can be seen that the apparatus did detect differences between the rate of heat loss in unpackaged turkeys (B) and the rate of heat loss in packaged turkeys (A). When the flow rate around the bird was decreased from .268 cubic feet per minute to .159 cubic feet per minute, there was a coincidental drop in the rate of heat removed from four turkeys immersed in this slower flow rate. However, under the conditions of these tests, the flow rate did not affect heat loss from turkeys as much as packaging did.

Each of three turkeys was held in the calorimeter for 60 minutes prior to further freezing in an air blast freezer at -20°F. When a substantial amount of heat was removed while a turkey was in the calorimeter, the time for the slowest cooling point to reach 20°F. was increased to six hours. Figure 13 shows the effect of the initial rate of removing heat on subsequent freezing times when placed in an air blast freezer maintained at 20°F.

In examining curves A and B it was noted that while there was an initial heat removal of 41 per cent from B and 36 per cent from A, turkey B required more time for the slowest cooling point to reach 20°F. This was not surprising because of the differences in weight together with difficulty in determining the slowest cooling spot. When the amount of heat removed initially was very small due primarily to a packaging effect as was the case with turkey C, the total freezing time was increased considerably.

The usefulness of this apparatus and the procedures pertaining to it, in measuring heat removal rates from turkeys, has been demonstrated. More detailed studies are indicated in establishing tempera-

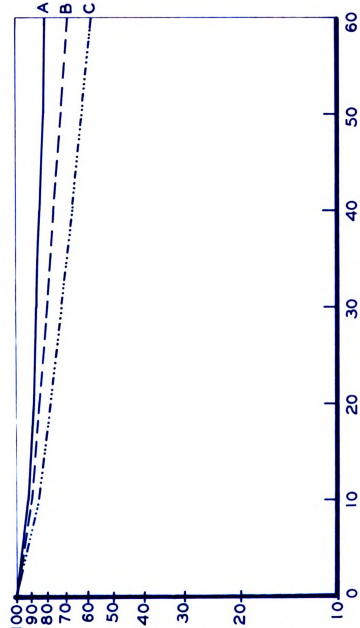

Fig. 12. Percentage of heat (B.T.U.) remaining in turkeys held up to 60 minutes in propylene glycol at 20° F. under three experimental conditions.
(A) - Packaged turkey - flow rate .268 cubic feet per min.
(B) - Unpackaged turkey - flow rate .159 cubic feet per min.
(C) - Unpackaged turkey - flow rate .268 cubic feet per min.

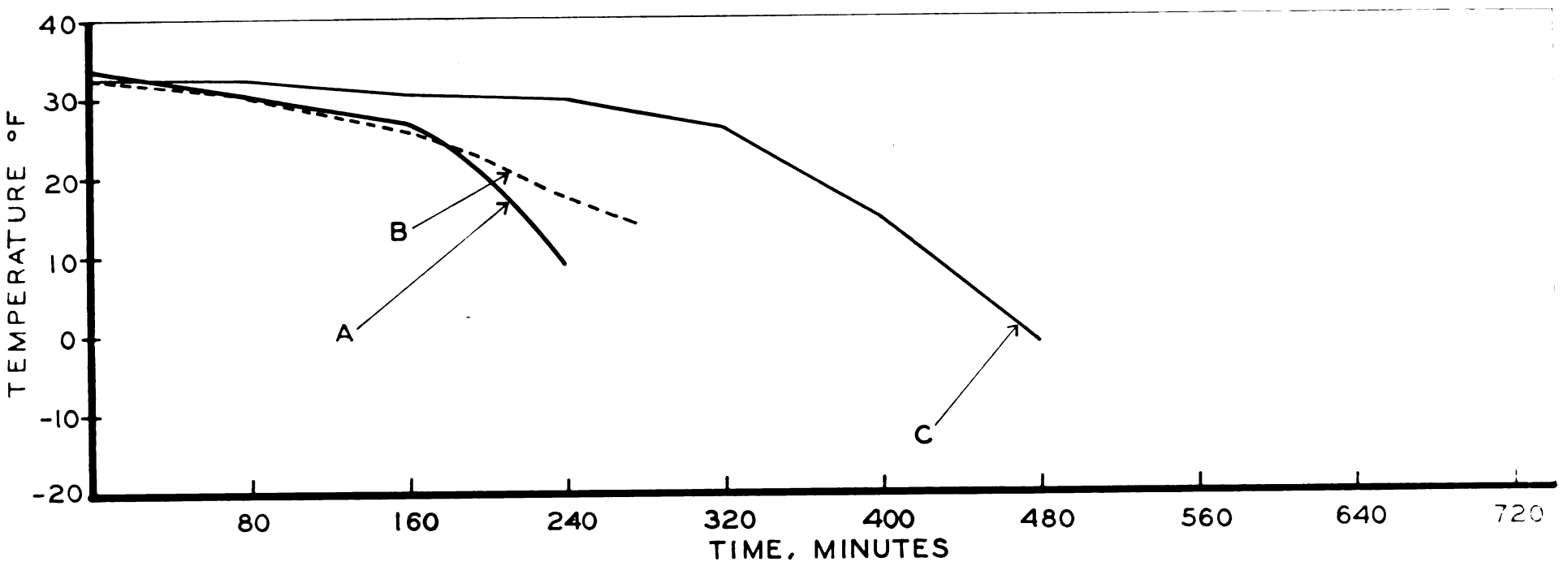

Fig. 13. The effect of rate of removing heat (B.T.U.) from turkeys held in propylene glycol at 2°F. for 60 minutes on subsequent temperatures of these turkeys when placed in a -20°F. air blast freezer.

A. Wgt. 11.75 - 36 per cent of total heat removed in first hour
B. Wgt. 13.75 - 41 per cent of total heat removed in first hour
C. Wgt. 12.00 - 16 per cent of total heat removed in first hour

ture distribution in turkeys, effects of packaging on heat transfer from the bird to a surrounding liquid, and the influence of flow rates on the rate of heat removal.

The present apparatus, while effective in measuring heat removal rates, could be altered to decrease the number of hand manipulations. A new refrigeration coil system would contribute to the speed of refrigeration adjustments in response to changes in liquid temperature. A different type of flowmeter which could be set for a wider range in flow rates would increase the scope of further studies and possibly approach the type of flow rate used in the commercial field. An automatic temperature recording device to measure temperature differential between the ingoing liquid and the outgoing liquid would alleviate the need for the operator to record two temperatures at one-minute intervals.

PART II: Histological Methods of Examining Frozen Turkey Muscle Tissue

PROCEDURE

To effectively preserve the original frozen structure of turkey muscle tissue, a method of fixation must be used which will minimize any changes during subsequent treatments. One standard method, two types of freeze drying methods, method A and method B, and a method of frozen fixation were evaluated in the laboratory.

Preparation of Turkeys for Freezing

Turkeys were slaughtered, eviscerated, chilled in ice for six hours and packaged in Cryovac bags which were air evacuated and sealed. Each turkey was placed in appropriate freezers or freezing medium until completely frozen. The turkeys frozen in an agitated liquid were immersed in the liquid by placing each turkey in a wire egg basket containing lead weights. Then, after complete freezing, the turkeys were removed from their respective freezers or medium and placed in a 0° F. air blast freezer room where all frozen tissue samples were cut and prepared for fixing. For standard comparisons fresh tissue samples were removed from a turkey five minutes after slaughtering.

All tissue samples were obtained from the breast muscle (pectoralis major). A small hand saw was used to remove a slice of muscle tissue which was then cut into smaller pieces. When necessary, tissue samples were separated for identification purposes by loosely wrapping in cheesecloth.

Method of Tissue Preparation

Standard Method[1]

Samples of tissue from the four turkeys were placed in tissue buttons and subjected to the following treatment in an autotechnicon:

10 per cent Formalin	6 hours
70 per cent Alcohol	1 hour
80 per cent Alcohol	1 hour
95 per cent Alcohol	1 hour
95 per cent Alcohol	1 hour
100 per cent Alcohol	1 hour
100 per cent Alcohol	1 hour
Equal parts of absolute alcohol and xylene	1 hour
Oil of Cedarwood	1 hour
Xylene	1 hour
Paraffin (50-52° C.)	1 hour
Paraffin (56-58° C.)	2 hours

After the above cycle was completed, tissue samples were removed from the autotechnicon, embedded in paraffin (56-58° C.), and allowed to solidify. After trimming, tissue blocks were sectioned on a Spencer No. 820 microtome. The ribbons of sections were floated on a water bath, and slides coated with egg albumin were immersed in the bath, permitting the sections to be floated onto the slides. The slides were then dried and stained.

Freeze Drying Method A (Modification of method used by Koonz and Ramsbottom, 1939)

In one test an insulated 1,000 ml. beaker was half filled with ethyl alcohol. A vacuum bottle was immersed in the beaker until the level of alcohol was approximately three-fourths of the way up the bottle. Dry ice was then added to the alcohol in the beaker and a vacuum line was attached to the bottle.

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[1]Armed Forces Institute of Pathology, Washington, D. C.

To prevent thawing, samples of tissues from the four turkeys were placed in a round bottom flask immersed in alcohol and dry ice. The bottle was then connected to the vacuum flask. When the vacuum was operating efficiently, the alcohol and dry ice bath were removed from the flask containing the tissue samples. This treatment was continued for 24 hours. Samples were removed from the vacuum bottle and held for two hours in paraffin at 50-52°C. and, for two additional hours, in paraffin at 56-58°C. The samples were embedded in tissue blocks, trimmed, and to prevent rehydration of the tissue sections they were placed directly on warm slides coated with egg albumin. The slides were then placed on a slide warmer for one hour. This permitted the section to flatten out on the slide and resulted in less distortion. After the slides were dried, they were stained in the manner described under Staining Procedure on page 50.

Freeze Drying Method B (Modification of Altman-Gersh method as cited by Hoerr, 1936)

In a second experiment an ordinary desiccator fitted with a stopper and containing P_2O_5 as a desiccant was placed in a 0°F. air blast freezer room. A vacuum hose was attached to the top of the desiccator and passed through a hole in the insulated wall to a vacuum pump operating at a warmer temperature. The tissue was cut in the same manner as previously described and placed in the desiccator. The vacuum pump was started and the tissues were dried in this manner for 24 hours. After the drying period, the stoppered desiccator was removed to the histology laboratory where the tissues were placed in a vacuum bottle containing paraffin. The paraffin was maintained at constant temperature by a water bath. The tissue was infiltrated under a vacuum until the tissue sank to the bottom of the vacuum bottle

indicating that the tissue was completely infiltrated. The tissue was then embedded, tissue blocks trimmed and sectioned. Slides were prepared in the manner described above for tissue fixed by freeze drying.

Frozen Fixation

Tissue samples were taken from the turkeys and placed in a 1:1 solution of 10 per cent formalin and absolute ethyl alcohol and held at 0° F. for 24 hours. After the process of frozen fixation, the tissues were removed from the fixative solution and placed in the autotechnicon starting at 70 per cent alcohol and through the same cycle as outlined in the standard procedure.

Staining Procedure

All slides prepared in this experiment were stained with a hematoxylin eosin stain as follows:

Xylene	3 minutes
Xylene	3 minutes
95 per cent alcohol	3 minutes
Hematoxylin	8-10 minutes
Running tap water	Rinsed well
Decolorized in acid alcohol	Dipped until sections were reddish
Rinsed immediately in tap water	
Dip in ammonia water until sections turned blue	
Tap water rinse	
Eosin Stain	1-2 minutes
Tap water rinse	
95 per cent alcohol	2 minutes

Absolute alcohol	2 minutes
Carbo-xylene	3 minutes
Xylene	3 minutes
Xylene (fresh)	3 minutes

RESULTS AND DISCUSSION

Histological Method for Studying Freezing Rates in Turkeys

The changes which take place in turkey tissues during freezing affect the appearance of the bird, the extent of tissue cell rupture, the amount and rate of moisture loss during thawing and the subsequent preservation of the product. Some of these changes are detectable by normal observation, while others can be detected only by microscopic techniques. These changes which take place in cellular structure, as a result of freezing, can only be accurately detected by examining the frozen tissue before it thaws and reverts back to its original appearance. The same effect can be obtained by treating the tissue to be studied in some way that will preserve the frozen tissue structure.

The use of standard techniques for examining tissue structure of frozen turkey muscular tissue permits the tissue to thaw before complete fixation. This results in tissue structure similar, although not identical, to that taken from an unfrozen turkey. Methods previously used for similar studies were not adaptable to the existing laboratory setup. A new method of frozen fixation was developed. This method is similar in some respects to that introduced by Cook et al. (1926) and the results obtained with this method compared with results obtained using a standard method and two methods of freeze drying.

Unfrozen Tissue Examination

The muscles attached to the skeleton of a turkey consist of

striated muscular tissue. In a preparation of fresh unfrozen muscle, the tissue usually appears to consist of long, cylindrical muscle fibers. When viewed in cross-section these appear to be large, multinucleated cells. Figure 14 is a photomicrograph of a cross-section of fresh, unfrozen tissue taken from the pectoralis major muscle of a turkey. Ordinarily the protoplasm of the unfrozen muscle fibers, when viewed in cross-section, is quite uniform and free from vacuoles of any kind.

When a turkey is frozen the moisture present in the muscular tissue begins to solidify as the temperature of the surrounding atmosphere reaches the freezing point of the liquid. The ice crystals formed as a result of freezing can and do change the physical structure of the muscular tissue from its original unfrozen structure. MacNeil et al. (1958) reported that the extent of this change and its effect on the turkeys while in market channels is directly related to the rate at which the turkey is frozen.

In describing the following photomicrographs, the term "ice crystal vacuole" will be used. The vacuole, as it appeared in the photomicrographs, was believed to represent the effects of shape and size of the actual ice crystal before the tissue was subjected to fixation.

Frozen Tissue Examination

Figure 15 shows a photomicrograph of a cross-section of muscle tissue taken from a turkey frozen at -20°F. in air and prepared by a standard method. As a result of freezing at this temperature, ice crystal vacuoles would be expected to occupy primarily the intracellular spaces. Small, irregularly-shaped vacuoles are evident within the muscle fiber; however, the general muscle fiber shape and

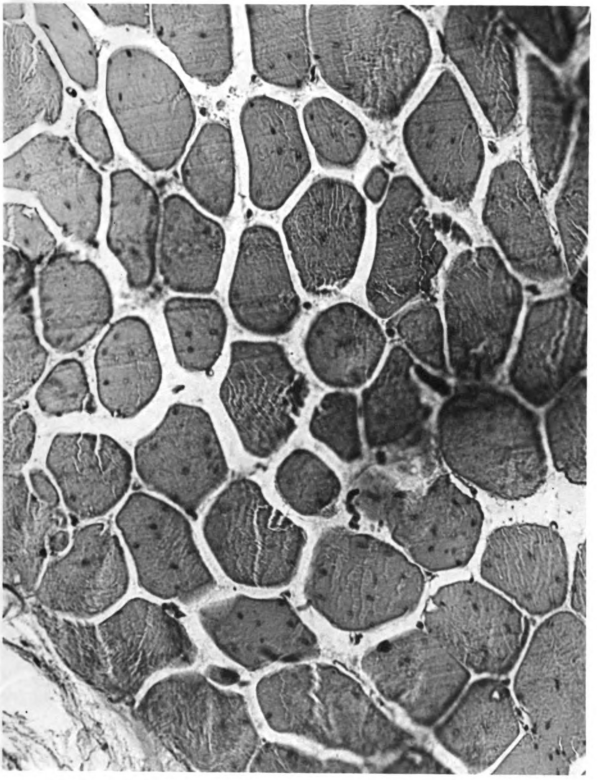

Fig. 14. Photomicrograph of a cross-section of muscular tissue taken from the pectoralis major of an unfrozen turkey and prepared by the standard method. 400X.

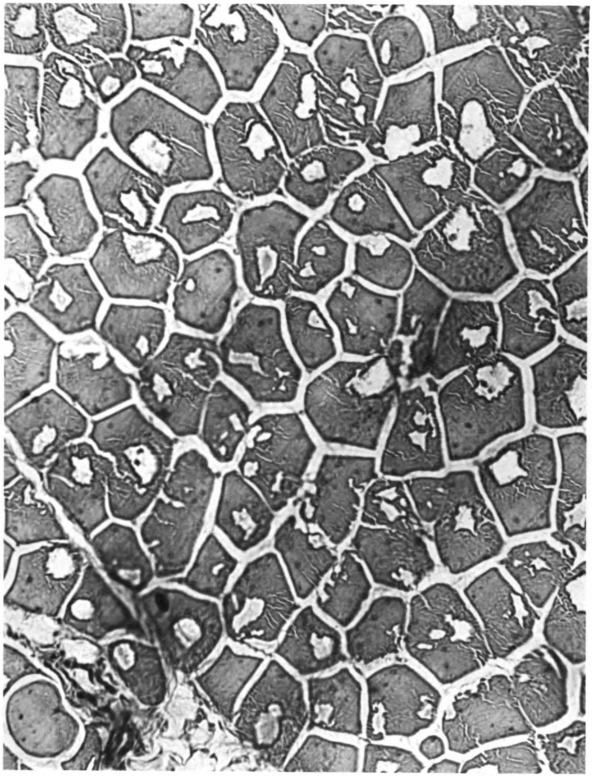

Fig. 15. Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen in a -20°F. air blast freezer and prepared by the Standard Method. 400X.

size is similar to that structure shown in the photomicrograph in Figure 14. The moisture content of turkey meat is 55 per cent (Winton and Winton, 1937); therefore, when all the moisture is solidified the ice crystals would be expected to occupy much more space than is indicated in this photomicrograph. Since this method of preparing a section of tissue for microscopic examination allows thawing of the tissue during section preparation, the true frozen structure became changed. The tissue structures reverted back to their original unfrozen appearance except for the small vacuole in the center of the muscle fiber. These vacuoles are probably a result of incomplete reabsorption. Because of these observations the standard method was not acceptable for examining the effects of ice crystal formation.

Figure 16 shows a photomicrograph of a cross-section of muscular tissue taken from the same turkey frozen at -20° F. in air. This section was prepared by the freeze drying method as outlined in the procedure on page 48. The nature of this method could possibly allow for some thawing, even though careful attention was given at this point. The alcohol and dry ice bath were removed from the side-arm flask containing the tissues after the vacuum pump was working effectively. Since the apparatus was set up in a 55° F. room, there was a chance for the tissue to come in contact with the glass bottle and, consequently, thaw; however, the tissues were wrapped in cheesecloth which helped in preventing contact with the sides of the side-arm flask.

Difficulties were encountered in obtaining satisfactory infiltration and, consequently, the tissue blocks were brittle. Improper removal of moisture could also account for poor infiltration. In order to obtain a satisfactory tissue for sectioning, the section

Fig. 16. Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen in a -20°F. air blast freezer and prepared by a freeze drying method. 400X.

thickness had to be increased from 7 to 11 microns. This photograph shows that there are a number of artifacts due primarily to the increased section thickness as well as tissue block crumbling. However, if the ice crystal vacuoles are examined closely, a general size and shape pattern can be seen even though the artifacts are present. There is evidence of some cell rupture but this was probably due more to the method of preparation rather than an effect of ice crystal formation.

Since both of these methods (standard method, freeze drying method) resulted in either the formation of artifacts or rehydration of the tissue during sectioning, attention was given to other methods which could be used in the laboratory. At the time, the laboratory or freezing rooms were not adaptable to the several methods cited in the literature. A method of maintaining a fixative solution at 0°F. to prevent thawing of the frozen tissue was then used.

Figure 17 shows a photomicrograph of a cross-section of muscular tissue taken from the same turkey (frozen at -20°F. in air) and prepared by the frozen fixation method as described under procedure on page 50. The ice crystal vacuoles in this case occupy the center of the muscle fiber with the fiber protoplasm distributed around the periphery. The general tissue structure in this photomicrograph is not unlike that shown in Figure 16; however, it is considered to be much clearer from the standpoint of artifacts and it was believed to give a more accurate representation of the tissue structure after freezing.

Comparisons between the freeze drying method and the frozen fixation method were made using a turkey frozen in an agitated liquid at -10°F. The freezing rate of similar-sized turkeys was much

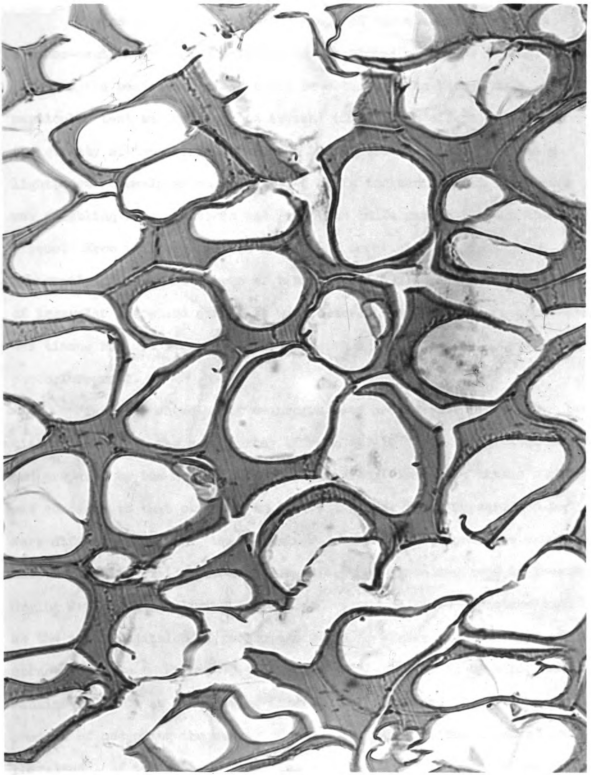

Fig. 17. Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen in a -20°F. air blast freezer and prepared by the method of Frozen Fixation. 400X.

greater in the agitated liquid at -10°F. than it was at -20°F. in air.

Figure 18 shows a photomicrograph of muscular tissue and is a cross-section which was prepared by the method of freeze drying. This was the best result that could be obtained even though this particular test was carried out several times in an effort to improve the quality of the tissue sections. During the sectioning process a light dust accumulated on the cutting knife indicating that the tissue was crumbling into powder as the microtome knife passed through the tissue. From this observation it was not surprising to find an apparently disorganized group of tissue particles separated by vacuoles of irregular shape and size. It was believed that while there apparently was tissue rupture it was not as extensive as that indicated by this photomicrograph.

Figure 19 shows a photomicrograph of a cross-section of muscular tissue taken from the same turkey (frozen at -10°F. in an agitated liquid) and prepared by the method of frozen fixation. When this tissue structure was compared to that observed in Figure 18, they first appeared to be very different; however, when examined in the light of previous experience, they were quite similar in many respects. The procedure used in freeze drying would not permit the observation of any delicate structure such as the thin projection of protoplasm shown in Figure 19. The type of structure shown in Figure 19 is similar to that reported by Hiner and Hankins (1946). As the rate of freezing was increased, the ice crystal, instead of occupying the center of the muscle fiber, formed on the peripheral edge of the fiber. These ice crystals are more numerous but of a smaller average size than those observed in Figure 18.

The extent of the fiber splitting determines the amount of drip

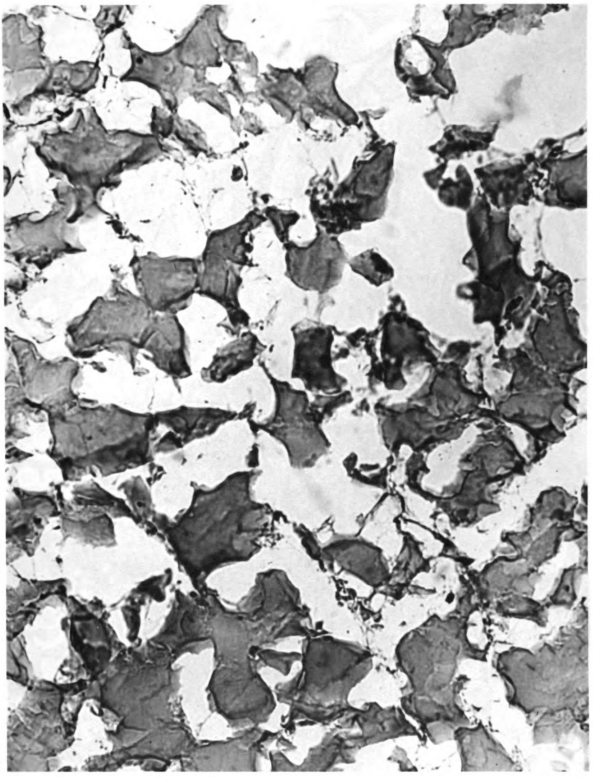

Fig. 18. Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen in an agitated calcium chloride solution at -10°F. and prepared by the method of freeze drying. 400X.

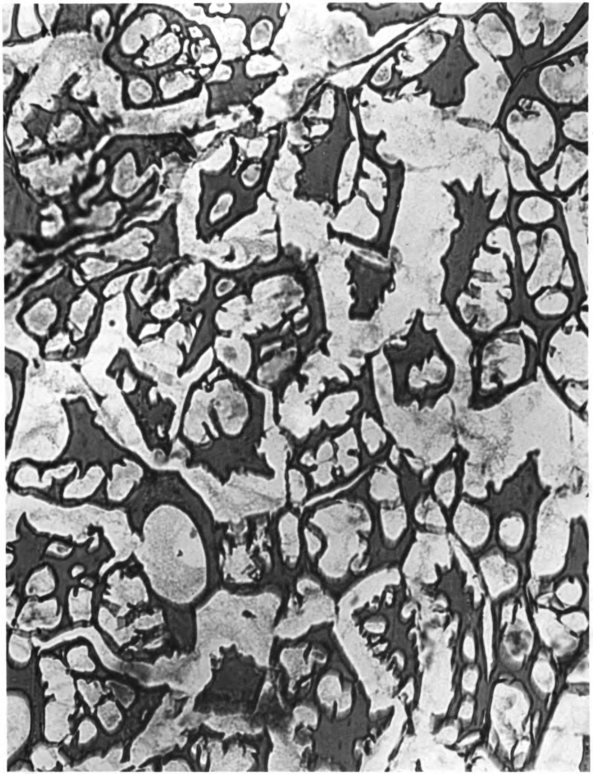

Fig. 19. Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen in an agitated calcium chloride solution at -10°F. and prepared by the method of Frozen Fixation. 400X.

loss upon thawing. The greater the fiber splitting the greater is the reabsorption of the tissue fluids, and conversely when the muscle fiber is not split, any fluids released from the fiber during ice crystal formation are not readily reabsorbed. Therefore, there would be much less drip loss in the turkey frozen at -10°F. in an unagitated liquid than there would be in one frozen at, say, -20°F. in air.

Comparison of Frozen Fixation and Improved Method of Freeze Drying

Koonz and Ramsbottom (1939) reported on a method of freeze drying which involved the use of a desiccator and vacuum pump. All the apparatus was placed in a freezing room where the drying procedures were carried out. Since a vacuum pump does not operate effectively at low temperatures unless a special oil is used, alterations were made in the experimental procedure as outlined under Procedure on page 49.

The process of infiltration used in connection with the former freeze drying method was felt to be the basic cause for many of the problems encountered. A method of vacuum infiltration, as described in the Procedure on page 49 was consequently used with method B of freeze drying.

Figure 20 shows a photomicrograph of a cross-section of muscular tissue taken from a turkey frozen at -40°F. in air and prepared by freeze drying method B. The ice crystal vacuoles occupied the center of the muscle fiber with the fiber protoplasm surrounding it. These sections were cut at 10 microns and there was also some evidence of crumbling although it was not as pronounced as that observed when using the other method of freeze drying. The general tissue structure,

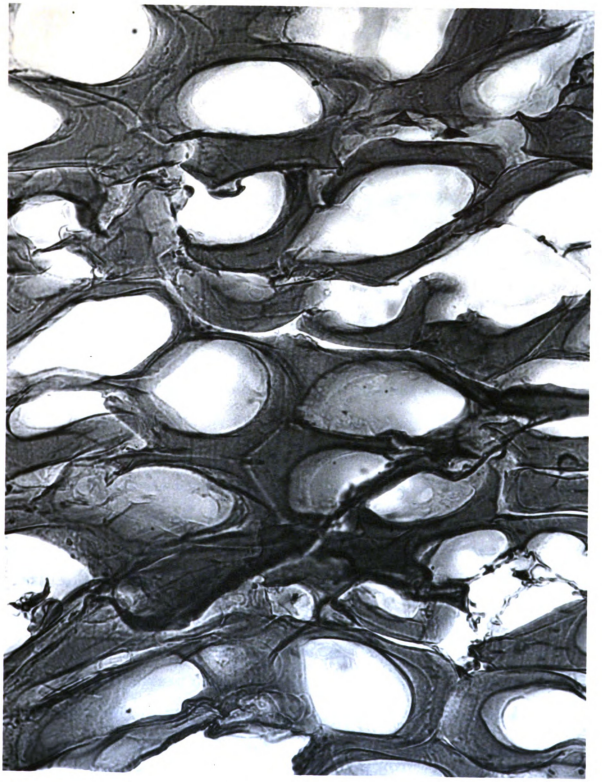

Fig. 20 . Photomicrograph of a cross-section of muscular tissue taken from a turkey frozen at -40°F. air blast and prepared by a freeze drying method. 400X.

as a result of freezing, can be examined using this improved method of freeze drying; however, the results of the frozen fixation method were believed to be still superior.

It should be pointed out that this method of frozen fixation can be used only for the examination of frozen tissue. When unfrozen tissue was prepared by this method, the tissue became frozen and consequently ice crystals were formed causing a disruption of the normal muscle fiber.

Figure 21 shows a photomicrograph of unfrozen tissue prepared by the frozen fixation method. This is also an example of quick frozen tissue showing the effects of a large number of small ice crystal vacuoles around the periphery of the muscle fiber. With an even faster freezing rate the ice crystal vacuoles would become smaller.

It was evident from these studies that a method of frozen fixation for frozen muscle tissue resulted in what was believed to be a true and accurate representation of the effects of ice crystal size and shape, when viewed microscopically. The use of a freeze drying technique accompanied by vacuum infiltration resulted in tissues which were comparable to those produced by frozen fixation; however, the personal attention and care required to prepare sections by freeze drying was greater than that required for the frozen fixation method. This method of frozen fixation, due to its simplicity, could be easily adapted to many laboratories lacking some of the more expensive pieces of apparatus commonly used today. Further use of this method would be as an added research tool in studying freezing rates of turkey immersed in a liquid.

Fig. 21. Photomicrograph of a cross-section of muscular tissue taken from an unfrozen turkey and prepared by the method of Frozen Fixation. 400X.

SUMMARY

This study was designed to develop equipment which would determine the rate which heat is removed from turkeys held in a liquid coolant under varying conditions, and to develop a method for evaluating the influence of different freezing rates on structure of muscle tissues.

A solution of propylene glycol and water, which has a low freezing temperature, was pumped, at temperatures as low as -10° F., from an insulated tank through an insulated calorimeter and back into the tank. The temperature of the liquid entering and leaving the calorimeter was accurately measured and recorded at frequent intervals. The volume of liquid passing through the calorimeter was accurately measured, and the mean temperature of the experimental product in the calorimeter was determined before and after each treatment. With these data, the amount of heat (B.t.u.'s) removed from the experimental product was accurately determined.

The accuracy of the apparatus was determined by comparing the heat lost as calculated from the calorimetric data during cooling of four propylene glycol models, with the calculated theoretical heat lost during a known temperature change in the models. It was found that an average of 96.5 per cent of the theoretical heat loss from the four models was measured by this equipment.

To demonstrate the applications of this apparatus, heat transfer studies were conducted in which the amount of heat removed from packaged and unpackaged turkeys was measured when the liquid coolant flowed at a constant rate for a specific length of time. It was found that with

this apparatus, differences in the rate of heat transfer from packaged and unpackaged turkeys could be accurately calculated. After one hour in a calorimeter through which propylene glycol was flowing at the rate of .268 cubic feet per minute, unpackaged turkeys still contained from 54 to 61 per cent of the heat to be removed to lower the temperature of the bird to 0° F. When birds were packaged in evacuated Cryovac bags this percentage was increased to from 75 to 85 per cent.

Differences in the amount of heat removed from turkeys subjected to different flow rates of the liquid coolant were calculated from data obtained from the calorimetric apparatus. The flow rate of the liquid coolant was decreased from .268 cubic feet per minute to .159 cubic feet per minute and four unpackaged turkeys were cooled in the liquid at this reduced flow rate. It was found that residual heat increased from a range of 54 to 61 per cent at the high rate of flow to 61 to 75 per cent at the lower rate.

Since standard methods of fixing tissue were found to be ineffective for studying, by microscopic technique, ice crystal formation in frozen turkey muscles, two additional methods were investigated. A freeze drying method followed by vacuum infiltration was evaluated. Microscopic analysis of sections of turkey muscles obtained by this method indicated that it was effective in observing the effects of ice crystal formation; however, the procedure required a considerable amount of personal attention throughout.

Another method was elaborated and labeled "Frozen Fixation." In this method, the frozen turkey muscle tissues were removed from the bird in a freezer maintained at 0° F. and placed immediately in a fixative solution maintained at 0° F. for 24 hours prior to removal

from the freezer for staining, imbedding, and sectioning. Due to the observation of slides of tissue frozen at different rates and prepared by this method of frozen fixation, it was believed that this method was superior to the others tested in showing the effects of ice crystal formation. This method does not require elaborate laboratory equipment and therefore can be used in a wide variety of research situations. With the development of both these methods (calorimetric and histological) of studying freezing rates, further studies could be carried out in which both methods would be used simultaneously on the same bird.

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