A Freezing Apparatus

for Cold-Tolerance Tests of Conifers

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SUMMARY

An apparatus was designed to permit freezing tests of needles and other tissue parts from coniferous trees under controlled rates of change in temperature with time. The design is based on the principle of a balanced system of refrigeration demand and heat input to achieve a steady temperature. The apparatus permits the operator to decrease temperatures from 0 deg C to -40 C at rates of 1, 2, 3, or 4 deg C per hour, to hold temperatures at the desired freezing level from 1 to 24 hours, and to increase to 0 deg C at rates of 1, 2, 3, or 4 deg C per hour. Temperatures achieved within the sample tubes are accurate to within ±0.9 deg C of the expected temperature.

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FREEZING APPARATUS FOR COLD-TOLERANCE TESTS OF CONIFERS

William J. Wessel and Richard K. Hermann[1]

INTRODUCTION

Frost may kill or damage trees and the degree of damage is greatly influenced by the ability of the trees to withstand low temperatures. This ability, termed cold tolerance, is determined by a complex of ecological, physiological, and genetic factors. It varies both between and within species. Knowledge about the cold tolerance of forest trees is essential to good silvicultural practices. The trend toward intensive management places a premium on individual trees because fewer trees will be grown on an acre. At the same time, a greater investment will be made in each tree because of the need for superior seed or seedlings. If we are to breed superior strains, we must consider the genotypic attributes of cold tolerance, or risk extensive losses of seedlings in nurseries and plantations and repeated injuries to trees throughout the rotation. Information concerning cold tolerance may be obtained through observations in the field or through experimentation in the laboratory. The principal approach to the testing of cold tolerance in the laboratory is artificial freezing of plants or of some of their tissues. Because of a limited market, equipment designed for such freezing tests is seldom available commercially. This paper describes an apparatus, which can be built at fairly low cost, that permits freezing with controlled rates of temperature change.

REVIEW OF LITERATURE

Equipment for tests on freezing of woody species varies in principle and performance. Most of the equipment has similar basic components, however: a freezing chamber, a refrigerant, a cooling medium, and a system for the measurement of temperature.

The equipment of Glerum, Farrar, and McLure (2)[2] consisted of an insulated fibre drum with a dry ice add-subtract method of temperature control. In the procedure of Irving and Lanphear (5), sample containers of styrofoam were transferred to freezers at successively decreasing temperatures in steps of 5-6 deg C. Temperatures in containers were measured at 2 1/2-minute intervals. Thawing was in plastic containers at room temperature and high humidity. Eguchi <u>et al</u>. (1) also relied upon a procedure of successive decreases in temperature, with steps of 2 1/2 and 5 deg C. Thawing was in air at 0 deg C. White and Weiser (10) placed flasks with samples in a freezer and lowered temper-

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[2]Numbers in parentheses refer to similarly numbered references cited.

ature at an approximate rate, then removed flasks at successive temperatures of decreasing values. The flasks were thawed in progressively increasing steps in cold chambers. Freezing tests were made by Siminovitch et al. (9) with a commercial apparatus that had manual temperature adjustment and an accuracy of 0.84 deg C. Thawing took place in air, at +3 C for 18-24 hours. Salt and Kaku (7) worked with a thermoelectric cooler and two concentric glass vials to slow the freezing rate to 1-3 deg C per minute.

Havis's (4) studies were made in an agitated methanol bath with samples contained in tubes. With this bath, temperature could be controlled to within 0.56 deg C. The equipment of Siminovitch and Briggs (8) consisted of a commercial apparatus with a dry ice refrigerant, from which a blower passed cold air through a manually dampened duct to a test chamber that contained a heater. The heater and blower were activated separately or together through manual control of a thermoregulator and relay. Control of temperatures from 0 to -70 C was achieved within ±1 deg C. The temperature was decreased by manually setting the regulator to successive temperatures in steps of 1 deg C each. Thawing was at -1 C or in water at room temperature.

Greenham and Daday (3) also worked with an air-medium chamber, with blown air cooled over refrigerator coils and heated by an electric fan motor. A second fan provided mixing of the air. Control was achieved by a 24-hour cam that regulated the position of one of a pair of contact points. The other contact point followed the rotary motion of a thermostat stem. When the contacts were open, the refrigerator operated, decreasing the temperature and turning the thermostat contact toward the cam-controlled contact; as they touched, the refrigerator shut off. The cam was programmed for a holding period, decreasing temperature period, holding period, increasing temperature period, then a holding period at starting temperature. This gave a standard program for comparative trials. Parker (6) had a refrigerated chamber, into which he placed flasks containing ethylene glycol and samples in six test tubes. After preliminary low-temperature equilibrium was reached, the refrigerator thermostat was set to a lower temperature in successive steps when the preceding temperature was reached in the test tubes. Thawing was accomplished by warming a flask with contents to room temperature, which gave a rate of thawing that was "faster at first."

DESIGN OF FREEZING APPARATUS

Design of the apparatus described here is based on the principle of a balanced system of refrigeration and heat input to achieve a steady state of temperature. The design permits us to program the decrease of temperature from 0 to -40 C at rates of 1, 2, 3, or 4 deg C an hour; the maintenance of the pre-selected lowest temperature for any period less than 24 hours; and, the thawing to 0 deg C at rates of 1, 2, 3, or 4 deg C an hour.

The principal parts of the apparatus (Figure 1) are: a copper drum, 12 inches deep and 12 inches in diameter, encircled by refrigerator coils and centered within a 3- by 3- by 3-foot cabinet of 3/4-inch plywood, filled with styrofoam insulation; a 1/3-horsepower motor and compressor, hermetically sealed and air-cooled; a stirring assembly; a strip chart recorder; and a time clock.

Measurement of Temperature

Temperatures are recorded with a Brown Electronic strip chart recorder with a range from 0 to -50 C and chart speed of 30 inches an hour. A 24-gauge, copper-constantan thermocouple with a fused junction is the temperature sensor. The junction is located in air inside one of 24 test tubes, and the wire passes through a rubber stopper. From this point, it is shielded over its 6-foot length to the recorder. If chart paper printed for 0 to -50 C is unavailable, chart paper printed for 0 to +50 C is also acceptable. With this paper, 0 deg C is read as -50 C and +50 C is read as 0 deg C.

The millivolt span for each range was accounted for graphically to check validity of interchanging the paper. Negligible differences were found across the temperature scale. Calibration of the recorder and thermocouple, which was done by freezing mercury (freezing point at -38.9 C) and salt solutions (freezing points at -1, -3, and -5 C), showed the accuracy to be ±0.3 deg C. The same accuracy was obtained with the complete apparatus system.

Stirring Assembly and Bath

The problem of the viscosity of glycerin was avoided by substituting a 60 percent ethylene glycol solution (freezing point near -50 C). The cooling curve for 18 liters of this solution, with refrigerator on constant demand, and the "free" warming curve are shown in Figure 2. The minimum cooling rate for the 18-liter solution was 3 deg C per hour near -45 C. Because the maximum desired freezing rate is 4 deg C per hour, this rate must be attained at the lowest operating range. This was accomplished by reducing the volume of the bath to 9 liters. The cooling rates obtained were a maximum of 15 deg C per hour near -5 C, and 4 deg C per hour near -45 C.

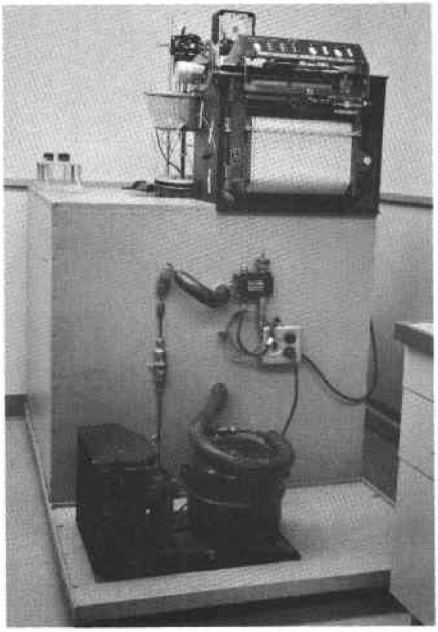

Figure 1. Complete freezing apparatus.

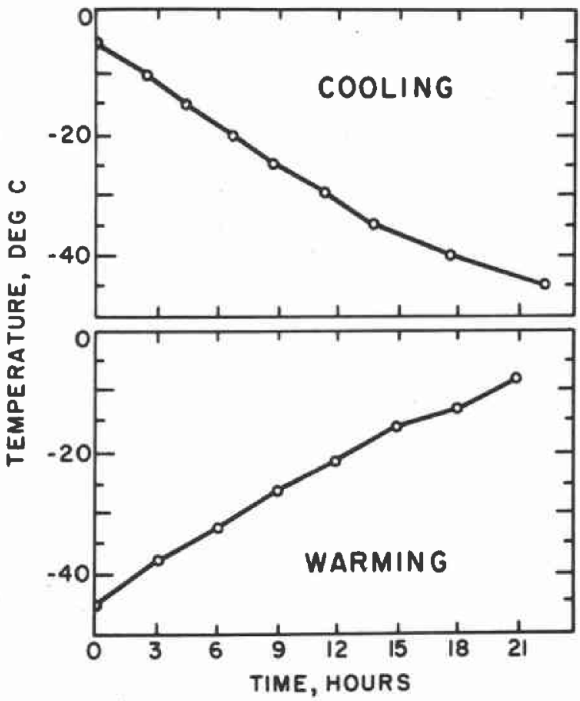

Figure 2. Representative curves for the bath.

Uniform temperature of the bath, as sensed in the test tubes, was achieved by a stirring assembly (Figures 3, 4, & Appendix). This assembly is mounted on the circular center section of a 4-inch-thick insulating cinder block, which fits into the 14- by 14-inch chamber. The weight of the assembly on its tapered sides provides a sufficient seal between the tapered faces. A small electric motor is mounted on the top of the assembly, which is made of composition board. A 1/4-inch extension shaft is sleeve-fitted to the motor. It extends through a 3/8-inch plastic (polyvinyl chloride) pipe, which reaches to the bottom of the drum. At this point, a 3 1/4-inch blade is bolted to the shaft. The blade circulates the solution downward over the walls of the drum (the system boundary) and upward onto and around the 24 Pyrex test tubes, 25 by 100 mm, for tissue samples. These tubes are sealed with rubber stoppers and placed in holes arranged in two concentric circles of 12 holes each in a 1/16-inch-thick, 7 1/2-inch diameter, aluminum tray. This tray also has an inner circle of smaller holes to allow upward circulation. A second aluminum disc, bolted at two points to the tray, has matching circulation holes. The tray assembly is threaded to the shaft housing. When in place, the entire tray assembly is immersed in the bath. Variation in temperature among test tubes will be discussed later (see accuracy of apparatus).

Regulation of Temperature

Regulation of the bath temperature, as sensed in an immersed test tube by the control thermocouple, is based on the principle that for a given point within the range from 0 to -50 C, a balance between cooling, by refrigeration, and heating will result in a constant temperature within the bath. The heat content of this 9-liter solution was calculated to be about 7,000 calories per degree centigrade. Cooling at a maximum rate of 15 deg per hour near -5 C, the refrigerator demands 29 calories per second. To balance this refrigeration demand, the maximum heat-input necessary is equivalent to 122 watts, or 8.13 watts per deg C.[3]

[3] The energy in calories required to raise the temperature of the solution 1 deg C would be the product of the density in grams per milliliter times the number of milliliters times the calories to raise the temperature of 1 gram by 1 deg C. For ethylene glycol in 9 liters of 60 per cent solution, this would be:

$$\left(\frac{1.1155 \text{ gm}}{\text{ml}}\right)\left(\frac{1{,}000 \text{ ml}}{\text{liter}}\right)(5.51 \text{ liters})\left(\frac{0.515 \text{ cal}}{\text{gm-deg C}}\right) = 3{,}140 \text{ cal/deg C}$$

For water in the 9 liters of solution:

$$\left(\frac{1.000 \text{ gm}}{\text{ml}}\right)\left(\frac{1{,}000 \text{ ml}}{\text{liter}}\right)(3.51 \text{ liters})\left(\frac{1.0122 \text{ cal}}{\text{gm-deg C}}\right) \; 3{,}540 \text{ cal/deg C}$$

For the entire 9 liters of solution, the sum is 6,680 cal/deg C or about 7,000 cal per deg C.

Cooling at 15 deg C per hour is equivalent to 0.00415 deg C per second, so (0.00415 deg C/sec)(7,000 cal/deg C) = 29 cal/sec.

Now, 1 calorie = 4.2 joules, so 29 cal/sec = (29)(4.2 joules/sec), which is 122 joules per sec, or 122 watts.

An immersion-type heating tape was placed in the bath around the inside of the drum. A variable autotransformer controlled the output of this heating tape (see list of parts, Appendix). From the maximum of 15 deg C per hour near -5 C, to the minimum of 4 deg C per hour near -45 C, the output of the heating element must change from 122 watts to about 33 watts, because 8.13 watts are needed for each change of 1 deg C. Expressed as voltages (based on tape resistance of 92 ohms), these values give a desired output from the autotransformer of 108 volts at -5 C and 54 volts at -45 C.[4] Figure 5 shows the refrigeration demand curve (expressed as rates, deg C/hr, determined by sampling segments of the cooling curve across the range) and the heater-output curve that results from matching the end-point voltages to the corresponding points on the temperature scale.[5] Fortunately, these curves are closely matched. The correctness of the method was verified by a satisfactory approximation of steady temperature in the bath with the values shown.

The need for a physical means of correlating the heater and autotransformer and the refrigerator with the actual temperature of the bath was satisfied by the travel of the pen carriage of the recorder. This pen became the linear equivalent of a rotating thermostat stem. To it was attached a T-bracket with two trippers mounted on it (Figure 6). The position of the tripper, and therefore, the temperature, was bounded by two microswitches on a carriage, one to control the refrigerator, the other to control the heater. At a given position on the scale, a temperature drifting upward (to the right, facing the recorder) causes the tripper to open the heater circuit, and the refrigerator reduces the temperature and returns the pen to position. When the pen crosses (right to left) the center point between the microswitches, the heater circuit closes and the refrigerator circuit opens. A similar operation occurs when temperature direction reverses. Any drift in temperature from imperfect matching of continuously operating cooling and heating systems is corrected by opening the circuit of the over-balancing element.

No physical connection exists between the trippers mounted on the pen carriage and the microswitch arms. The light resistance to pressure of the arms is negligible and does not affect the recording pen. An errant pen is free to exceed both microswitch arms, in either direction, without damage to any component.

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[4] Watts = (volts)(amperes), or $P = EI$,
and volts = (amperes)(ohms resistance), or $E = IR$,
so $P/R = I^2$.
Then, $122/92 = 1.33$, and $I = 1.17$ amperes.
Also, $33/92 = 0.35$, and $I = 0.59$ amperes.
When $I = 1.17$ and $R = 92$, $I = 108$ volts,
and when $I = 0.59$ and $R = 92$, $I = 54$ volts.

[5] The change from 108 volts to 54 volts, corresponding to the change from -5 C to -45 C, requires an arc of about 134° to be turned by the autotransformer brush, which has a linear relation between volts and degrees of arc. The voltage output through the arc, projected to a straight line of corresponding degrees centigrade, namely the temperature scale, results in the curve shown for the heater.

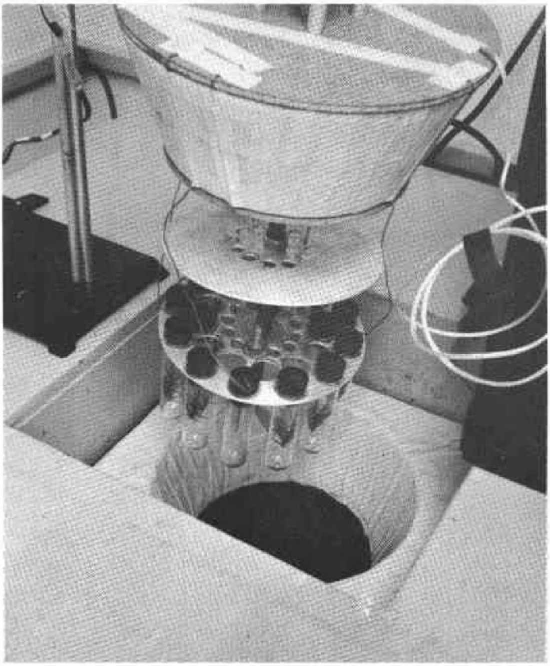

Figure 3. Stirring assembly.

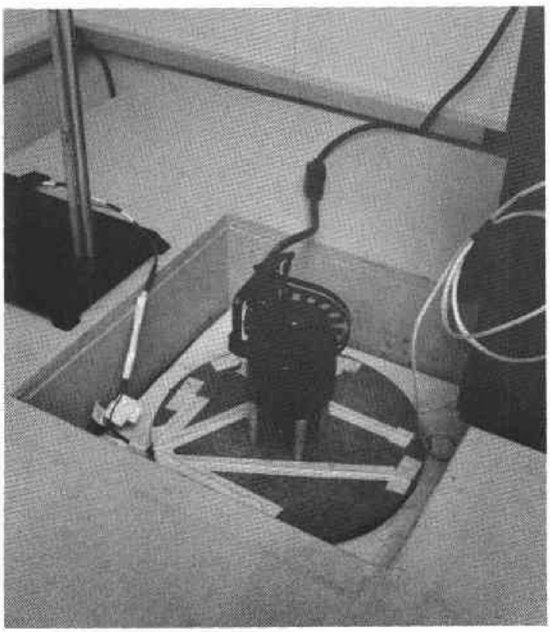

Figure 4. Stirring assembly in place.

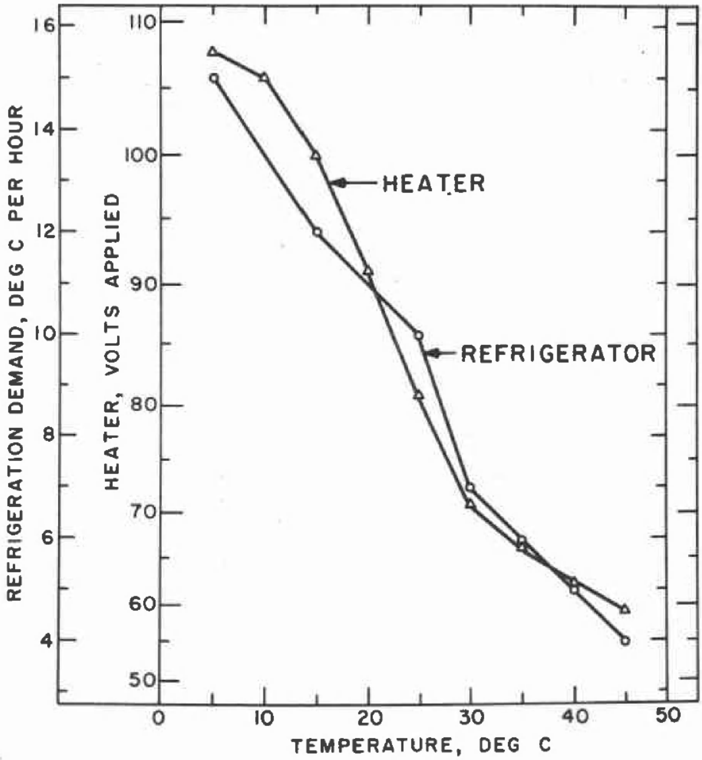

Figure 5. Matched heater voltage and refrigeration demand as a function of bath temperature. The theoretical relation between the values is shown by the scales on the left; the curves represent actual measurements.

The microswitch carriage is geared to a helically cut shaft, and it slides on a guide bar. These components are attached to supporting brackets beyond each end of the chart paper, which allows full-scale travel of the center point of the microswitch carriage. The geared shaft is driven by a motor and gear assembly (called the rate-control assembly) mounted at the left side of the recorder (Figure 7 & Appendix). When the shaft rotates, the microswitch carriage slides across the guide bar. By placing the appropriate gear on the extension of the drive shaft, the speed of the carriage across the temperature scale can be varied at 1, 2, 3, and 4 deg C per hour. The pen carriage "follows" the microswitch carriage, staying within the bounds of the two microswitches, except for a minimal over-travel. The oscillating deviation of the temperature from the linear rate will be discussed later.

The versatility of temperature control is achieved by the sequential operation of the reversible rate-control motor and a time clock, both of which are controlled by limit switches (Figure 8). The first step entails setting the microswitch carriage at -1.5 C, which is the upper temperature limit of the system, as defined by the upper limit switch attached to the recorder at the right edge of the chart paper. With a freezing rate selected and the proper gear in place, the rate-control motor drives the carriage across the chart until it reaches the lower limit switch. This switch slides on the guide bar and is secured by holding notches at each degree from -5 C to -45 C. The microswitch carriage trips the lower limit switch at the temperature set previously and thus turns the rate-control motor off and switches to the time clock, which has been set to a pre-selected period up to 24 hours for holding the temperature. When this period has elapsed, a specially installed microswitch in the time clock is tripped and thus turns off the time clock and switches to the reversed direction of the rate-control motor. The microswitch carriage then is driven back across the scale until the upper limit switch is tripped and the rate-control system is turned off. The rate-control system has a power switch and directional indicator lights on the control panel.

Two additional circuits are present in the apparatus (Figure 8). Each has a power switch and indicator light on the control panel. The refrigerator circuit is a simple "off-on" system, controlled by the carriage microswitch. The heater and autotransformer circuit is slightly more complex. The direct correlation of the autotransformer to the temperature scale and refrigerator is achieved by a rigid arm connected to the microswitch carriage assembly (Figures 6 and 9). This arm moves across the scale with the carriage. As it moves, the free end slides in a slotted arm that is attached to the autotransformer control shaft. In this way, the linear motion of the pen carriage is translated to the rotary motion of the autotransformer brush, as previously described.

Accuracy

The rate-control assembly was tested for accuracy by running the mechanism across the full scale. The teeth on the gears for changes of 2 deg C and 4 deg C are whole-number multiples of the number on the gear for 1 deg C, so accuracy of one means accuracy for the others. Error, proportional to the number of revolutions, is more obvious in a longer trial. In a 45-hour trial of the gear for 1 deg C, a change of 44.8 deg C was produced rather than the expected change of 45 deg C. The difference is considered negligible. The gear for 4 deg C produced a change of 42 deg C in 10 1/2 hours, for an average of 4 deg C per hour. The gears for 1, 2, and 4 deg C are considered to be error free. The gear for 3 deg C produced an average rate of 2.97 deg C per hour over a 14-hour period. It is also considered error free.

The oscillating deviation of the temperature from the linear rate of position change of the microswitch carriage was determined for increasing and decreasing temperatures at 1 deg C and 4 deg C per hour. This variation was established by the distance between the microswitches. It was a consistently oscillating motion, which alternated high and low around the theoretical linear trace. Maximal variation occurred for the slower rate when temperature was decreased. The faster rate varied most as temperature increased. Variations from held temperatures

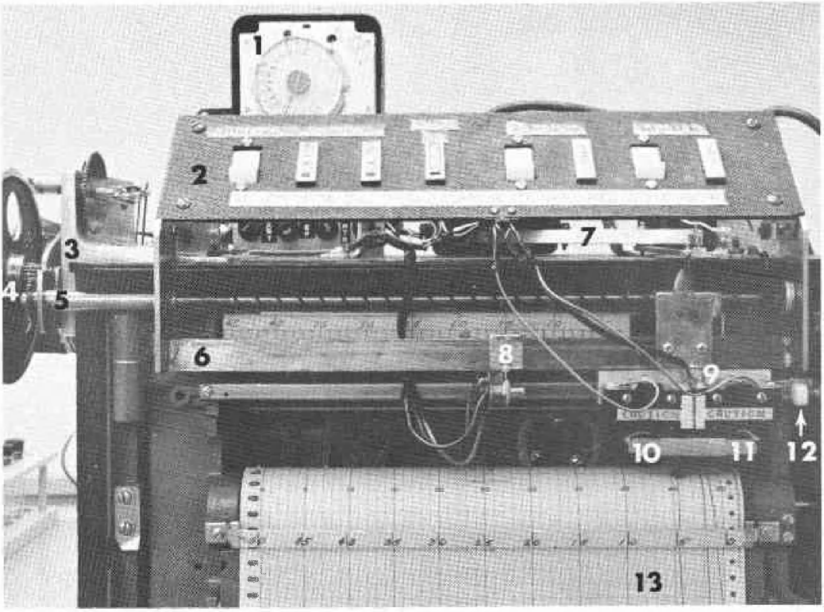

Figure 6. Recorder and temperature control apparatus:

1. Time clock;
2. Control panel;
3. Gear storage;
4. Rate-control gear train;
5. Microswitch carriage drive shaft;
6. Guidebar;
7. Slotted arm connecting autotransformer to microswitch carriage assembly;
8. Lower limit switch;
9. Microswitch carriage assembly;
10. Refrigerator microswitch tripper;
11. Heater microswitch tripper;
12. Upper limit switch;
13. Recorder.

at various points across the scale were also determined. Variations were always greatest at temperatures below -40 C, where displacement from ambient temperature is greatest. For this reason, the apparatus is best for temperatures not less than -40 C. Maximal variation with a decreasing temperature was ±0.4 deg C. Maximal variation with an increasing temperature was ±0.6 deg C. The maximal variation in holding temperature was ±0.3 deg C. To these figures must be added the accuracy of ±0.3 deg C determined by calibration of the recorder. The overall statement of accuracy is then ±0.9 deg C. Freezing and thawing rates are as selected, with achieved temperature at any given point within ±0.9 deg C of the expected temperature.

The reproducibility of these conditions became evident over the period of development of the apparatus. Various changes in switches and

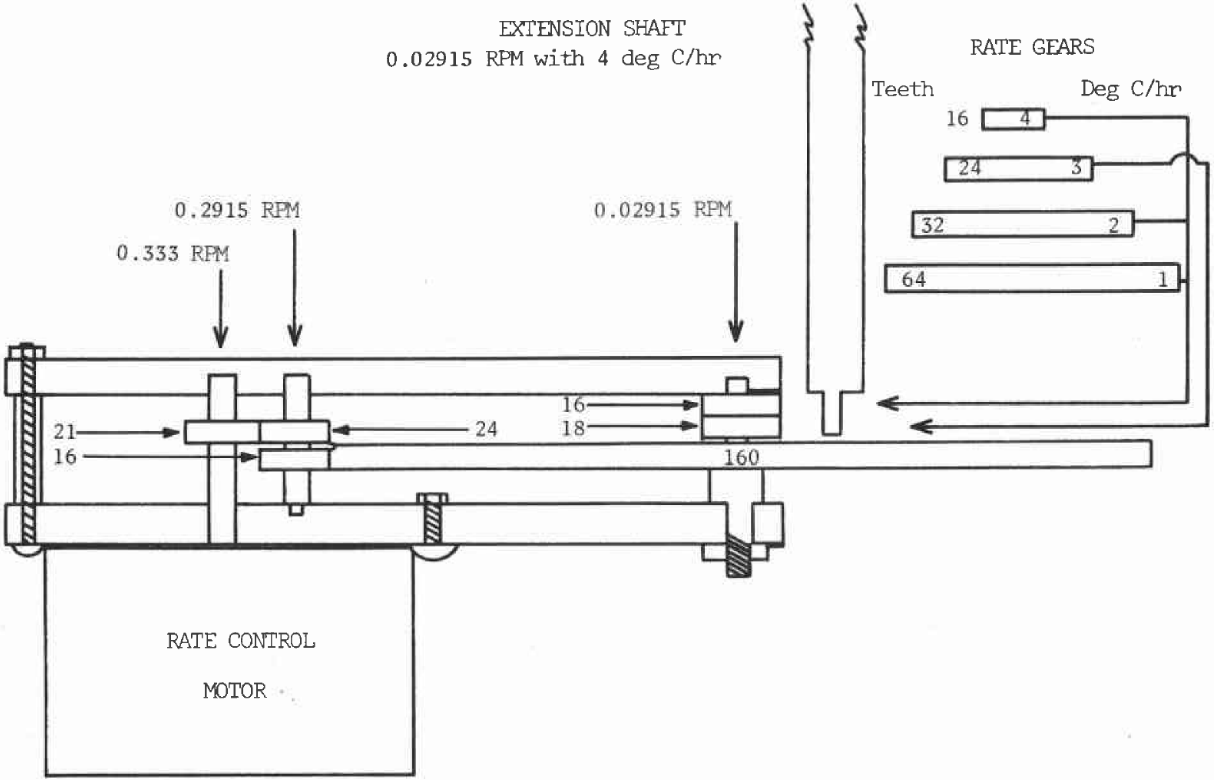

Figure 7. Diagram of rate control assembly.

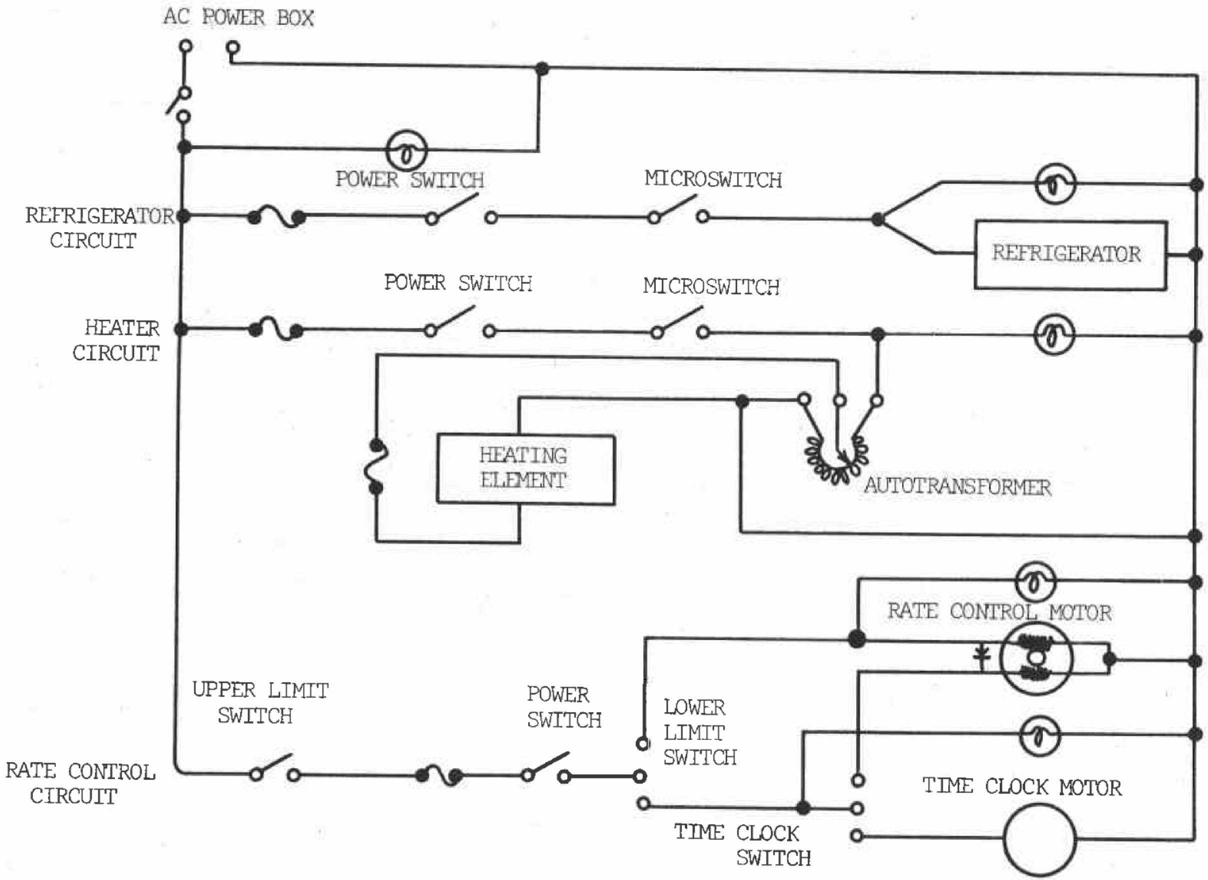

Figure 8. Diagram of circuit. Metal components are grounded to safety line.

Figure 9. Temperature-control apparatus:

1. Time clock;
2. AC power box with system AC switch;
3. Refrigerator-heater plug-in box;
4. Variable autotransformer;
5. Slotted arm connecting autotransformer to microswitch carriage assembly;
6. Control panel;
7. Fuse panel;
8. Sliding adjustment for gear accommodation;
9. Mounting block;
10. Assembly pivot;
11. Rate-control assembly.

autotransformer settings were the causes for remeasuring variation. At no time were values greater than those stated for the apparatus in final form.

Variation between test tubes was determined by randomly selecting four tubes for thermocouple insertion. An automatic switch assembly was used to convert the recorder to a twelve-point recorder. Temperature of each tube was recorded at three consecutive positions, for a total of about 30 seconds. Work with the apparatus indicated that maximum differences could be expected at 4 deg C per hour. Variation among tubes was less than ±0.2 C within and between the two circles of tubes with decreasing and holding temperatures. With increasing temperature, the outer circle proved to be of higher temperature only while temperature changed and only below -40 C. This variation is acceptable.

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APPENDIX

List of Parts

A. Heating tape, silicone rubber embedded, 115V, 144W max. (Briscoe Manufacturing Company, Columbus, Ohio, #BS 6 1/2)
B. Variable autotransformer, 120/132V, 1.75Amp. (Staco Incorporated, Dayton, Ohio, 175BU)
C. Synchronous reversible motor, 1/3 RPM, 5W (Hurst motor, Allied Electronics, #41#7154C)
D. Miscellaneous lights and switches.
E. Time clock with microswitch.
F. Gears as noted (specialized to individual need)

Components

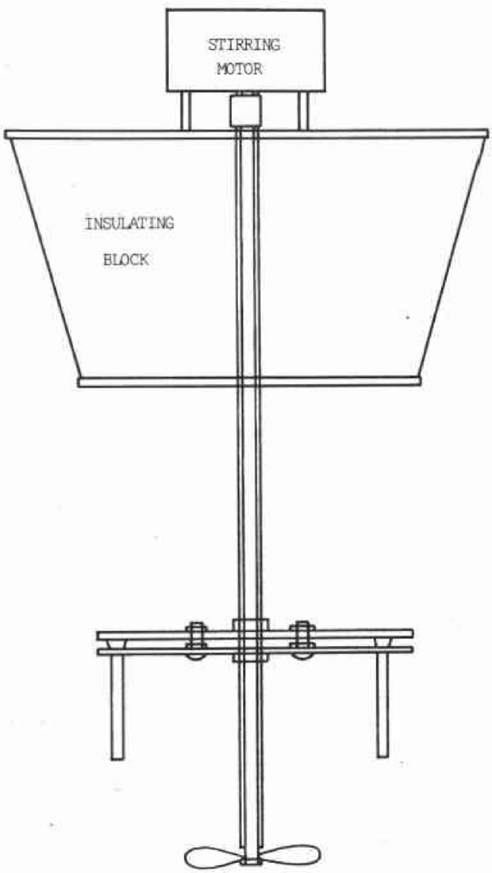

Figure 10. Diagram of stirring assembly.

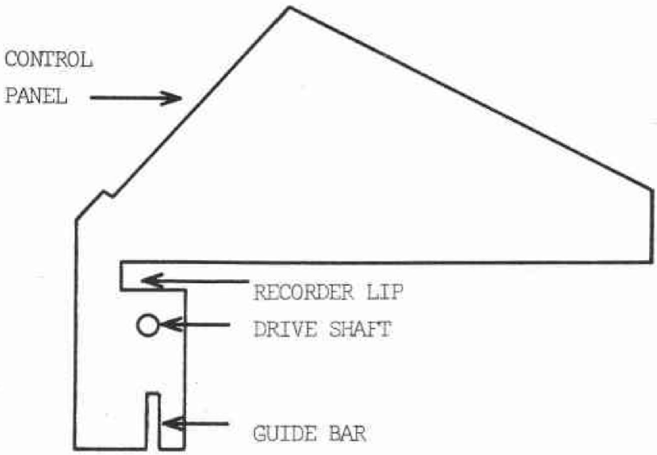

Figure 11. Drive shaft and guide bar support.

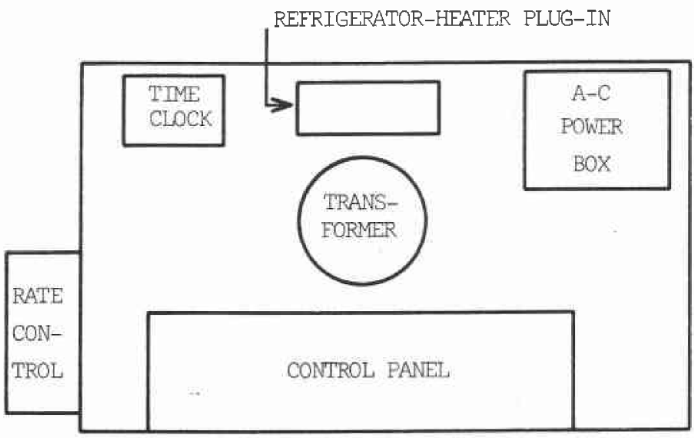

Figure 12. Control apparatus, top view.