LETHAL INTERNAL TEMPERATURES FOR THE CHICKEN, FROM THE FERTILE EGG TO THE MATURE BIRD

By

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INTRODUCTION

Vertebrate animals are divided into two classes in regard to the relation of their body temperature to their environment. The "cold-blooded" or poikilothermic animals are those whose body temperature varies with that of the environment, and the "warm-blooded" or homoiothermic animals are those whose body temperature is largely independent of the environment. Mammals and birds are classified as homoiothermic, since they possess an elaborate heat-regulating mechanism which enables them to maintain, within normal temperature ranges and conditions, a relatively constant body temperature. Some homiothermic mammals hibernate, and during this period when they are no longer able to compete with the cold, they are classified as poikilothermic. The chicken has at times also been classified in this two-stage manner, since at various points in its early development it exhibits characters of a poikilothermic nature. These characteristics were noted as the temperature of the domestic fowl was observed and studied over a long period of time.

Interest in the body temperature of the chicken probably developed some time after man began to domesticate this animal, with a resulting attempt to induce artificial incubation. No doubt, when it was observed that the average body temperature of birds is quite a bit higher than that of other vertebrates, especially mammals, the study of birds' body temperature and its regulation was stimulated. As interests in the domestic fowl grew, the role of various factors concerned with natural and artificial incubation was investigated along with their influences on the chicks produced. Among the factors first studied, temperature during the incubation period was of prime interest.

Investigations were made on the physiological aspects of the chicken both as biological material and as a source of food. Among these developments, interests centered on the influences of low and high temperatures on embryonic development. The ability of the organism to withstand a deviation from its normal or optimum environmental temperature was investigated from many different viewpoints. The storage temperatures as well as incubation temperatures of the chicken eggs were found to be very important, while the effects of variations in environmental temperature on the growth and performance of the chick and adult bird were also recognized.

In the study of lethal body temperatures, it should be understood that the notion of lethal temperature depends on the idea that one has of the mechanism of death. Therefore, this point is susceptible to many interpretations (Luyet and Gehenio, 1940). There is no possible definition of the death point or of the survival point. When animals are exposed to cold, there is no specific body temperature above which life persists and below which death always occurs (Jackson and Alonge, 1934). In the work reported in the following pages, an attempt has been made to eliminate as many variables as possible. All possible efforts have been made to reach a lethal temperature, which is not to be interpreted as a definite point, but as a range of temperatures in which death predominates.

REVIEW OF LITERATURE

Since the potentiometer, employed in the work to be reported, recorded temperatures in degrees Fahrenheit, all temperatures are expressed in this manner. In order to facilitate a clear understanding of the work reported in degrees Centigrade by other authors, temperatures have been converted to degrees Fahrenheit and stated to the nearest degree.

The minimum temperature for embryonic development in the domestic fowl was concluded by Edwards (1902) to be 68.9°, and by Funk and Biellier (1944) and Kaufman (1948) as 80°. Needham (1931) quotes the work of Koestne which shows the minimum temperature for embryonic development to be 82° with an optimum temperature of 95° to 102°. The maximum temperature at which development will take place was stated to be 109°. Dareste (1877), Alsop (1918-1919), and Kaufman (1948) reported abnormal embryo development from unusually high or low temperatures. However, the minimum temperature to which the egg can be exposed and still be induced to maintain an embryo is of further interest.

Colasanti (1875) studied the effects of low temperatures on hatchability of the hen's egg by immersing eggs in an ice bath ranging in temperature from 25° to 14° for one to two hours. When removed from the bath, the eggs were reported to be practically frozen through. However, these eggs showed apparently normal embryonic development after eight days of incubation as compared with control lots of eggs which had not been treated in the ice bath.

Raboud (1899), put 30 sets of 18 eggs each in an ice-salt mixture at -0.4° for a half an hour, after which time most of the shells were cracked. Some of the frozen eggs were incubated immediately at 100°, a second lot was kept in a cool chamber and incubated after one day, and a third lot was kept for three days and then incubated. After three days of incubation, all eggs were opened. About one-third of the eggs in each of the different series contained embryos, all of which were found to be abnormal. The author reported an apparent proliferation of cells without distinct differentiation in the remaining two-thirds of the eggs. The rate at which the eggs thawed did not seem to have any effect on embryonic development. Grodzinski (1933, 1934) also observed that eggs exposed to a temperature of 27° for four days previous to incubation developed germinal discs which lacked embryos or blood vessels.

Mancini (1908) obtained results which were somewhat similar to those obtained by Colasanti (1875). He immersed eggs in a refrigerating mixture varying between 25° and 14° for a period of two hours. The fact that the contents of the egg were found to be transformed into a solid mass did not injure the vitality of the germ nor its power to develop. He concluded that the germ while dormant is less sensitive than after development began.

No detrimental effect of low temperature on hatching eggs could be found by Elford (1921) who exposed eggs in cases to temperatures which varied from 14° to 20° for periods of fifteen minutes to five hours. Mauro (1923) reported no appreciable effects on hatching power when eggs were held at 33° for 24 hours. However, when the eggs were held by the same worker for 48 hours at 33° the capacity of the embryo

to develop was considerably reduced, while after 72 hours it was entirely destroyed. Lowering the temperature of fertile eggs to 32° for six hours (Mussehl and Bancroft, 1924-1925), did not lower their hatching power or result in an unusual number of crippled or otherwise abnormal chicks. No detrimental effect on hatchability could be found by Dougherty (1926-1927) who exposed hatching eggs in cases to temperatures of 28° to 32° for one to three successive nightly periods of 14 hours each, plus a continuous period of 38 hours. Exposure to the same temperatures for four successive nightly periods plus a continuous period of 38 hours however did result in a reduction in the percent of eggs that hatched. A holding time of seven days at 32° was used by Phillips (1945) with no apparent effect upon hatchability or observed fertility. Scott (1933) reported no reduction in hatchability when eggs were held up to six days at a slightly higher temperature of 36°. It was found by Funk (1947) that holding eggs between 32° and 38° for 48 hours was not harmful to hatchability, but that holding for 96 hours or longer at this temperature depressed the hatching power until a zero point of hatchability was reached at 196 hours. Hatching eggs were stored for two to four days and six to eight days prior to incubation at temperatures of 30°, 40°, 50°, 60°, and 70° by Olsen and Haynes (1948). The highest hatchability (78.6%) was obtained after storage for six to eight days at 50°, and the lowest (2.2%) in eggs stored for the same length of time at 30°.

Moran (1925) investigated the effect of room temperatures between 24° and 61° on the fertile egg of the fowl. He reported that below a 32° internal temperature the eggs quickly lost their fertility; in

fact, he concluded that the germination power is probably destroyed immediately at internal temperatures of approximately 21° to 19°. At higher temperatures the point at which death occurs was found to take an increasingly longer time. Some eggs were still capable of developing after having been at internal temperatures of 24° for 47 hours and 27° for 118 hours. The limit of time that eggs could be stored at a temperature of 33° was 10 days and at 51°, 34 days. Jull, McCartney, and El-Ibiary (1948) reported that subjecting chicken hatching eggs in cases to -1° for as long as 10 hours, with the internal temperature reaching approximately 30.2° did not seriously impair hatchability. Chicken eggs whose internal temperature was approximately 3.2°, were reported to have produced some chicks.

Thus far the work reviewed has dealt only with eggs treated before incubation. The next problem is to study the results of cooling eggs to very low temperatures during the incubation period. Chick embryos from virile stock were reported by Lamson and Kirkpatrick (1918) to be able to stand four to five hours exposure at a temperature of 50°. They found that after the first 24 hours of incubation, and from this point to the tenth to twelfth day of incubation, the time could be increased up to fifteen hours. After the seventeenth day, continued exposure to 50° for more than six hours caused death to the embryo before the normal hatching time. Moran (1925) made the general observation from his results that incubated eggs were less resistant than fresh eggs to low temperature storage. Parker (1929) reported that chilling failed to alter markedly the course of yolk absorption of the embryo. Eggs which had been incubated from nine to 14 hours were found by Grodzinski (1934) to have resulted in a considerable number

of dead and defective embryos as a result of being subjected to 27° for 30 to 120 hours. He noted further, however, that there were also some normal embryos after this exposure. Kaufman (1934) reported normal hatches from incubated eggs that had been exposed for 24 hours to a temperature of 54°. The critical minimum temperature was reported by this worker to rise directly with advancing age of the embryo to the 18th day. Romanoff (1939) studied the specific effect of short exposures to extreme temperatures on the development of the chick embryo. Various lots of eggs were exposed to 106° or 84° at 0, 4, 8, 12, and 16 days of incubation for 24 hours. He concluded from the data obtained that the effect of temperature is greatest in the early stages, the growth of the embryo being accelerated by the high temperatures and retarded by the low temperatures. With the advancement of incubation, Romanoff (1939) noted that the temperature effects are lessened, and from about the tenth day, both high and low temperatures slightly inhibited the development. The susceptibility of the embryos, as shown by total mortality, was greatest from the exposure to high temperature during the early periods and greatest from the exposure to low temperature during the latter periods of incubation.

The effects of temperatures above the normal incubation range on hatching eggs also provides interesting results. The range in temperatures which fertile eggs and developing embryos can withstand is not as wide at temperatures above the normal as that range which has been shown for the low temperatures. It appears that this upper range has not been given as much attention as have the temperatures in the lower range. This lack of attention may be explained further by

the fact that high temperatures are not as frequently a problem before and during incubation as are low temperatures. Pritsker (1940) as quoted by Landauer (1948) reported a favorable effect on hatchability as a result of preheating eggs at 118° for 30 to 60 minutes before the beginning of regular incubation. Temperatures at the level of the blastoderm were recorded as high as 112°.

Devitalizing of the germ in fertile eggs was carried out by Funk (1943) by immersion in water for 35 minutes at 120°, for 15 minutes at 130°, for 10 minutes at 138°, and for 5 minutes at 140°.

The fact that the chick exists in a poikilothermic state during a portion of its period of embryonic development is generally accepted (Pembrey, Gordon, and Warren, 1895; Romanoff, 1939), but the time at which the chick commences to react as a homoiothermic animal has not been clearly defined. It has been stated by Pembrey, Gordon, and Warren (1895) and Edwards (1902), that the developing chick during the greater part of the incubation period responds to changes in external temperature in a similar manner to that of a cold-blooded animal, and commences to assume its homoiothermic state at the time of hatch. Romanoff (1939 and 1941), on the other hand, stated that the chick behaves as a cold-blooded animal only until the tenth day of incubation. In a review of low temperature studies, Luyet and Gehenio (1940) observed that embryos of birds were always more resistant than the adults. Randall (1943) found that after hatching, the body temperature of the chick increases from a temperature identical to that of its environment (incubator at 100° to 102°) to about 106° ten days after hatching, after which time it approaches and remains within the limits of the diurnal variation of the adult. A pronounced rise

in the body temperature of the chick during the first week after hatching, particularly during the first four days, was noted by Lamoreux and Hutt (1939), while Card (1921) found morning and mid-day body temperatures of the chick tended to fall through a five-day period and evening temperatures tended to rise. Glazener and Phillips (1949) noted that at ten days of age the chick's body temperature was no longer influenced by a 30 minute exposure to an environmental temperature of 38°. It was demonstrated by Moreng and Phillips (1950) that the poikilothermic characteristic of the chick at hatching time enables it to withstand severe chilling for short periods.

The body temperature of the average normal chicken has been recorded by Fronda (1921 and 1925) as 106.7° with a variation from 104.6° to 109.4°, and an individual diurnal variation of 1.6° to 4.8°. The highest point of the temperature curve was observed by Heywang (1938) to be reached during the hottest part of the day, which was 12 Noon to 4 P. M.; however, body temperatures were the lowest at 2 A. M., while the coolest part of the day was reached at 6 A. M.

Randall (1943) noted that in the chick, shivering ceases at approximately 68° and breathing stops at 59°. By suspending birds in a water bath at 43° to 53°, Sturkie (1946) studied the tolerance of the adult chicken to hypothermia. The lethal body temperature of hens ranged from 73° to 75°, while that of males ranged from 67° to 72°.

Dramatic effects of high temperatures on the hen have been described when the rectal temperature reached 113°. Yeates, Lee, and Hines (1941) and Lee, Robinson, Yeates, and Scott (1945) state that the bird shows marked distress through deep sighing respirations and symptoms which correspond to true heat-stroke in man, all of which

represent a failure of the central nervous system in the period shortly before death. It was observed in one chicken by Randall and Hiestand (1939) in a study of panting and temperature regulation, that when the bird's body temperature was raised to 117° and maintained for a brief time, the bird died.

The preceding review has demonstrated the interest shown in the effects of extreme environmental temperatures on fertile eggs, developing embryos, and the adult chicken. However, in most cases, there has been little indication as to the lethal temperature of the subject when time is not a factor. These studies of the effect of low temperatures on eggs can be criticized in that there were very few data collected on the internal temperature of the egg during exposure which is the most important temperature. The study herein reported is an attempt to determine the high and low lethal internal temperature, eliminating as much as possible the effect of time. In this study, an investigation has been made on the lethal internal temperatures of the fertile egg, for the embryo during development, and for the chicken from day of hatch to maturity. It is felt that a continuous study of this type will also supply valuable data in determining at what point of development the chicken changes from a partially poikilothermic animal to a true homoiothermic animal.

MATERIALS AND PROCEDURE

The experiment consisted of a series of studies on approximately 920 chickens ranging in age from day of hatch to maturity and 3,000 eggs in various stages of incubation. The procedure was designed to carry out the purpose as stated in the preceding paragraph. All birds and eggs used were from the Maryland Experiment Station strain of New Hampshire chickens. Birds chosen for study were battery-grown stock fed the station broiler mash and were selected as being normal representatives of their particular age group. Fresh (not over 7 days of age), sound hatching eggs were selected on the basis of shape and weight so that they were in a 24 to 25 ounce classification. It was felt that uniform size eggs would result in more uniform changes in temperature. All eggs were incubated in a Jamesway forced-draft incubator.

Temperature of birds and eggs was measured in degrees Fahrenheit by means of copper-constantan No. 25 gauge wire thermocouples, which were attached to a recording potentiometer made by the Brown Instrument Company. Thermocouples were made and calibrated following, in part, the techniques described by Warner and Estabrook (1930) and Tuttle and Janney (1948). This was done by twisting the two wires together after their ends had been thoroughly cleaned of all insulation and soldering them securely so that a dew-drop point was formed. It was found that this blunt type of a point would not puncture the internal organs of the birds. Care was taken that the wires did not cross at any other point. All wires were silk insulated; any fraying of the insulation

was repaired by the use of lacquer and tape. Lacquer was also used to protect insulation two inches back of the junction, to insure that the two metals were only in contact at the one point. The apparatus was mounted on a movable table so that it could be rolled to the desired place of work. A special terminal switch box connected with the potentiometer made it possible to record temperatures from twelve different thermocouples by merely switching from one to the other. The recorder used may be described as a high speed, multiple point instrument which operates on a system of continuous balance. The minimum printing cycle is one second, which is attained when consecutive temperature records are not further apart than approximately 20 percent of full scale. The manufacturer's statement of accuracy of this instrument is $\pm$ 1°. The instrument was checked and calibrated at frequent intervals by comparing it with a standard laboratory thermometer. This was done by wrapping a thermocouple around the thermometer and placing them both in an ice bath. At times they would read exactly the same, although occasionally, the recorder would be about a degree too low or too high but never greater, when the variation of the instrument was checked.

In order to determine the low and high lethal internal temperatures for chickens of various ages and eggs from zero to 21 days of incubation, a procedure was designed which would best use facilities available at the poultry department. Three different cooling compartments were available, 55°, 36°, 32°, and -10°. To eliminate the time factor as much as possible from these experiments, it was decided to use the walk-in type deep-freeze unit at -10° and obtain maximum cooling in all studies. Preliminary tests in other chambers

showed that this choice was the most desirable, since a rapid rate of cooling could be obtained. It was felt that this air temperature would not be too low to induce any great degree of shock to the birds or the embryos as may be induced if lower temperatures, or more rapid rates of cooling attained by the use of a water bath. The object was to keep the birds in as natural condition as possible in order to keep any effects of shock at a minimum.

In the first portion of this experiment the lethal body temperatures for chickens from day of hatch to maturity was determined by exposing the birds to an ambient temperature of -10°. Chicks were placed in wire pedigree baskets so that each chick was contained in a separate compartment thus huddling was eliminated and maximum normal radiation obtained. From 12 to 24 chicks of the same age group were employed in each test, with the body temperatures of half of these chicks recorded. The thermocouple was inserted to a depth of one inch into the body cavity by passing it through the naval of the young birds. This proved to be an easy source of entry to the body cavity and could be re-opened by puncturing with a pair of forceps. In older birds, entry was obtained by surgical methods and sutured closed with metal wound clips. Thermocouples were usually inserted in young birds, after results of exposure had caused the birds to become inactive, thus making it easier to keep the thermocouples in place. Individual thermocouples were checked frequently to assure that the movements of the bird had not worked a thermocouple out from the desired depth in the body cavity.

Chicks up to ten days of age were placed in the wire pedigree baskets and the basket was kept in its normal horizontal position

during exposure; however, for older birds the basket was placed in a vertical position on its side which availed more head room for the bird in a compact but reasonably comfortable appearing cage. This type of cage kept the action of the chick to minimum without actually restraining the bird to a great extent. Adult chickens were kept in larger cages where free movement of the bird was possible. The potentiometer was rolled into position in front of the deep freeze and the thermocouple wires run into the chamber through a hole which had been previously drilled in the wall, as may be seen in Plate 1.

The body temperature of each individual was checked periodically as it fell to the range in which preliminary tests had indicated lethal to the bird. When this area was reached, the temperature of the birds was recorded every five minutes and a close check kept on their physical condition. The birds were then removed and put in a forced-draft incubator at 99° to re-warm and recover if possible. After approximately one hour of re-warming, the birds were removed and checked for signs of life, and their sex and body weight recorded. Since a definite death point could not be expected for all birds, it was felt that when approximately 50 percent of the birds failed to recover, the body temperature at which they had been removed from the cooler was lethal.

The length of exposure to the -10° environment was based upon preliminary trials, lethal temperature for other age groups, and by the use of a two-phase trial. In the first phase, the birds were held until their temperature reached what was considered to be the lower limit of possible recovery. The second phase was based largely on the results of the first phase and was run the same day on birds from the

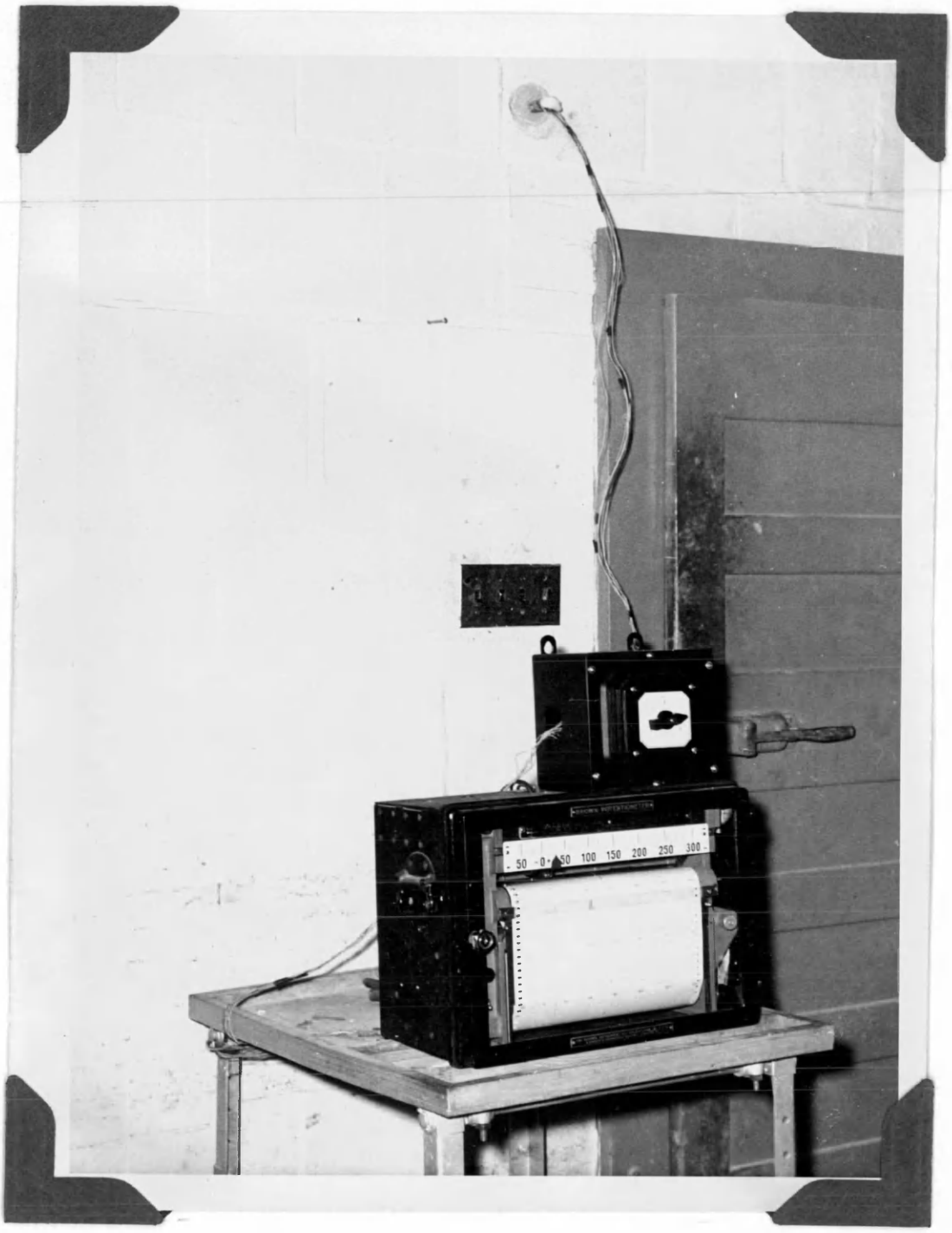

Plate 1. - Recording potentiometer on movable mount, in position in front of deep-freeze locker. Thermocouple wires may be seen entering hole in wall, after which connections were made with eggs or chickens.

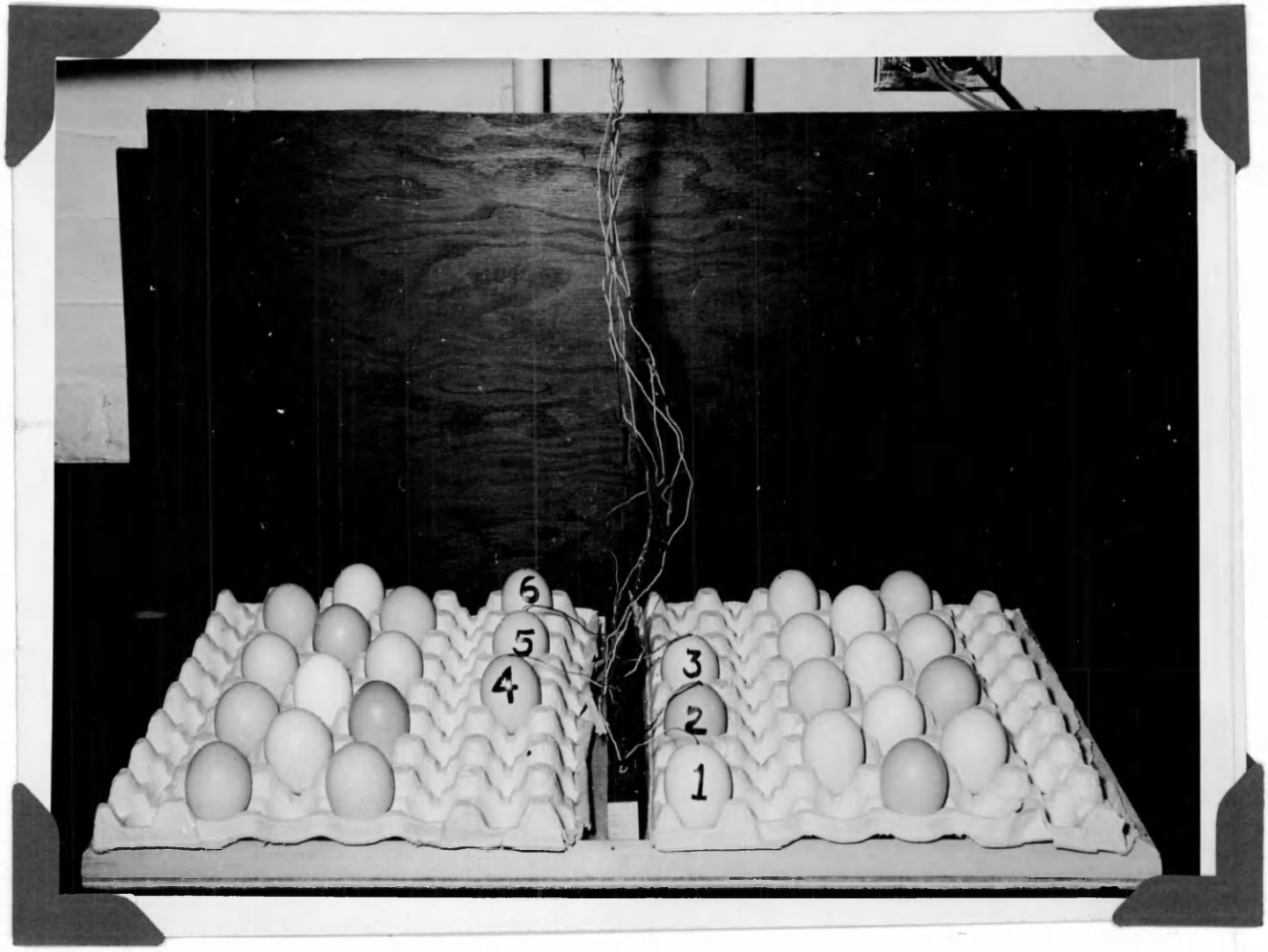

Plate 2. - Arrangement of eggs used in the study of low lethal internal egg temperatures for the embryo. Numbered eggs contain thermocouples.

same pen as those in the first phase. In this second portion of the trial, a more exact time of removal was possible. If the first phase resulted in the desired percentage of live birds, the second phase was carried out to obtain further conclusive evidence. The whole method of determining the lethal temperatures was dependant upon a series of trials which employed a bracketing technique.

At various points throughout the series, special tests were made as check points on the method employed in determining the time of death. One man was stationed in the deep freezer with a speaking tube to the outside, where a second man was posted at the potentiometer. When a bird died, notation was immediately made to the operator on the outside who recorded time and body temperature. A definite muscle spasm accompanied by a few deep gasps for breath usually were clear indications of death. Temperatures recorded by this method were in most cases the same, or a few degrees below those found when recovery was obtained. In addition, a number of these birds were removed and re-warmed to verify that their lethal temperature was reached. A stethoscope was used to check heart beat and time of death. This correlation of physical appearances with the heart beat served as a further indication that the bird was dead.

Records of low lethal body temperature as previously described were made on chicks daily from day of hatch (denoted as 0 days) to 21 days of age. After this three-week period, recordings were made on various numbers of birds at various ages as follows: 12 birds, 4 weeks old; 2 birds, 5 weeks old; 12 birds, 6 weeks old; 2 birds, 10 weeks old; 2 birds, 12 weeks old; 4 birds, 16 weeks old; and three mature hens. Cases where two birds were used served mostly as intermittent check

points in the series. It was found necessary to clip a large portion of the body feathers on the older chickens and the mature hens to increase their rate of heat loss, since their heavy insulation of feathers enabled them to withstand the cold environment for a number of days without any appreciable change in body temperature.

The study of low lethal temperatures for the developing embryo was carried out by exposing the eggs to an environmental temperature of -10°. Data were collected on eggs from zero to 20 days of incubation. Eggs were chosen for uniformity as previously described. An attempt was made to set eggs at approximately the same hour each setting day, and to coordinate their exposure so that the incubation time would be in a whole number of days. In order to assure a rapid rate of heat loss, an arrangement was used as shown in Plate 2, employing two egg flats mounted next to each other on a wooden tray, so that each egg was one space from the next. Thirty eggs were used for each trial. Six of these eggs had thermocouples inserted to record internal temperature. It should be noted that these eggs were used only for recording temperature since the insertion of the thermocouple caused physical damage to the egg which would soon result in the death of the embryo. These six eggs may be seen in Plate 2 and are denoted by their numbers. It was felt that their average temperature represented the average temperature of the remaining twenty-four eggs. Thermocouples were inserted through a small hole bored in the large end of the egg, so that the point at which the temperature was measured was at the center of the egg.

Although some other workers exposed eggs to temperatures as low as those studied herein, it is felt that they omitted important

information in their failure to record internal temperatures of the eggs. When eggs were exposed in cases, it can be readily assumed that all eggs were not of a uniform external temperature and subsequently were not of uniform internal temperatures.

Preliminary experiments were carried out to determine the approximate temperature and exposure range in which the work should be done. On the basis of these preliminary trials, eggs were exposed to the -10° environment and the internal temperature recorded. When the lethal temperature-time range was reached, six eggs were removed every five to ten minutes, the internal temperature being recorded each time. This method allowed for a bracketing of the temperature range with four sets of six eggs. Eggs were then marked and incubated for 24 hours after which time they were removed from the incubator, broken out in petri dishes, and the number of dead and live embryos recorded. Eggs treated at zero, one, and two days of incubation were held and broken out on the fourth day, since heart beat in the live embryo could be clearly seen at this time. The temperature and time necessary to kill 50 percent of the embryos was classified as lethal. Time was found to play an important role in the determination of the lethal temperature since the temperature of the eggs remained constant for a period of about 55 minutes when the eggs were in the process of freezing.

The second portion of the experiment consisted of determining the high lethal body temperature for chickens from day of hatch to maturity, and the high lethal internal egg temperature for the embryo from zero days to 20 days of incubation. The procedure followed was somewhat similar to the one followed in determining low lethal temperatures

except that in this case high environmental temperatures were used. A laboratory oven was employed for this purpose with its temperature set at 160°.

High lethal temperature was obtained by placing a chicken in a wire pedigree basket in the oven. Only one bird was exposed at a time and the internal body temperature recorded by inserting a thermocouple into the body cavity by the same means described previously. Length of exposure and temperature at death were recorded in all cases. Data were obtained on six birds in each of the following age groups: day of hatch, 3, 6, 9, 12, 15, 18 days of age, 4 and 8 weeks of age, and on two hens at maturity.

Lethal temperatures for the developing embryo were studied in the same oven with the same ambient temperature of 160°, as was used for chickens. Thirty eggs were arranged in an egg flat, as shown in Plate 3. Thermocouples were inserted in six of these eggs and each egg numbered in conjunction with its thermocouple number. Since the size of the oven limited allowing one space between eggs, the eggs with the thermocouples were numbered and placed at various points throughout the flat. The arrangement of the eggs is shown in Plate 3. The internal temperatures as measured in these eggs were said to represent the average temperature of the lot. In order to insure maximum rate of warming, holes were cut in the flats so that air could circulate freely around the eggs. Circulation of air was increased by the use of a small fan placed at the bottom of the oven; the complete arrangement is shown in Plate 4. This means of circulating the air assured a relatively uniform air temperature throughout the oven in addition to increasing the rate of warming.

Plate 3. - Arrangement of eggs used in study of high lethal internal egg temperatures for embryos. Numbered eggs contain the thermocouples, and are arranged on flat to obtain temperatures representative of all eggs concerned.

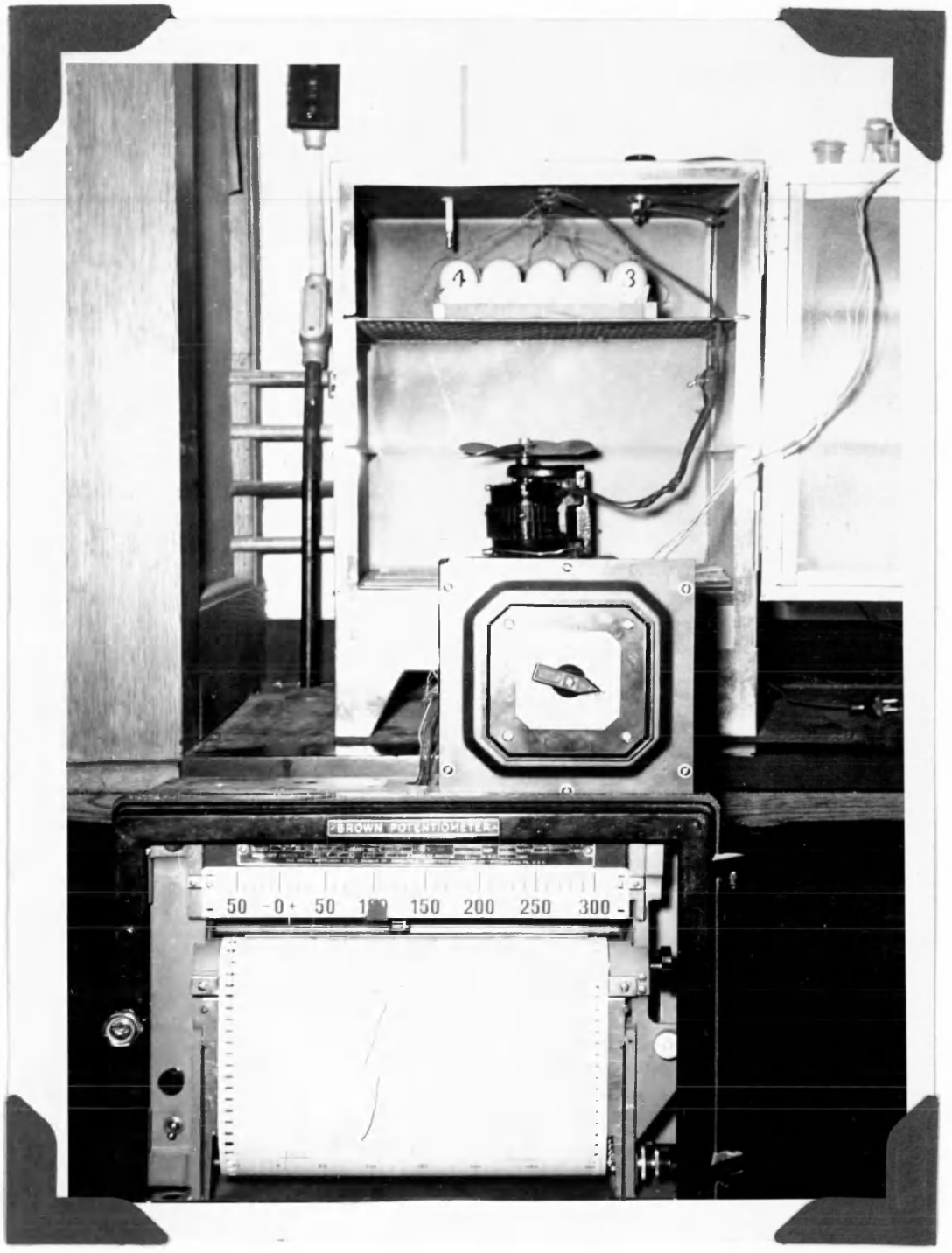

Plate 4. - Arrangement of apparatus used in the determination of high lethal embryo temperatures in eggs. Potentiometer with thermocouple wires in eggs may be noted. Fan placed beneath the eggs increased circulation of air within oven.

Once again preliminary experiments were used to determine the range in which to work. When this range had been found, the same bracketing technique was followed as had been employed in the low temperature studies, except that temperature was the factor of most concern. As the temperature rose, eggs were removed at intervals of approximately 4°. Eggs were allowed to cool at room temperature for about 15 minutes before being returned to the incubator. About 24 hours later, they were broken out in petri dishes and the numbers of dead and live embryos recorded. When 50 percent of the embryos were recorded as dead at any one time, it was considered as the lethal temperature for the age group concerned.

After the lethal temperature for the incubation period had been determined, it was felt that an interesting addition could be made to the data if the time could be obtained that eggs might be held at approximately nine to five degrees below the lethal temperature. This was done by raising the temperature of the eggs to 110° in the oven having an ambient temperature of 160° and then maintaining the temperature of the eggs in a second oven set at 110°. Lots of thirty eggs each were so treated at: 0, 4, 7, 9, 13, 14, 15, 18, 19, and 20 days of incubation. At various periods of time, four to six eggs were removed and broken out immediately, the dead and live embryos were recorded and lethal time noted. When a number of dead embryos began to appear, all remaining eggs were opened. Eggs exposed at zero days of incubation were incubated for three days before being broken out. Trials were repeated until the lethal holding time was obtained.

RESULTS AND DISCUSSION

A. Low lethal body temperatures of the chicken

The average low lethal body temperatures obtained for chickens from day of hatch to twenty-one days of age are recorded in Table 1. It will be noted that in this period the lethal point rose from an average of 60° for the chick on the day of hatch to 67° at three weeks of age. In the period from three weeks of age to the mature hen, as shown in Table 2, only a slight rise in lethal body temperature can be noted. In general, there was usually a difference among individuals as to the temperature at which they died, the variation being about three degrees in most cases. This difference in lethal temperature was also accompanied in different birds by a difference in exposure time, both of which may be attributed in part to individual variation. The records indicate no difference in lethal temperature due to sex, while it was observed that lighter weight birds in general succumbed first.

The length of time that the chick could withstand exposure to the environment at -10° increased progressively from 30 minutes for the baby chick to 130 minutes at six weeks of age. After this point, exposure time increased rapidly and was not recorded as it was felt that it no longer was an important phase of the experiment. It was noted that the growth of feathers on the bird's body during the period of development was an important factor in the increase in resistance to exposure. Clipping the feathers, as practiced on the older birds, reduced the exposure time to eight to twelve hours, while previous to

Table 1. - Low lethal body temperatures of chickens, day of hatch to twenty-one days of age. Body temperature of half of the birds in each trial was recorded

	Age in days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Average body temperature at death	60	61	61	61	62	64	64	65	65	68	67	68	65	66	67	68	66	67	67	67	67	67
Average exposure time in minutes to -10°F.	30	33	35	40	38	40	43	44	42	45	45	43	53	55	55	55	60	70	70	75	75	75
Number of trials*	2	3	2	2	2	2	2	3	2	4	4	4	2	2	3	1**	3	2	2	2	2	4

* Each trial consisted of twelve birds.

** This trial consisted of 18 birds, all of whose body temperature was recorded.

Table 2. - Low lethal body temperature of chickens by weeks, from day of hatch to the mature hen

	Age									
	Day of hatch	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	10 weeks	12 weeks	16 weeks	Mature hen
Average body temperature at death	60	65	67	67	67	67	67	67	69	69	72
Average exposure time in minutes to -10°F.	30	44	55	75	80	120	130	--	--	--	--
Total No. birds involved	24	36	36	48	24	2	12	2	2	4	3
Number of trials	2	3	3	4	2	1	1	1	1	2	2

the feather removal birds withstood the environment up to one week, after which time they were removed.

The steady rise in lethal body temperature has been graphed in Figure 1, for the first 20 days of age. This period of growth is the most interesting to study since in the first 10 days the bird apparently passes through a stage of development in which its lower lethal temperature rises seven degrees. After this rise is obtained, the temperature remains constant. A drop follows on the twelfth and thirteenth days, after which the temperature returns to the previous level and remains at this level up to ten weeks of age. This dip in temperature can be noted in Figure 1, and the lethal temperatures following are seen in Table 2. In an additional period of six weeks, or to 16 weeks of age, lethal body temperatures rose five degrees. This change was followed by an increase of one to four degrees in the period from 16 weeks of age to the mature hen. A further note of these contrasting changes may be made in observing the data in Figure 2, where the lethal temperatures by weeks have been graphed. The rapid rise in exposure time can be seen graphically in Figure 3, which shows that the increase in exposure time accompanies the raise in the lethal body temperature. This graph indicates two steps in the gradual increase in exposure time. The first may be seen to go from day of hatch to about six days of age when a leveling off period is noted on the tenth and eleventh day. On the twelfth day, a second noticeable rise in seen followed by a second leveling off period from the fourteenth to the sixteenth day, when once again an upward trend can be seen to continue on to the nature bird, as shown in Table 2.

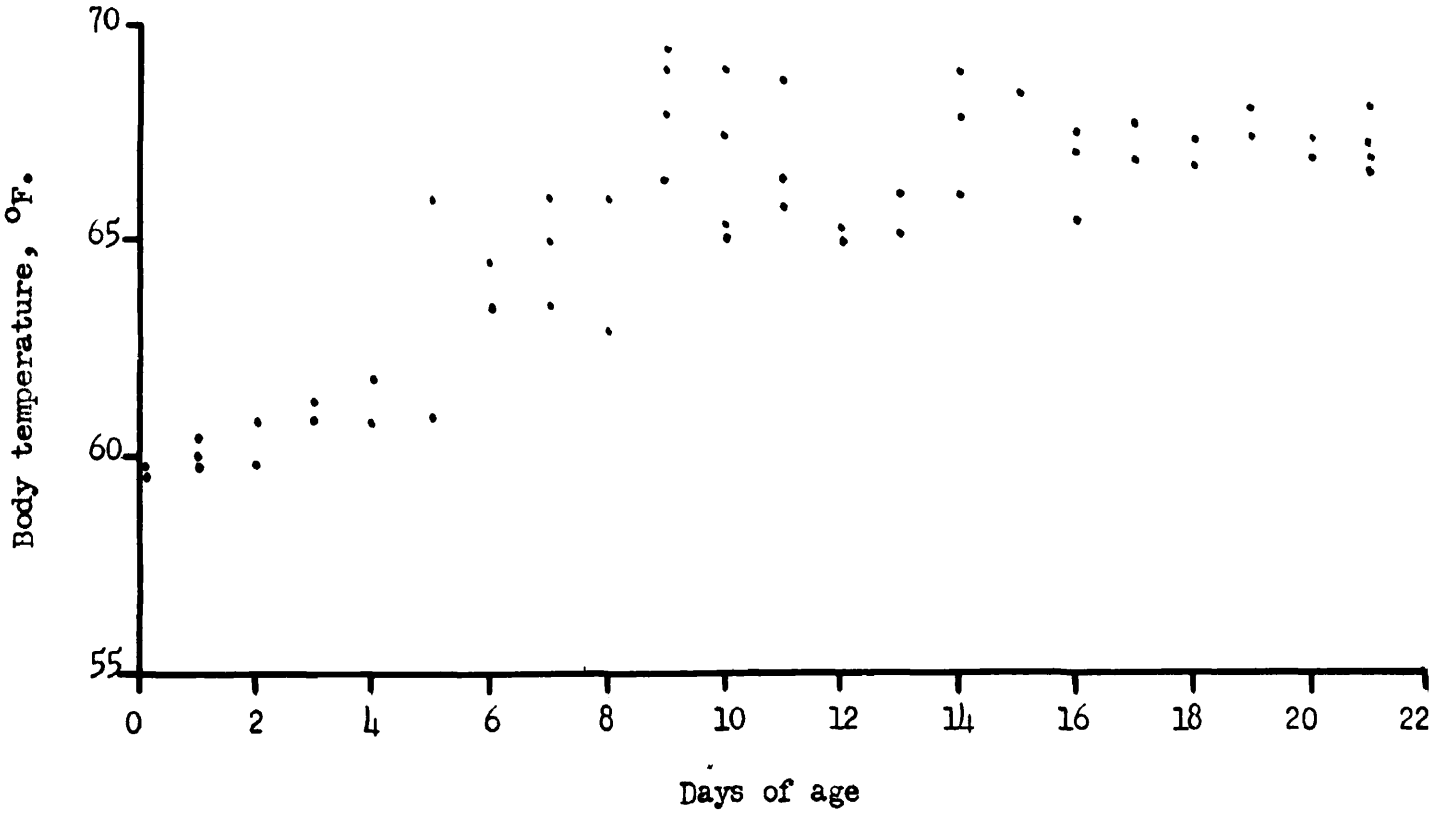

Figure 1. Low lethal body temperature for the chick, day of hatch to twenty-one days of age. Each point represents data collected on 12 chicks.

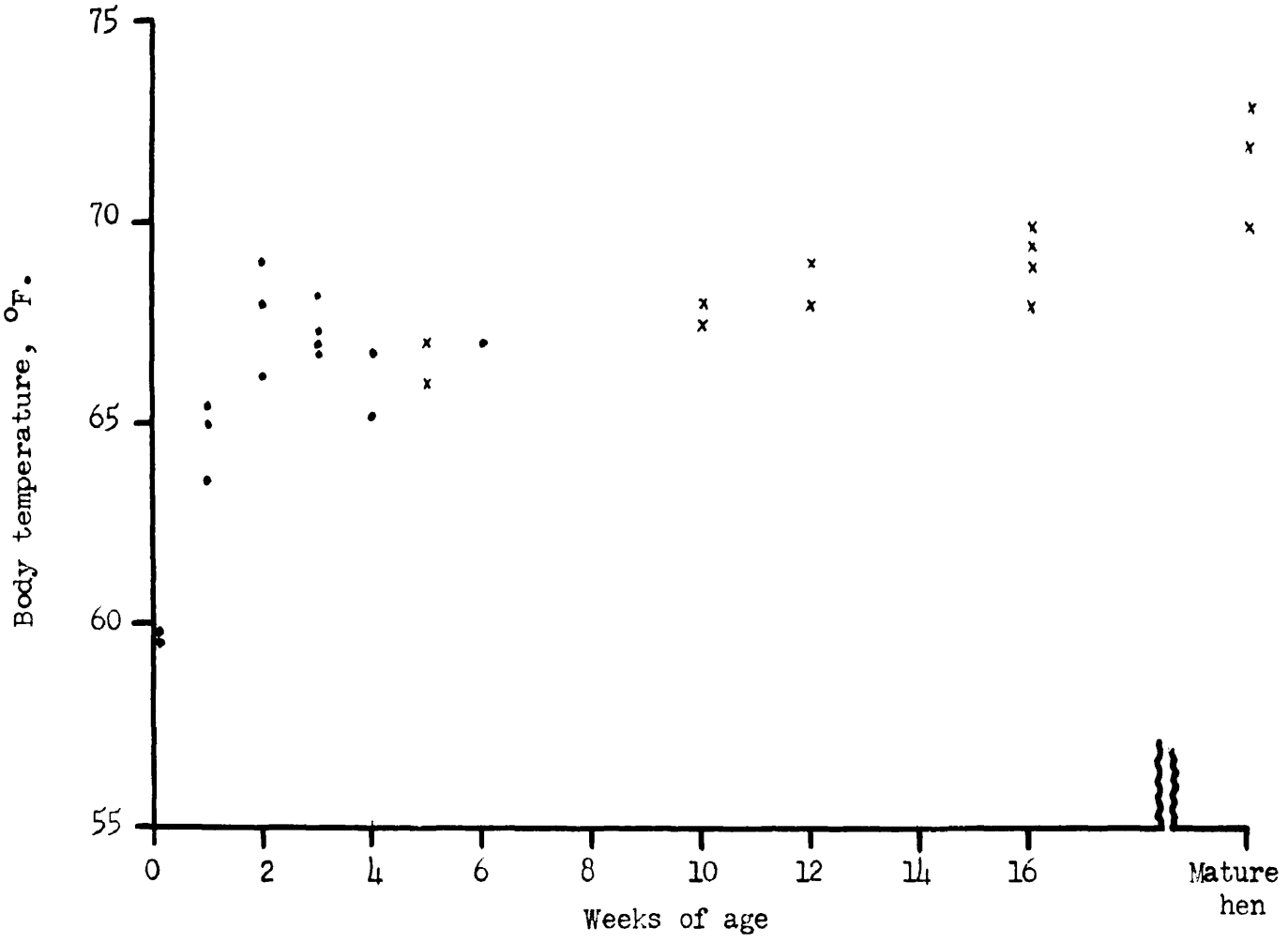

Figure 2. Low lethal body temperature for chickens, day of hatch to the mature hen. Each dot represents data collected on twelve birds; each cross, data from individual birds.

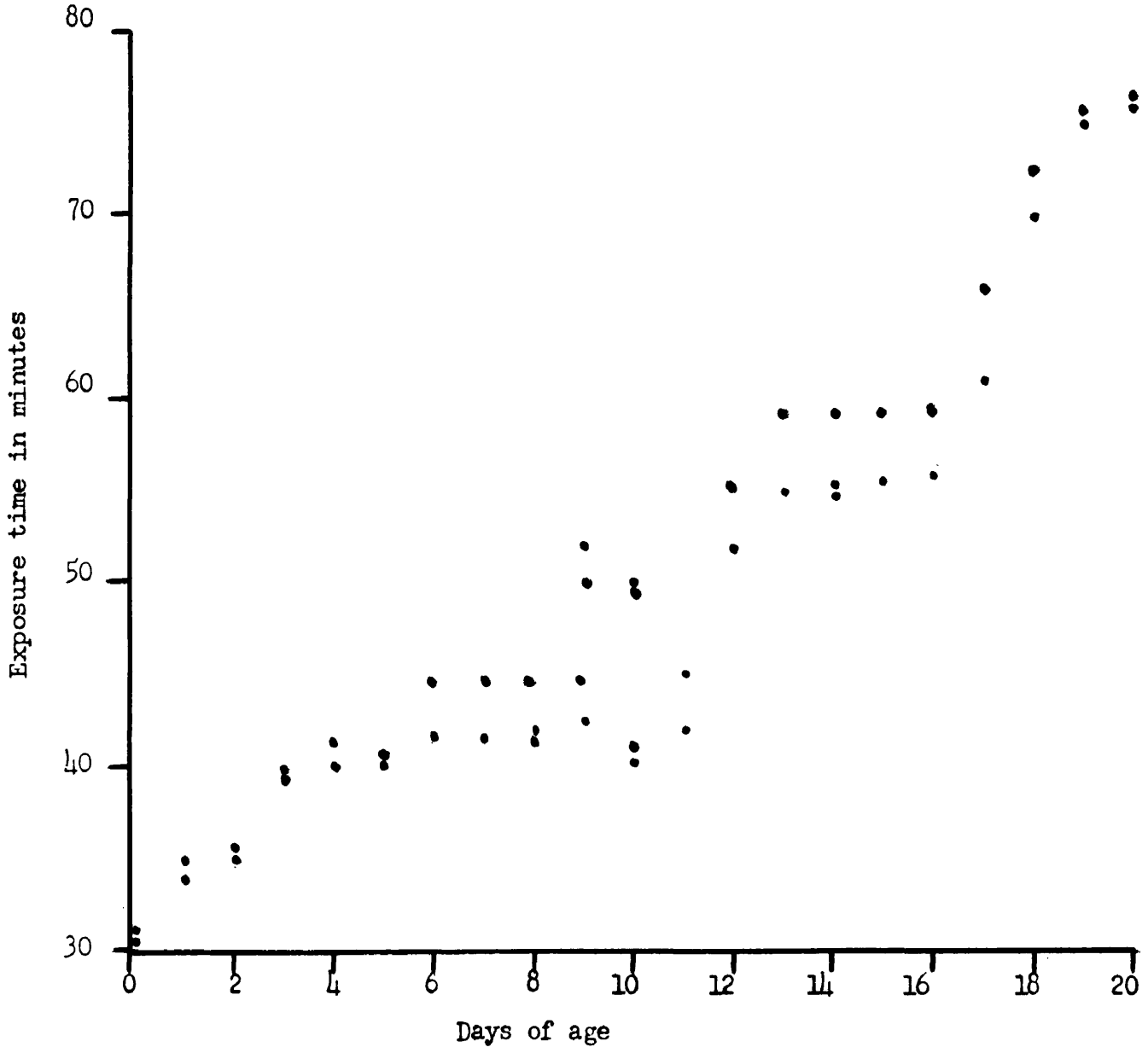

Figure 3. Average lethal exposure time for chicks, day of hatch to twenty days of age, at -10°F. Each dot represents data collected on 24 chicks.

The low lethal body temperature obtained here for the adult hen is similar to the lethal temperature of about 73° reported by Sturkie (1946). The lethal temperatures determined for the chicken are also remarkably close to those reported for the house wren by Baldwin and Kendeigh (1932). Low lethal temperatures for the young house wren that had not yet developed temperature control was 47°, with a general trend in lethal temperatures that follows very closely with that of the chicken. These workers reported the lowest temperature for the adult eastern house wren recorded was 74.6° when recovery was allowed, and 71.0° was found to be the death point.

The rapid rise in low lethal temperature the first ten days after hatch coincides with the rise in body temperature during the same period, as shown by Lamoreux and Hutt (1939) and Kandall (1943). About this time (15 days of age), a peak in basal energy metabolism was recorded by Barott, Fritz, Pringle, and Titus (1938). These facts may be said to indicate that the chicken at this stage is beginning to function more completely as a coordinated unit, with a more complete development of the thermoregulatory mechanism. The rapid development of the nervous system during the first ten days may be the reason that the birds lack the ability to withstand the same low body temperature at ten days of age as they did at one day of age. The fact that the nervous system, including the thermoregulatory mechanism, is still in the developing stage, enables the young chick to be more resistant to temperature shock which is known to accompany any appreciable lowering of the body temperature (Wiggers, 1949).

In order to demonstrate the absence of the ability of the chick to control its body temperature at -10°, the cooling rate of a chick two

days old was recorded and is presented in Appendix Figure 2 B. The rapid rate at which the young chick cools can be seen here, and can be further exemplified by comparison with the rate of cooling recorded for the egg in Appendix Figure 2 C and D, where a close similarity can be noted. The comparison of these two charts may be said to show that neither the heat production of the embryo nor that of the young chick was effective in maintaining any degree of body temperature at an ambient temperature of -10°.

The low lethal body temperature of the chicken when compared to some other animals is found to be somewhat similar. Simpson and Herring (1905) and Britton (1922) report recovery in the cat after the rectal temperature had been lowered to 61°. The body temperature of the monkey was lowered to 57° with recovery and to 61° without recovery by Simpson (1902). The lethal body temperature of the guinea pig was recorded as 57° by Britton (1922). The lethal body temperature for the rabbit has been reported as 61° by Troedsson (1939) and 66° by Jackson and Alonge (1934). The lethal temperature of dogs was observed by Penrod (1949) to be 68°, and Wiggers (1949) states that mammals in general have a lethal body temperature of 68°. In summarizing the lethal temperatures of animals in general, Juyet and Gehenio (1940) state:

> Invertebrates were found, in general, to be killed when frozen at temperatures of a few degrees or of some ten degrees below zero. . . . . The cold-blooded vertebrates died when their internal temperature dropped a few degrees below zero. They could support the formation of some ice in their body. . . . . The warm-blooded animals died at above-zero temperatures except if, as in the case of hibernators, they had developed some adaptative properties.

The low lethal body temperature of the chicken appears to be somewhat higher than that reported in earlier papers for most other animals. In general, it may be said to be approximately the same as that reported in more recent studies for mammals, being in the range of 68°.

B. Low lethal internal egg temperatures for the embryo

The low lethal temperature for the developing embryo from zero days of incubation to 20 days of incubation is recorded in Table 3. As stated previously, time was found to be an important factor in determining the low lethal temperature since the temperature remained about constant during the process of freezing. Eggs which had been exposed to low temperatures at zero days of incubation showed signs of early embryonic death when opened on the fourth day of incubation. Evidence of the development of the germinal disc was seen at this time. Similar development was also noted by Rabaud (1899) and by Grodzinske (1933 and 1934). It may be seen from the data in Table 3 that lethal temperature varied from 28° to 30° with exposure time ranging from 70 to 95 minutes throughout the study. The pattern which time and temperature follow during the incubation period are rather similar. Indications are that the embryo is less resistant from the eighth to the fourteenth day, since during this period the lethal temperature was highest at 30° and was accompanied by a short exposure time of 70 to 80 minutes to -10°. A slight rise in exposure time can be noted from the fifteenth to eighteenth day, with an abrupt drop just prior to hatching on the twentieth day. The developing embryo appeared to be most resistant during the early part (zero to five days) of the incubation period, since at this time the lowest lethal temperature was recorded at 28° to 29° and was accompanied by the longest period of exposure, which was 95 minutes. The second but lower peak near the end of the incubation period is marked by a slight

Table 3. - Average of low lethal internal egg temperatures for the developing embryo, zero to twenty days of incubation

	Days of incubation																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Average lethal internal temperature	29	29	28	29	29	28	30	30	30	30	30	30	30	30	29	30	30	29	30	29	29
Average exposure time in minutes to -10°F.	95	95	95	95	95	95	95	85	75	75	70	75	75	75	70	80	80	80	83	85	80
Number of trials*	5	3	3	5	3	1	1	2	2	2	1	1	1	1	1	1	1	1	2	2	1

* Each trial consisted of thirty eggs.

rise in exposure time of 80 to 85 minutes with a corresponding temperature range of 29° to 30°.

Figure 4 presents the exposure time graphed by days during the period of development. It can be seen here that there is a very prominent drop on the fifth day in the time which the embryo can stand the lower temperatures. A difference of twenty minutes in lethal exposure time can be noted between the sixth to the eighth day. After this period, the time remains constant until the previously mentioned rise near the end of the embryonic growth period.

The peak of chicken embryo mortality has been shown by Payne (1919-1920), Byerly (1930), and many others to be generally at the beginning and at the end of the normal incubation period. Neither the lethal temperatures nor the exposure times reported shown in Table 3 seem to be related to these mortality curves. The difference in peaks of mortality indicates that death was probably due to the treatment and not to other factors which have been found to contribute to mortality during the incubation period.

An extremely interesting point brought out in the data is the demonstration of the ability of the embryo to withstand the lower temperatures for the longest period of time at the beginning of incubation. It can be said to add further evidence as to the exact time the chicken embryo changes in its behavior from a cold-blooded animal. Pembrey, Gordon, and Warren (1894-1895) suggested that this change in behavior takes place at hatching time, while Romanoff (1939) disputed the fact; his data indicating that the change takes place after the tenth day of incubation. The data obtained in this experiment may be said to agree in part with both hypothesis. The fact

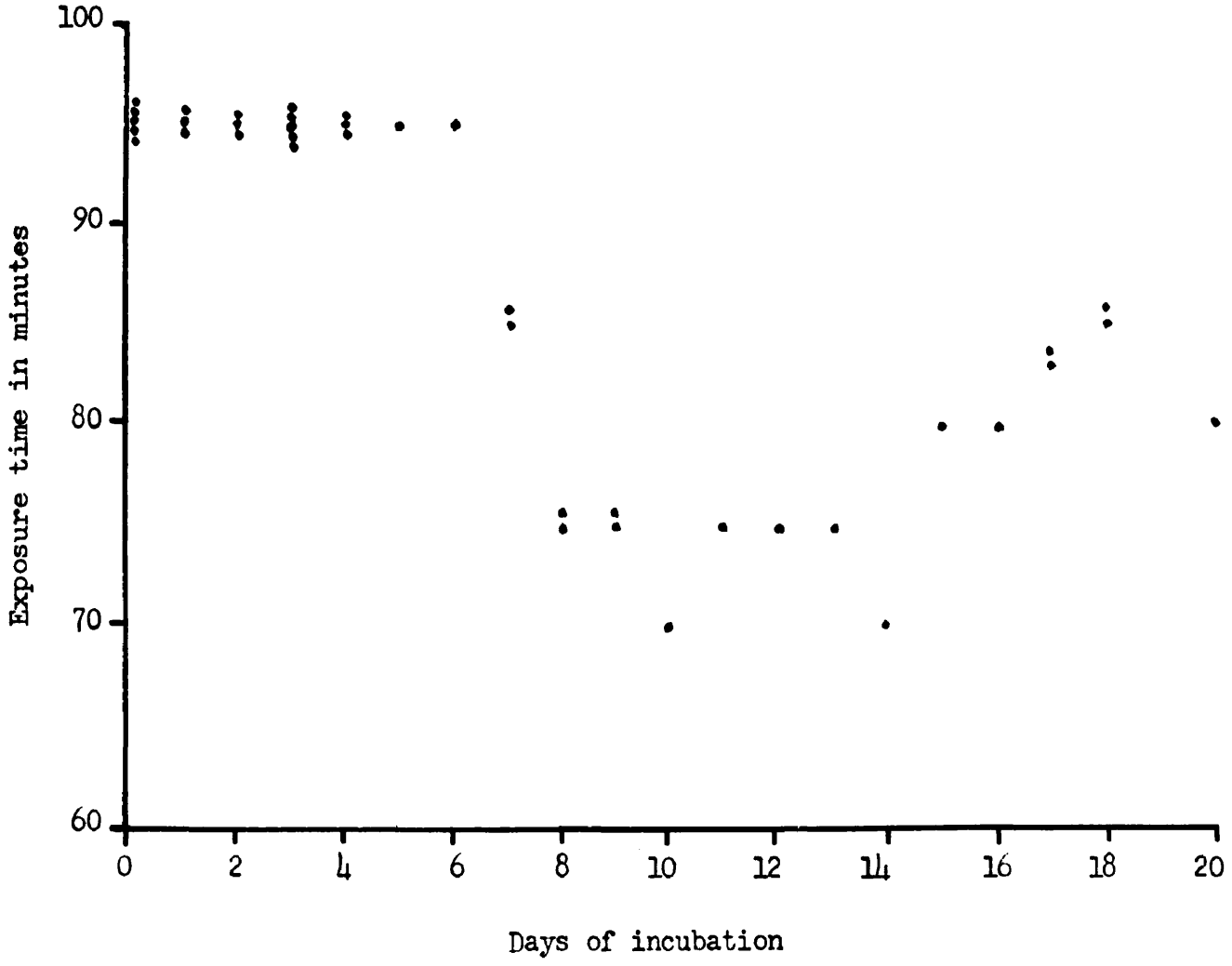

Figure 4. Average lethal exposure time for the developing embryo, zero to twenty days of incubation, to -10°F. Each dot represents data collected on 30 eggs.

that there is an abrupt change in the amount of time that the embryo can withstand exposure to -10°, from 95 minutes on the sixth day to 75 minutes on the eighth day of incubation, indicates that the embryo undergoes a definite change in its development at this time. The fact that the embryo will withstand a much lower temperature than the chick suggests that another very definite change also takes place at hatching time. It is felt that the alteration which takes place at this time is actually the greatest change the embryo undergoes, and may be said to show a more positive shift from a partially poikilothermic condition.

A record of the internal temperature of an egg taken from the incubator after two days and subjected to -10° may be seen in Appendix Figure 1. This continuous recording made with the recording potentiometer provides an interesting picture of the eggs' temperature during the process of freezing. As can be seen from the chart, the eggs cooled very rapidly, reaching the freezing point in about 55 minutes. In accordance with the rate of cooling as recorded, we may say that the embryo was actually exposed to a temperature between 29° and 30° for roughly two-fifths of the total exposure time or about 30 to 40 minutes.

In some cases, as shown on the chart, the temperature dropped below the freezing point to 26°. This was probably due to the phenomena of supercooling or subcooling. The supercooled state existed for only a short time before the temperature returned to 30° and later 29° and 28°, where it remained for about 65 minutes during the process of freezing. The cooling of the egg may be said to follow the cooling curve of a solution. When ice begins to form, after supercooling

passes, the temperature rises but does not quite reach the true freezing point of the egg. This is due to the fact that separation of ice leaves the solution somewhat more concentrated than it was initially (Gucker and Heldrum, 1942). The preliminary drops from 30° to 29° and from 29° to 28° may be due to the increasing concentration of the liquid in the egg as the ice separates. It may be seen from Appendix Figure 1 that after almost two hours at approximately 29°, during which time freezing took place, the temperature curve once more commenced to fall. This fall indicates a cooling of the solid state, until a temperature equal to that of the environment was reached at -10°.

A state of subcooling did not appear in all cases when internal temperatures of cooling eggs was recorded. Appendix Figures 2 C and D show records of cooling rates of eggs when no evidence of subcooling was demonstrated. No evidence was produced, however, that indicates any difference in exposure time as influenced by the brief period of supercooling. It may be added further that, under controlled conditions, Moran (1925) reported keeping an egg yolk subcooled for a week at -11°C. (12.2°F.), which is more than 10°C. below the freezing point.

C. High lethal body temperature for the chicken

When the chicken was exposed to an ambient temperature of 160°, it quickly lost its ability to regulate body temperature. The temperature of the body cavity rose rapidly in young chicks soon after the bird was subjected to the high temperature. Older birds maintained their body temperature somewhat better than the chick, but only for a short period. Table 4 summarizes the results obtained on the study of high lethal body temperatures. Average body temperature at death was 116° for the chick at day of hatch and was remarkably uniform for all age groups studied from three days of age to the mature hen. This temperature correlates with that observed by Randall and Hiestand (1939) at the death of one chicken at 117°. Individual variation was noted, but only ranged from 116° to 118° in all age groups except on day of hatch, where the variation was from 115° to 118°. Baldwin and Kendeigh (1932) reported an approximate high lethal body temperature for the adult eastern house wren regardless of age to be 116.3°, which is comparable to the lethal temperature of the chicken.

A general trend toward an increase in average exposure time is the only difference which could be determined between the age groups. Exposure time to 160° increased with age after four weeks; however, up to this time, an average range of 10 to 13 minutes was all that could be endured. A general increase in exposure time after four weeks of age may be attributed to a more developed cooling system in older birds. The main function of this mechanism being evaporation of moisture through panting, in conjunction with an added insulating

Table 4. - High lethal body temperatures for chickens, day of hatch to maturity

Age	Average body temp. at death	Average exposure time in minutes to 160°F.	No. of birds involved
Day of hatch	116	13	6
3 days of age	117	10	6
6 days of age	117	11	6
9 days of age	117	10	6
12 days of age	117	10	6
15 days of age	117	11	6
18 days of age	117	12	6
4 weeks of age	117	19	6
8 weeks of age	117	20	6
Mature hen	117	33	2

value from the more abundant plumage.

An increase in panting rate accompanied the increase in body temperature. At a point a few degrees before the bird died, the very rapid panting rate fell off sharply and ceased as the bird quickly lost its ability to function as an organized body. Death was accompanied by a brief muscle spasm, which in all cases made the determination of this point quite distinct.

D. High lethal internal egg temperatures for the embryo

The average high lethal temperature as determined for the fertile egg and the developing embryo may be seen in Table 5. The lethal temperature ranged from a high of 119° for the fertile egg to about 108° on the fourth and fifth days of incubation. There was a marked change in lethal temperatures observed during the incubating period. The data obtained on this phase of the experiment when graphed by trials is shown in Figure 5. An interesting change in lethal temperatures may be noted at two points of the graph. The first change comes after one day of incubation, at which time the embryo became very susceptible to high temperature. Before incubation, the fertile egg withstood an internal temperature of 119°, but after an incubation period of one day, the lethal temperature dropped to about 108°, as can be seen in Figure 5.

An average temperature of 107° to 110° is recorded in Table 5. This table represents the combined data which have been graphed by trials in Figure 5. A second point may be noted by the increase in the lethal internal body temperature between the fifth and sixth days of incubation when it rose seven degrees from 107° to 114°. This fact seemed to indicate that a drastic change has taken place in the development of the embryo at this point, which in turn increases its resistance to heat. During the remainder of the period of development, the lethal temperatures remained quite constant with variations ranging from 114° to 118°. This variation may be contributed to individual resistance of the different embryos. A continuous recording

Table 5. - Average high lethal internal egg temperatures for the developing embryo, zero to twenty days of incubation

	Days of incubation																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Average lethal internal temperature	119	109	110	109	109	107	114	114	118	118	116	117	114	116	114	116	116	117	118	118	117
Average exposure time in minutes to 160°F.	37*	9	9	8	8	6	11	10	11	11	10	10	11	12	9	16	11	17	11	10	11
Number of trials**	2	2	2	4	3	3	2	2	2	2	2	2	1	1	1	1	2	1	2	1	1

* These eggs were warmed from approximately 55°F. temperature at which they had been stored; in all other cases, initial temperature of the egg was about 90° - 95°F.

** Each trial consisted of thirty eggs.

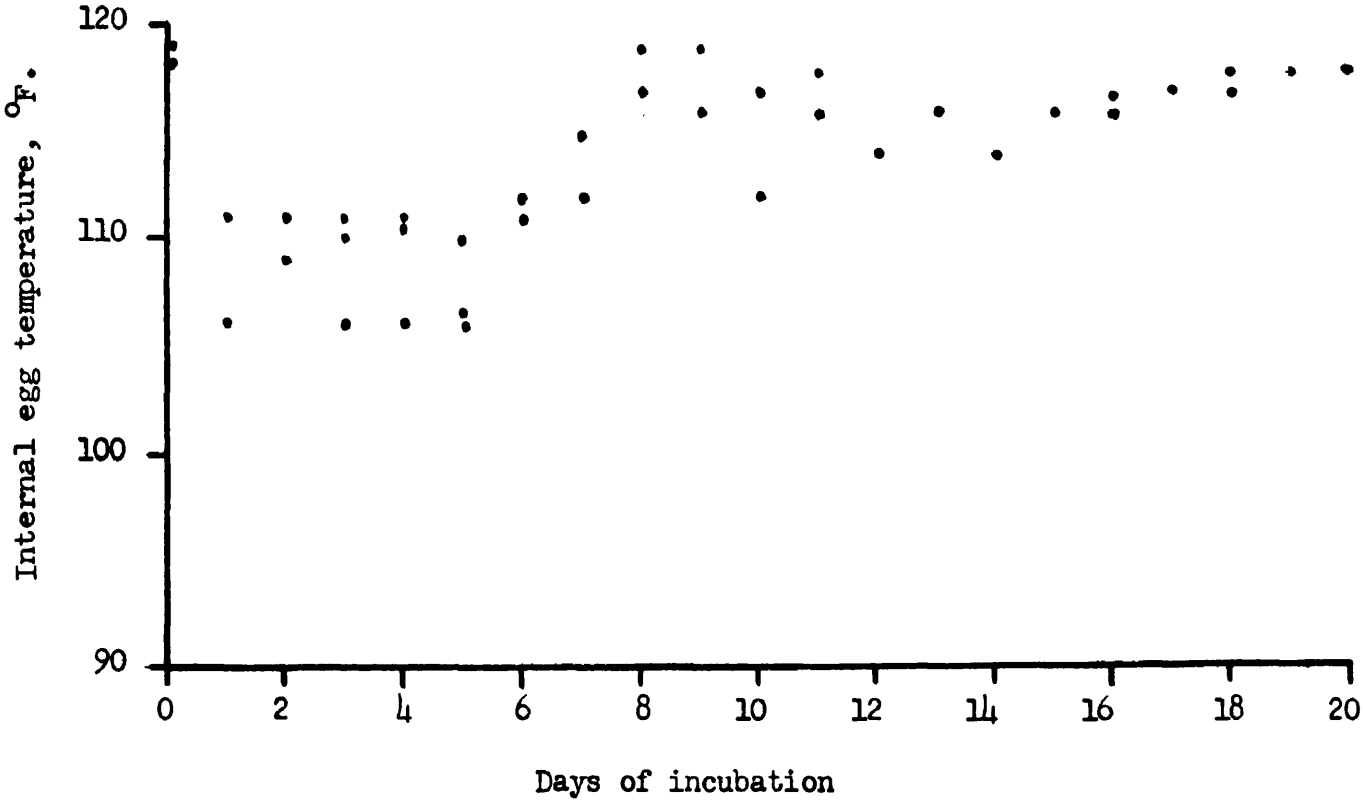

Figure 5. High lethal internal egg temperatures for the developing embryo, zero to twenty days of incubation. Each point represents the data obtained from 30 eggs.

of the rate at which the egg warmed at an ambient temperature of 160° can be seen in Appendix Figure 2 A.

Exposure time was relatively constant in most cases. The variations in lengths of time required by eggs heated to the same temperature may be said to be due to a difference of a few degrees in the eggs' initial temperature. Although an attempt was made to transfer eggs from the incubator to the oven as soon as possible, delays or differences in room temperature made it difficult to start exposure with all lots of eggs having the same internal temperature. At zero days of incubation, the noticeably longer period of exposure is due to the fact that these eggs were taken from the cooler in which they were stored at 55° and brought directly to the oven. Had they been warmed slowly at room temperature, embryonic development would have started and a true lethal temperature of the fertile egg before incubation would not have been possible. The importance of this point is emphasized by the great change in lethal temperature in evidence after one day of incubation.

Eggs exposed to high temperature at zero days of incubation and subsequently incubated for five days showed evidence of cell proliferation. Usually this growth of the blastoderm appeared to cover an area of about one centimeter in diameter, thus presenting evidence of some development followed by early death. In a few cases, development had progressed as far as the blood-ring stage, but this was a rare occurrence. A number of the embryos were also observed to appear weak and retarded in their growth, although still alive.

The lengths of time which the embryo could withstand high internal egg temperatures was studied to further clarify the differences found

Table 6. - Lethal exposure time for the developing embryo at an internal egg temperature of 110°F.

Days of incubation	Holding temperature degrees F.	Average lethal exposure time (hrs.)	Number of trials*
0	110	2	1
4	110	1/6	1
4	106	30	2
7	110	9	1
9	110	11-1/2	2
13	110	6	1
14	110	7	1
15	110	7	1
18	110	8-1/2	1
19	110	5-3/4	2
20	110	8-1/2	1

* Each trial consisted of thirty eggs, except on the twentieth day when forty eggs were used.

in lethal high temperatures at various stages of embryonic development. Eggs were heated rapidly to an internal temperature of 110° and then held at this temperature. The data on exposure time as recorded in hours can be seen in Table 6. The pattern followed by these data is similar to that shown by the data on high lethal temperatures as graphed in Figure 5. The fertile egg at zero days of incubation was mildly resistant, with an average exposure time of two hours. On the fourth day of incubation, the time dropped to ten minutes and then increased again on the seventh day to remain at an average level of about eight hours for the rest of the incubation period. When the internal temperature was maintained at 106° on the fourth day of incubation, exposure time rose sharply to 30 hours. This, in turn, demonstrates that a difference of four degrees apparently marks a critical point in the lethal temperature of the embryo. This critical point may be a direct result of a sudden increase in metabolic rate observed on the third day of development (Noyons and Pascal de Hesselle, 1939). The data in Table 6 supports the variations observed in the lethal temperatures during the period of embryonic development. The pattern shown by the data in Figure 5, compared with lethal temperature at death in relation to age is somewhat similar to the trend of the data in Table 6. A drop of four to nine degrees in the internal temperature of the egg below the lethal temperature enabled the embryo to withstand a temperature of 110° for extended periods of time. It is felt that this drastic change demonstrates that time was eliminated as an important factor in the determination of the high lethal temperature.

E. General observations on the data

In an over-all study of the data, a number of interesting additional observations can be made. It appears that at hatching time, a definite change takes place which enables the chick to begin some regulation of its body temperature. In a study of the data in Table 4, it can be seen that the day-old chick takes a little longer to reach the high lethal temperature than did the embryo in the period just before hatching, yet it may also be observed that the chick has about the same lethal temperature as that of the embryo.

Further indications of a change at hatching time may be seen by a comparison of data in Tables 1 and 2. A more definite alteration in the susceptibility of the embryo to low temperatures takes place at hatching time. During the period of development just prior to hatching, the embryo withstood an internal egg temperature of 29° to 30° with a total exposure time of 80 minutes to -10°. However, at hatching time the resistance of the chick changed sharply, the lethal temperature jumped to an average of 60° and the young bird withstood an exposure time to -10° for only 30 minutes. As the thermoregulating mechanism develops after hatching, the chick becomes more resistant to the low environmental temperature and is able to maintain its body temperature for an increased period of time.

The changes in the general condition of the chick which takes place during the first week after hatching can be observed from the data in Table 1 and Table 2. A rapid rise in lethal body temperature is shown during the first seven to ten days; however, evidence of this

change is not demonstrated by the data in Table 4, since high lethal temperatures for chicks were quite consistent for all ages.

The changes undergone by the embryo as it develops and the resulting effects of these changes on lethal temperatures may be clearly seen from the data in Tables 3 and 5. The definite change in exposure time shown by the data on low lethal temperatures recorded in Table 3 indicates a change about the sixth and seventh days. Differences in high lethal temperatures between the fifth and sixth day can be seen from the data in Table 5. A second interesting change can be seen from the data in this table, as the lethal exposure time decreases sharply after embryonic development commences. The embryo has been shown by many workers to be extremely sensitive during the early part of the incubation period. This sensitivity is shown again in the data by the much lower range in lethal high temperatures during the first five days of incubation.

SUMMARY

1. Studies were made to determine what high and low extremes in internal egg temperatures the embryo in various stages of incubation could withstand. Data were collected on over 3,000 eggs from zero to twenty days of incubation.
2. The high and low lethal internal body temperatures of approximately 920 chickens of various ages were studied, and average temperatures around which most birds died were determined.
3. Temperatures in the body cavity of the chicken and at the center of the egg were measured by the use of copper-constantan thermocouples connected to a recording potentiometer.
4. In order to eliminate as many factors as possible, especially time, extremes in ambient air temperatures were used. Low temperature exposure was -10°F. (-23.3°C.), while high temperature exposure was 160°F. (71.1°C.).
5. When eggs were removed from the incubator and exposed to -10°F. for about 55 minutes, their internal temperature reached about 30°F. At this point, the egg commenced to freeze and the internal temperature remained constant for about 65 minutes, after which it approached the temperature of the environment.
6. Supercooling of the egg at -10°F. was demonstrated, as the internal temperature was lowered to 26°F. for a short period of time, after which it returned to 30°F.
7. Total lethal exposure time to -10°F. was approximately 95 minutes, only about 40 minutes of which the egg's internal temperature was

as low as 29° or 30°F.

8. High lethal internal egg temperature for the embryo ranged from an average of 106° to 119°F., in general, increasing as the embryo development progressed.
9. The average low lethal body temperature for chickens was found to be 60°F. on day of hatch and progressively increased to 72°F. for mature birds.
10. The high lethal body temperature ranged from an average of 116° to 117°F. for all age groups.
11. The data collected indicates that a change in the poikilothermic characteristics of the embryo takes place about the seventh day of incubation. At this point, the lower limit of time considered lethal to the embryo decreases, while the high lethal internal egg temperature for the embryo rises.
12. A definite change is also indicated at hatching time when the chicks' low lethal temperature rose from an average of 29°F. (80 minutes exposure to -10°F.) in the egg, to a lethal body temperature of the chick of 60°F. (30 minutes exposure to -10°F.).

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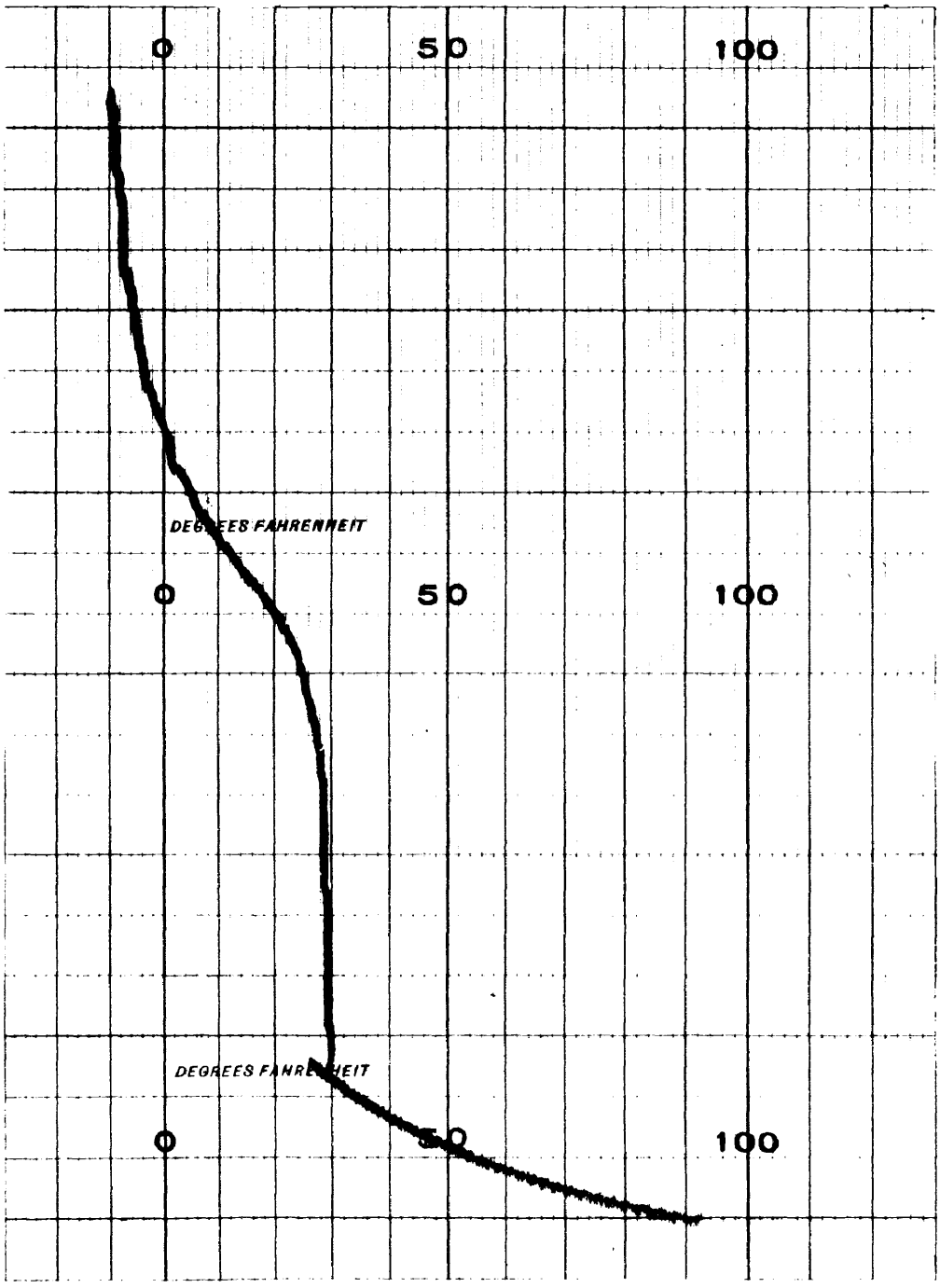
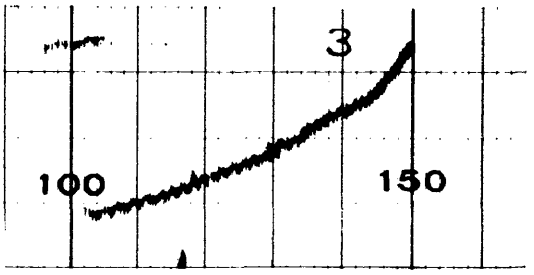
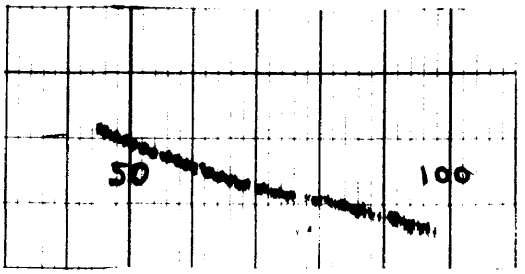
APPENDIX



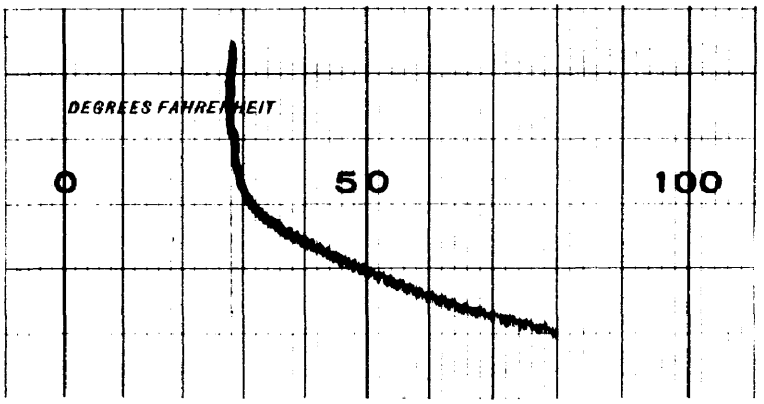
Appendix Figure 1. Continuous record of internal temperature of an egg containing two-day-old embryo as it cooled at -10°F. Chart rate one inch per hour. Supercooling is demonstrated by the break in the line when the temperature reached 26°F.



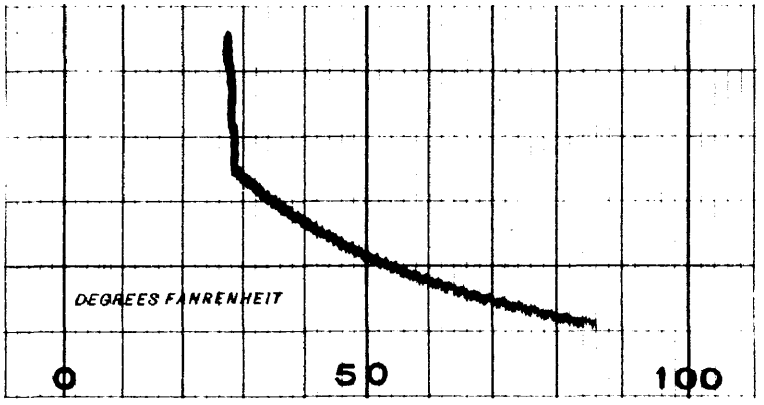
A. Rate of warming of egg 160°F. 6 days of incubation.



B. Cooling rate of chick at -10°F. 2 days old.



C. Cooling rate of egg of -10°F. 19th day of incubation.



D. Cooling rate of egg at -10°F. 5th day of incubation.

Appendix Figure 2. Continuous records of warming and cooling rates made on chicks and eggs. Chart rate one inch per hour.