EFFECT OF TEMPERATURE, HUMIDITY, AND OTHER FACTORS ON HATCH OF HENS' EGGS AND ON ENERGY METABOLISM OF CHICK EMBRYOS

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INTRODUCTION

In recent years the increasing use of incubators in hatching chickens for the replenishment of poultry flocks has made desirable a more complete knowledge of factors that influence hatching results. The 1930 census indicates that the number of chicks hatched annually in this country is in excess of 1,100,000,000. To produce this number, owing to the high mortality of the embryos during the incubation period, requires approximately 1,800,000,000 eggs of average hatchability. The failure of 700,000,000 eggs to hatch represents an annual monetary loss of at least $14,000,000.

As long ago as 1750, according to Réaumur (*34*),[2] it was realized that factors prevailing during the incubation of hens' eggs markedly

affect the development of the embryos. Some of the factors which are of paramount importance are temperature, humidity, and the amount of carbon dioxide and oxygen in the atmosphere surrounding the eggs. As late as 1840 some investigators still maintained that a fertile egg did not respire and could develop normally with all air excluded. Since that time it has been fully established that long before the lungs begin to function in the chick embryo, gaseous interchange takes place through the porous shell. The amounts of various gases in which the egg is incubated, therefore, would affect appreciably this gaseous interchange and thus the development of the embryo. Each of these factors affecting hatching results must be controlled with precision if the best hatch of vigorous chicks is to be obtained.

Research on the effect, during incubation, of the temperature, humidity, and amount of carbon dioxide and oxygen in the atmosphere surrounding the eggs has yielded many results, yet information concerning the optimum conditions for normal development of the embryos is indefinite and unsatisfactory. It was the aim, in the investigations reported in this bulletin, to ascertain the optimum condition for each of these factors for embryonic growth and for hatching. It was also desired to ascertain the specific effect on embryonic growth and on hatch of a change in any one of these factors.

With increased knowledge of the underlying principles governing the growth of the embryos during incubation, it should be possible to reduce materially the large economic loss in the United States each year.

REVIEW OF LITERATURE

EFFECT OF VARIOUS FACTORS ON HATCH

Many investigators, namely, Burke (7), Cadman (9), Chattock (10), Covalt (11), Dareste (12), Eycleshymer (17), Philips (31), Philips and Brooks (32), and Skinner (38) have attempted to determine the optimum temperature, during different stages of incubation, to produce the greatest number of vigorous chicks from a given number of eggs incubated. In most cases little was known concerning the physical conditions other than temperature. Variation in humidity or the gaseous constituents of the atmosphere surrounding the eggs may have so affected the growth of the embryo that results attributed to temperature were caused in part by other factors.

The temperatures of incubation were invariably measured above the eggs. Because of the temperature gradient involved, there is doubt as to the actual temperature of the air surrounding the incubating egg. Atkinson (2), Burke (7), Cadman (9), and Chattock (10) found differences in temperature of 4° to 10° F. at the top and bottom of incubators. Philips and Brooks, who placed the thermometer in a celluloid egg set among the other eggs, found that in this position it registered 2° lower than a thermometer hanging directly above the eggs but not touching them. They obtained the best hatches at temperatures of 99° to 100°, measured by the thermometer in the celluloid egg, the hatch decreasing at temperatures either higher or lower. The optimum temperature as given by investigators whose works were reviewed ranged from 95° to 104° just above the egg.

It is rather difficult to determine the humidity of the air surrounding the eggs in the limited space in incubators ordinarily used for

experimental purposes. The mammoth-type incubators have seldom been used. When a wet- and dry-bulb hygrometer is used in an egg chamber, the humidity of the air surrounding the wet bulb will always be higher than that in the rest of the chamber unless the air velocity is at least 9 feet per second. The rate of evaporation from the wick, therefore, will be greatly decreased and the thermometer will read too high. The spread in temperature of the wet and the dry bulbs will be much reduced and the humidity computed from the readings too high. A comparison of humidities made by Day (13), obtained by using a wet- and dry-bulb hygrometer, fanned and un-fanned, showed that, between 50 and 75 percent relative humidity, fanning lowered the reading of the wet bulb sufficiently to account for a difference of 10 to 15 percent in humidity.

The results on the effect of humidity on hatch obtained by several investigators, namely, Cadman (9), Chattock (10), Day (13), and Dryden (15), show wide variations. Little was known relative to incubation conditions other than temperature and humidity, and unquestionably all conditions varied considerably during the incubation period and even more from experiment to experiment. As careful an analysis of each investigator's results as could be made seemed to show that he obtained better hatches between 50 and 70 percent than at either higher or lower humidities.

Lamson and Kirkpatrick (22) obtained results by use of their modified incubator which cover the entire practical range of humidity. They determined the humidity by gravimetric measurements. Such measurements are subject to errors in the determination of the temperature and pressure of the air at the time measurements are taken, the temperature at which the humidity is computed, condensation of moisture in the connecting tubes, and other factors. They also determined the loss of weight of the eggs. Loss of weight can be very accurately obtained as it involves only simple weighings. Since loss of weight depends on humidity, it provides a means of computing humidity as a check on actual measurements. The author, using loss of weight as determined by Lamson and Kirkpatrick, has computed humidity, and these computations are from 5 to 15 percent higher than those reported by them. The values computed by the author show that the best hatches were obtained at from 55 to 65 percent relative humidity and that they fell off rapidly above or below these percentages.

Many investigators, notably Burke (7), Cadman (9), and Dareste (12), made measurements to determine the amount of carbon dioxide gas under sitting hens, on the assumption that the optimum for incubation would be present. The results showed 5 to 25 parts of carbon dioxide in 10,000 by volume for the first 10 days, 20 to 50 parts in 10,000 at the end of 2 weeks, and 50 to 80 parts in 10,000 by the end of the incubation period. As shown by these results, the carbon dioxide gas in the air under a sitting hen is variable in amount depending, for instance, on how closely the hen confines the eggs, on whether or not the nest admits air freely, and on the carbon dioxide content of the air surrounding the nest. In various incubators the amounts of carbon dioxide were comparable to the amounts under hens except that owing to increased ventilation they were somewhat lower at the end of incubation.

The experiments of Lamson and Edmonds (21) are the only ones observed by the author which show the effect of carbon dioxide concentration on hatch. Over a 5-year period they incubated approximately 10,000 eggs with carbon dioxide ranging from 0.5 to nearly 5 percent. The best hatch was obtained when the carbon dioxide content was 0.5 percent. The hatch decreased as carbon dioxide content increased; with a 4.5-percent concentration the hatch was less than one-sixth that with 0.5 percent.

Although little work has been done on the effect of carbon dioxide on hatch, still less is reported on the effect of varying percentages of oxygen. Wesselkin (39) incubated eggs from 24 to 72 hours in vessels through which a current of air, containing varying percentages of oxygen, was run. He states that in an atmosphere containing 5 percent of oxygen the embryo lived 48 hours; in 10 percent and in 15 percent life persisted 72 hours. In all cases development was subnormal, the degree in backwardness increasing with decrease in the amount of oxygen. Various morphological deformities were observed.

ENERGY METABOLISM OF INCUBATING EGGS

So far as is known, respiration is a universal characteristic of all animals. Many experiments, notably those of Aggazotti (1), Düsing (16), and Mitrophanow (26), have shown that the chick embryo is no exception to this rule.

In making their experiments, some investigators coated the surface of the eggshell partially or wholly with varnish or other material; others incubated eggs in atmospheres of hydrogen, nitrogen, or carbon dioxide. They proved that restricting the gaseous interchange between the embryo and the external air has a decided effect on the growth of the embryo. If the interchange is only partially restricted, development is below normal and many monstrosities appear. If the interchange is completely restricted, no development will take place after the first few hours, during which time the oxygen in the air cell and in the egg content is sufficient to supply the amount necessary for life.

Experiments on complete energy metabolism were conducted by Bohr and Hasselbalch (5, 6), and by Hasselbalch (19), as early as 1900–1903 by use of a specially designed thermostat. Their results on energy metabolism began slightly below zero the first day of incubation and gradually increased at a nearly uniform rate to the tenth day, at which time the oxygen consumption was 9 liters per 100 eggs per day, the carbon dioxide elimination 5 liters per 100 eggs per day, and the heat elimination 21 calories per 100 eggs per day. From this time the increase was much more rapid, but uniform, until the seventeenth day, at which time the oxygen consumption was 40 liters per 100 eggs per day, the carbon dioxide elimination 30 liters per 100 eggs per day, and the heat elimination 190 calories per 100 eggs per day. On the seventeenth, eighteenth, and nineteenth days there seemed to be some retardation in the rate of metabolism as indicated by the carbon dioxide elimination. The oxygen consumption and heat elimination were too erratic to show this trend definitely. The results obtained by these investigators on the twentieth day were 52 liters of oxygen, 40 liters of carbon dioxide, and 240 calories of heat per 100 eggs per day. The respiratory quo-

tients were very erratic, varying from more than 1.00 to about 0.50, with an average of 0.72 for the 21 days of incubation.

The data on energy metabolism of incubating eggs obtained by Bohr and Hasselbalch have been widely used. In fact, the results on heat elimination have been the only ones available.

Carbon dioxide elimination of incubating eggs was determined by Atwood and Weakley (4), Murray (28), and by Harcourt (18). The results obtained by Atwood and Weakley and by Murray were practically the same. Both obtained data that paralleled those of Bohr and Hasselbalch but were approximately 35 percent higher. The results by Harcourt were practically the same as those obtained by Bohr and Hasselbalch.

Lussana (25) determined the gaseous metabolism of the growing embryo during the last week of incubation. The results he obtained are much lower than those obtained by other investigators.

Henderson (20), in his studies on the effect of temperature on rate of growth of chick embryos, found that "temperature exerts a profound influence on the growth rate." As metabolism is closely associated with growth rate, one would expect an agreement between the two. He found that the development was much arrested at low temperatures (95° F.) and that an increase of temperature above 95° favored development until a temperature of 102° was reached. This temperature gave optimum development, whereas at higher temperatures the development was again arrested, although proceeding at a more rapid rate for the first 2 weeks of incubation.

Romanoff (36) studied the effect of three humidities on growth and mortality of the chick embryo. The growth was optimum at 60 percent whereas at 80 and at 41 percent there was little effect until after the second week. During the last week of incubation both the high and the low humidities showed detrimental effects. The mortality during the third week was also greatly increased at both the high and the low humidity, but the effect was most pronounced at the high humidity.

Romanoff and Romanoff (37) also studied the effect of air composition on growth and mortality of the chick embryo. In their studies "The increase in carbon dioxide * * * was at the proportional expense of the * * * oxygen." Consequently, the results they report are a cumulative effect of oxygen deficiency and carbon dioxide surplus. They found that 0.4 percent of carbon dioxide and 20.8 percent of oxygen affected the growth very little, if at all. With increasing amounts of carbon dioxide and decreasing amounts of oxygen the growth was retarded in proportion to the increase in carbon dioxide and the mortality also increased. With 6 percent of carbon dioxide and 19.6 percent of oxygen, no embryo was able to survive more than 2 weeks.

APPARATUS AND METHODS

The respiration calorimeter which was used as an incubator in the experiments conducted by the author is ideally adapted to the study of the factors affecting the development of the chick embryo. In the form used it affords a means of controlling, with precision, all the physical factors involved and of varying any one factor in any way desired while keeping the others unchanged. Accurate deter-

minations of the heat elimination and gaseous exchange in the growing embryo for the entire incubation period can be made.

The essential features of the respiration calorimeter are as follows: For the measurement of heat produced in the chamber the device is a constant-temperature, continuous-flow, water calorimeter, in which the calorimetric features provide for preventing the passage of heat through the walls of the chamber and for taking up the heat by a current of water as fast as it is generated in the chamber. For the determination of gaseous exchange the device is a gastight
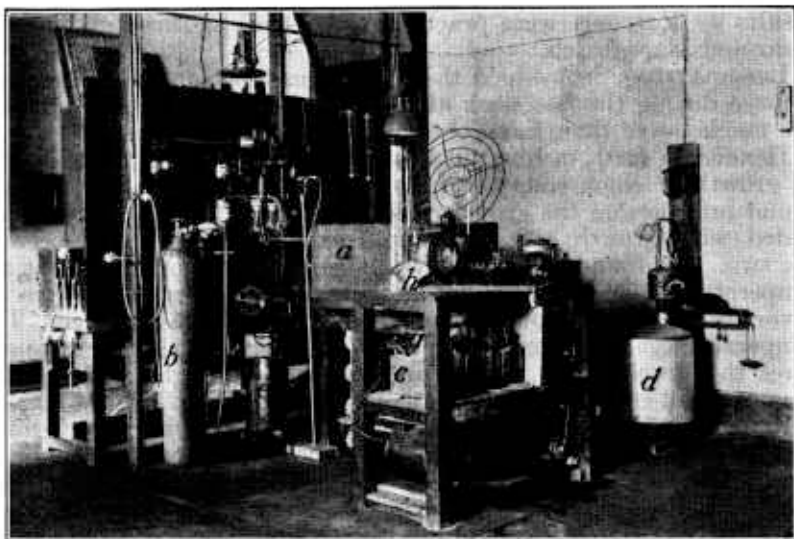

FIGURE 1.—Respiration calorimeter and accessory apparatus: *a*, Calorimeter; *b*, oxygen supply; *c*, absorption train; *d*, water tank and scale; *e*, spirometer for measuring oxygen; *f*, flasks for absorption of water vapor; *g*, canister for absorption of carbon dioxide; *h*, hygrometer.

chamber connected to a system of gas absorbers in a closed circuit. The gas confined in the circuit is kept in circulation, the gaseous products imparted to it by the growing embryos are constantly removed, and oxygen is constantly supplied to replace that used.

Figures 1 and 2 show the assembly of the apparatus. All measurements and control were from the table (fig. 2) at which the observer sat with all control apparatus within reach. Use was made of two calorimeters, operating independently, to eliminate errors which might arise owing to equipment. Both calorimeters were constructed on the same principles. One calorimeter, however, had a square base and the other was round, a point which is mentioned for identification later in connection with data.

THE CALORIMETRIC SYSTEM [3]

The calorimeter consists essentially of a double-walled, copper chamber (fig. 3, *a*) in the form of a cylinder, 65 cm in diameter and 15 cm deep. The walls are separated by an air space 1 cm wide.

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[3] Detailed description of respiration calorimeters from which the apparatus used in this investigation was developed is given by Langworthy and Milner (*23, 24*), and Atwater and Benedict (*3*).
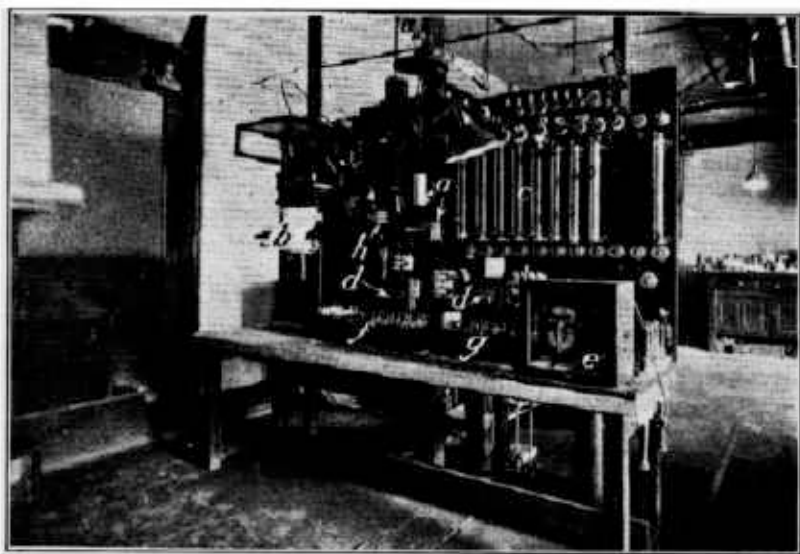
FIGURE 2.—Instrument table and control board for calorimeter: *a*, Parts of galvanometer system; *b*, recorder; *c*, control rheostats; *d*, multiple switch; *e*, automatic controller; *f*, Wheatstone bridge; *g*, potentiometer; *h*, controller.

FIGURE 3.—Respiration calorimeter and accessory apparatus: *a*, Absorber coil; *b*, covers to calorimeter; *c*, heating coils for top section; *d*, cork insulation against temperature changes in surroundings; *e*, electrical conductivity apparatus for gas analysis; *f*, oxygen supply control apparatus.

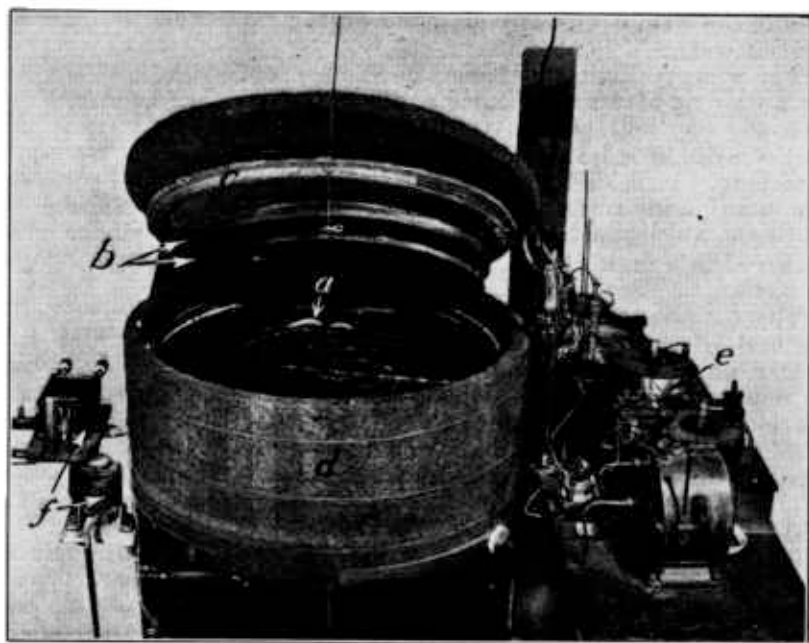The total volume is approximately 50 liters. The entire top is re-movable and is hermetically sealed when in use.

This double-walled chamber is surrounded by sheet insulation as thin as is consistent with mechanical strength, providing an air space 1 cm wide in which are inserted three electric heating coils, uniformly distributed over the entire surface, one over the top, one under the bottom, and one around the sides, of the outer copper chamber. By means of these coils heat can be supplied to the air space to control the temperature of the outer copper wall. A cover-ing of cork board 5 cm thick gives protection against any fluctuations in temperature in the surroundings.

The chamber is designed to hold 89 eggs. These are supported on a perforated copper tray suspended from the side walls, 5 cm above the bottom. Each egg rests horizontally in a loop of a spe-cially designed wire turning device in the form of a horizontal wheel, which turns the eggs by rolling them on the perforated tray. The de-vice is turned by a central shaft which projects out of the chamber.

DETERMINATION OF HEAT PRODUCED

Heat is given off by the eggs in two ways: As latent heat of water vaporized and as sensible heat liberated to the air from the surface of the eggs. Both the latent and sensible heat must be determined.

The water vapor leaves the chamber in the outgoing air and is collected in the flasks (fig. 1, f). The quantity of heat leaving the chamber as latent heat in any given period is determined by multi-plying the weight of water absorbed during the period by the latent heat of water.

The energy eliminated from the eggs as sensible heat is absorbed by a current of water which circulates through the chamber in the heat-absorber coil (fig. 3, a) suspended 3 cm above the eggs. The coil consists of a spiral of thin-walled copper tubing approximately 6 m long. It is so wound that the coldest and the warmest water are in adjoining turns of the coil. Water is supplied to the absorber coil from a constant head tank and after passing through the coil is collected in a tank (fig. 1, d) and weighed to an accuracy of 1 part in 3,000.

The temperature of the water entering the heat-absorber coil is maintained constant by electric heating. The final control is by a heater which comprises a water channel made of thin brass tubing. In one end of the flattened channel is inserted an electric resistance thermometer and in the other end is an electric heater. Both are of similar construction and are of the Dickinson and Mueller (14) type. The coil of the resistance thermometer forms one arm of a special Wheatstone bridge connected to an automatic controller (fig. 2, h) which keeps the inflowing water at the desired temperature.

The increase in temperature of the water passing through the absorber coil is of fundamental importance in the measurement of heat generated in the chamber and must be determined with extreme accuracy. The difference between the temperatures of the inflowing and outflowing water is determined by the difference in resistance of the two specially designed identical platinum resistance thermome-ters of the Dickinson and Mueller (14) type. These two thermom-eters form the opposing arms of a special Wheatstone bridge. A

slide wire is connected between the two thermometer coils. This slide wire is incorporated in the mechanism of a recorder (fig. 2, *b*), which automatically balances the bridge for inequalities in resistance of the thermometer coils and at the same time records by a graph the balancing operations in terms of temperature difference between the two thermometers to an accuracy of 0.01° C. The difference in temperature of the water as it enters and leaves the chamber was determined frequently by a multiple-junction differential thermocouple inserted in the water channel, as a verification of the readings of the thermometers.

The weight, in kilograms, of water that flows through the absorber coil during a given period multiplied by the difference of temperature, in degrees centigrade, of the water as it enters and leaves the chamber represents the quantity of heat removed during the period.

The rate at which heat is removed from the calorimeter is regulated to prevent fluctuations in the temperature of the air in the chamber, as the temperature falls if the rate is too fast and rises if it is too slow. By proper control of the temperature of the water as it enters the chamber and of the quantity of water which passes through the absorber coil, the removal of heat may be made to accord with its production in the chamber within very narrow limits. In practice, a constant rate of flow is maintained whenever possible and the temperature of the water is varied according to the quantity of heat to be removed.

Any interchange of heat between the calorimeter and its boundaries is prevented by keeping the outer copper chamber surrounding the calorimeter in thermal equilibrium with the copper calorimeter. This condition is determined by a system of differential thermocouples installed between the two copper walls with the junctions in thermal contact with each copper wall but electrically insulated from it. The thermal condition of the couples was determined by readings of a galvanometer. The sensitivity of the system was such that a difference in temperature as small as 0.001° C. was easily detected. In actual practice the walls were always practically in thermal equilibrium. The control was by means of electric heating elements placed in the air space just outside the outer copper chamber. Independent heaters were provided for the top, the bottom, and the sides to correspond with the thermocouple system distribution.

Provision was also made against loss or gain in heat in the circulating air. A differential thermoelement was installed with one end in the incoming air at the point where it enters the chamber and the other end in the outgoing air just as it leaves the chamber. Any difference in temperature between the incoming and outgoing air was corrected by means of an electric heating coil on the inflow air tube.

When the passage of heat into or out of the chamber through the walls or in the ventilating air current is prevented, the sum of the latent heat and sensible heat removed equals the amount of heat actually produced in the chamber, except for the two following minor corrections: A correction for any change in temperature of the calorimeter or contents, and a correction for the small amount of heat lost by leakage.

Heat leakage by conductivity through the various outlets containing wires and pipes, which could not be entirely prevented but which was very uniform for given conditions of outside and inside temperatures, was determined by holding the calorimeter in thermal equilibrium 10° to 15° C. above the temperature of the room and measuring the amount of electric energy necessary to be supplied to keep the temperature of the calorimeter constant.

DETERMINATION OF HYDROTHERMAL EQUIVALENT

The hydrothermal equivalent (heat capacity) of the calorimeter can be calculated from the weights and specific heats of the various materials entering into its construction. The difficulty with the computation is the exact determination of the boundaries of the calorimeter thermally. Therefore, in this investigation, an actual measurement was made by supplying sufficient electric energy to raise the temperature of the chamber several degrees. During the interval of heating, the system was kept in thermal equilibrium. The amount of electric energy supplied, which was accurately measured, divided by the rise in temperature in degrees, gave the hydrothermal equivalent. The hydrothermal equivalent of the eggs was assumed to be 0.80 of an equivalent weight of water.

The temperature of the copper walls of the calorimeter was determined by the use of specially designed nickel resistance thermometers, which were held firmly against them.

The temperature of the air in the calorimeter was determined by means of a platinum electric resistance thermometer of the Dickinson and Mueller (14) type, the resistance of which was measured on a precision Wheatstone bridge of the Mueller (27) type (fig. 2, f). In an apparatus of this type, there are slight variations in the temperature of the air in the chamber even under the most favorable conditions. This variation was determined by a series of six differential thermocouples placed within the chamber. One junction of each couple was in juxtaposition with the resistance thermometer, and the other six junctions were distributed variously around the interior of the calorimeter. Under conditions of actual use the variations in temperature at the coldest and hottest spots were not greater than 0.25° C.

THE RESPIRATORY SYSTEM

The copper calorimeter comprises the respiratory chamber in which the eggs incubate. The respiratory system, which is of the closed-circuit type, hermetically sealed to the external air, comprises the chamber, the gas line, and the absorption train. The gaseous medium surrounding the incubating eggs is circulated through this system by a positive-pressure rotary pump. The air leaves the chamber at the center and above the eggs, from which point it is passed by the pump back to the chamber at the center and below the eggs. The inlet pipe is bent into a circular ring, and the air leaves the pipe through a series of holes, graduated in size, in the outer circumference of the ring.

The atmosphere of the empty chamber contains nitrogen, oxygen, carbon dioxide, and water vapor in the same proportions as air.

When the embryos begin to develop, the proportions begin to change because of the consumption of oxygen and the elimination of carbon dioxide and water vapor. The removal of the excess carbon dioxide and water vapor and the restoration of the oxygen used, in such a manner that the quantity may be accurately measured, form the basis of the determination of the respiratory exchange in the chamber. The movement of the air past the eggs is fixed and constant, once the speed of the pump is adjusted; the air is uniformly distributed under the eggs, thus keeping the gas of uniform composition throughout the egg chamber.

THE ABSORPTION SYSTEM

The absorption system (fig. 1, c) is a shunt line off the main air-circulating line and has two flasks (fig. 1, f) containing sulphuric acid for absorption of water vapor. These were especially designed by Langworthy and Milner (24). The flow of air through them was maintained at such a rate that moisture was removed from the air in the chamber at the same rate that it was imparted to the air by the embryos. In this way a constant humidity was maintained in the chamber. The volume of air to be passed through the absorbers was determined by readings of the hygrometer (fig. 1, h).

The air from the flasks passes next through a canister (fig. 1, g) containing soda lime, which removes the carbon dioxide gas. The dry air from the water absorbers removes moisture from the soda lime in passing. This water vapor is collected by two absorbers (flasks) containing sulphuric acid. The humidifying flask is of the same kind as the flask used for water-vapor absorption except that it is filled with water instead of sulphuric acid. The increase in weight of the absorbers in a given period shows the quantities of water vapor and carbon dioxide carried out of the chamber. These quantities, corrected for the change in composition of the gas within the chamber, determine the quantity produced during the period. In a ventilation system of this type, as fast as any gas is removed some other gas is introduced to maintain atmospheric pressure in the chamber; in this case, oxygen to replace that used by the embryos is supplied automatically (fig. 3, f) from a calibrated spirometer through a regulating valve operated by electrical control. The quantity supplied is measured accurately for the period. This quantity, corrected for any change in composition of the gas in the system, equals the quantity of oxygen used by the developing embryos during the period.

ANALYSIS OF GAS IN THE CHAMBER

The composition of the gas in the chamber at the beginning and end of each period was determined by both gravimetric and volumetric analyses.

For the determination of the quantities of moisture and carbon dioxide in the chamber by gravimetric measurement, a small absorption train (fig. 4) was connected in series with a wet gas meter in a shunt on the gas line, and a measured quantity of gas, usually 2 liters, was passed through this absorption train where the water vapor and carbon dioxide in the gas were removed. The absorption train was composed of two specially designed water absorbers (fig. 4, a) of such size that they could be weighed on an analytical balance,

two modified zinc chloride tubes (fig. 4, *b*) containing soda lime for carbon dioxide absorption, and two water absorbers (fig. 4, *c*) for recovering the water imparted to the gas by the carbon dioxide absorbers. This train effectively removed the water vapor and carbon dioxide from the air passing at the rate of 1 liter per minute. The absorbers were weighed to an accuracy of 0.001 g. The increase in weight of the units showed the quantity of water vapor and carbon dioxide absorbed from the volume of gas passed through the train. This volume was measured to an accuracy of 0.001 liter.
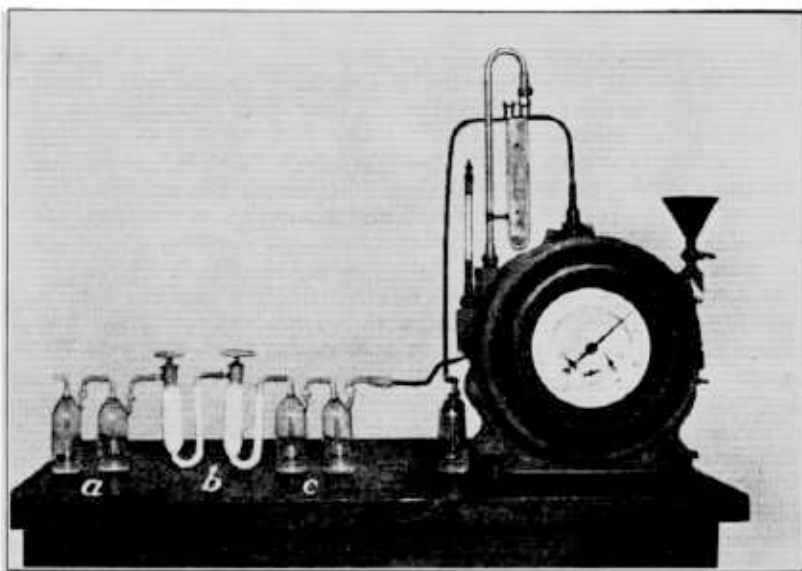

FIGURE 4.—Train for gravimetric gas analysis and meter for measuring gas to be analyzed: *a*, Flasks for absorption of water; *b*, tubes for absorption of carbon dioxide; *c*, flasks for absorption of water eliminated by soda lime in *b*.

Samples of gas from the chamber were analyzed volumetrically to an accuracy of 1 part in 10,000 for carbon dioxide and oxygen. The gas was passed from the sampling tube into a 100-cc measuring burette graduated in 0.1 cc and easily read to 0.01 cc. The pressure was adjusted to equal that in a compensating burette whose volume was equal to that of the measuring burette. Between the two burettes was a small mercury manometer. Equality of pressure between the measuring and compensating burettes was determined by an electrical contact in the manometer, which lighted a signal lamp when equality of pressure was established. The measuring and compensating burettes and manometer were placed in a water bath to maintain constant temperatures during the analysis. The carbon dioxide and oxygen were removed by absorption pipettes which contained potassium hydroxide solution for absorption of carbon dioxide and pyrogallic acid solution for absorption of oxygen.

To determine the quantities of nitrogen, oxygen, and carbon dioxide in the chamber by these methods, the volume of the system

must be accurately known. It may be computed from the dimensions of the chamber, the absorbers, and other equipment. The volume so obtained was verified by ascertaining by analyses the proportion of carbon dioxide in the chamber, then admitting a measured quantity of this gas and subsequently determining the increase in the carbon dioxide content of the gas in the chamber.

TESTS FOR ACCURACY OF CALORIMETER AND RESPIRATORY SYSTEMS

The apparatus as a calorimeter and as a respiratory chamber was tested at frequent intervals to determine the accuracy of the measurements. To test the instrument as a calorimeter, electric energy was converted into heat in a resistance coil within the chamber. The quantity of heat produced in a given period of time was accurately determined from measurements of current flowing in the coil and from the voltage drop across the coil. Measurements of the heat produced by the electric heating element agreed with the calorimetric measurements of this heat to within 1 percent in six tests of 8 hours each.

To test the accuracy of the apparatus as a respiratory chamber, ethyl alcohol was burned in the chamber in such a manner as to insure complete combustion. The quantity of oxygen used in the combustion was measured, and the products of combustion, consisting of water vapor and carbon dioxide, were collected in an absorption train and weighed. The quantity of oxygen required to burn the alcohol and the quantities of carbon dioxide and water vapor resulting from its combustion were computed from the chemical equation for the reactions occurring in the combustion of ethyl alcohol. The computed and measured amounts agreed to within 1 percent in three tests of 8 hours duration each.

EXPERIMENTAL PROCEDURE AND EGGS USED

The experiments reported herein were made from March 1924 to April 1928, during which period 4,000 eggs were incubated and forty-four 3-week tests were conducted.

Eggs used in these experiments were obtained from a selected flock of White Leghorn fowls kept at the National Agricultural Research Center at Beltsville, Md. The feeding and handling of the birds were such that eggs of high fertility and hatchability were produced. The hens were trap-nested and the eggs collected every 3 to 4 hours. The number of the hen and the date laid was marked on the egg. Only eggs less than 7 days old were used in the experiments, and all the eggs were kept under favorable conditions before incubation. They were selected for uniformity of size and shape and for freedom from fine cracks and defective shells. The eggs were weighed to the nearest 0.01 g, and the outline of the air cell was marked on the shell of each egg. They were then put in the calorimeter, which was immediately sealed, and the conditions which were to prevail during incubation relative to temperature, humidity, and other physical factors were established as quickly as possible, 3 to 4 hours being required. Observations of physical conditions were begun immediately and taken every few minutes for the incubation period

of 3 weeks, except for interruptions due to candling. Heat elimina-
tion and gaseous exchange were determined for each 8-hour period,
the results giving a mean value for each period.

The temperature, humidity, and concentration of oxygen and car-
bon dioxide in the air around the eggs in a typical experiment are
shown in figure 5. The temperature was measured with a resistance
thermometer placed inside the calorimeter. For this particular ex-
periment the mean temperature was 102° F., with a mean variation
of only 0.007°, the maximum variation from the mean temperature
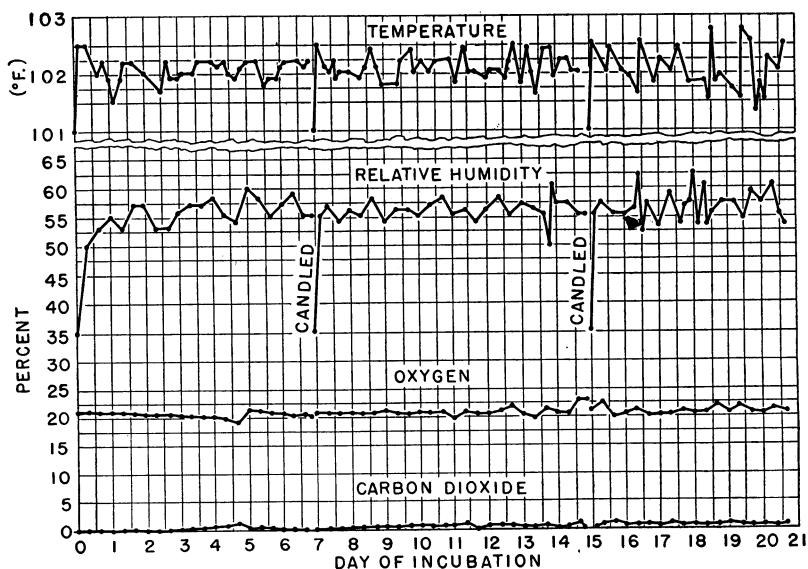to the eighteenth day being 0.5°. The relative humidity measure-

FIGURE 5.—Conditions prevailing in calorimeter during incubation in a typical experiment.

ments were made with a hair hygrometer placed in the air surround-
ing the eggs. The mean humidity was 56 percent; the mean deviation
was 0.1 percent, and the maximum deviation from the mean humidity
was 3.0 percent for short intervals.

The oxygen and carbon dioxide concentrations were obtained from
the volumetric gas analyses. The mean oxygen concentration was 21
percent, the mean deviation was only 0.1 percent, and the maximum
deviation from the mean was 2 percent but only for very short
periods. The mean carbon dioxide concentration was 0.4 percent, the
mean deviation was only 0.1 percent, and the maximum variation
from this value was 1.0 percent at three points for a few hours each.

In determining the effect of any one factor, all the factors except
the one under investigation were held constant. For example, in the
series of tests to study the effect of different temperatures on hatch,
the air around the eggs was kept at a constant humidity and at con-
stant concentrations of oxygen and carbon dioxide, whereas a differ-
ent temperature was used in each experiment.

EFFECT OF VARIOUS FACTORS ON HATCH

EFFECT OF TEMPERATURE DURING INCUBATION

To determine the optimum temperature for incubation a series of tests was conducted at temperatures of 96°, 98°, 99°, 100°, 102°, and 103.5° F. In all these tests the relative humidity was kept at 60 percent, the concentration of oxygen at 21 percent, and the carbon dioxide below 0.5 percent. The air movement past the eggs was approximately 12 cm per minute.

The results in terms of percentage of fertile eggs hatched are shown in figure 6. Each point plotted represents the hatch obtained from
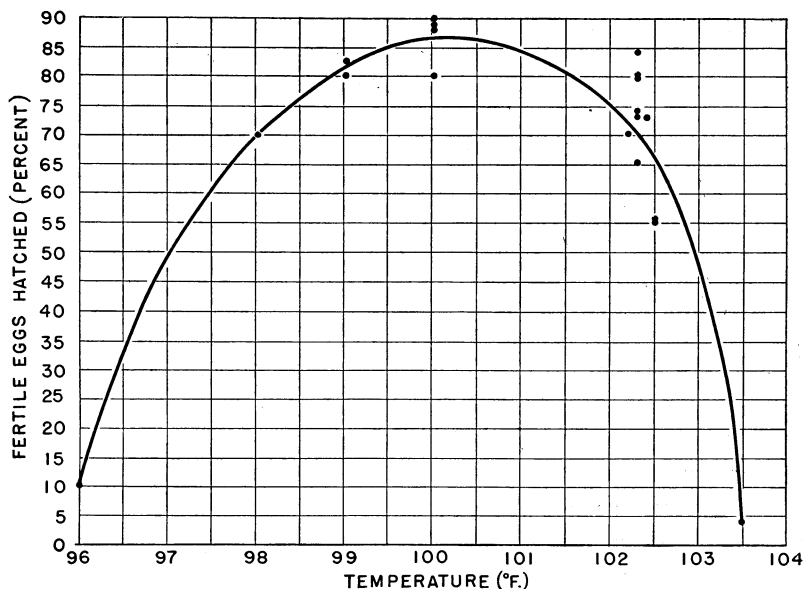

FIGURE 6.—Effect of temperature of incubation on percentage of fertile eggs hatched (relative humidity 60 percent, oxygen 21 percent, carbon dioxide below 0.5 percent).

approximately 90 eggs. The best hatches were obtained at 100° F., which indicates that this is about the optimum temperature for incubation. The hatch decreased at higher and at lower temperatures, slowly at first, then more rapidly as the deviation from 100° increased, until at 96° and at 103.5° nearly all the embryos died in the shell.

The appearance of the chicks varied considerably with the incubation temperature. At 102° F. they were not so large, fluffy, and lively as at 99° or 100°. There were also many abnormalities. The most common troubles were crooked toes and sprawling legs. In the latter case the chicks were unable to stand. Crooked necks also appeared frequently, the chicks never having been able to straighten them after emerging from the shells. Practically no abnormalities occurred at an incubation temperature of 100°, crooked necks never appearing and crooked toes very infrequently, and the chicks were strong and vigorous.

EFFECT OF HUMIDITY DURING INCUBATION

When the investigation was undertaken, it was the consensus of opinion that 102° F. would be found to be the optimum temperature for incubation. Therefore a series of experiments was conducted at this temperature with the relative humidity ranging from 8 to 87 percent. The oxygen content was kept at 21 percent, carbon dioxide below 0.5 percent, and the air movement at 12 cm per minute past the eggs. Later, when it had been determined that the optimum temperature for incubation was about 100°, experiments, with conditions as previously stated except that the temperature was kept at 100°, were conducted at humidities of 30, 44, 61, and 85 percent, to determine whether the effect of humidity on hatch was the same at both
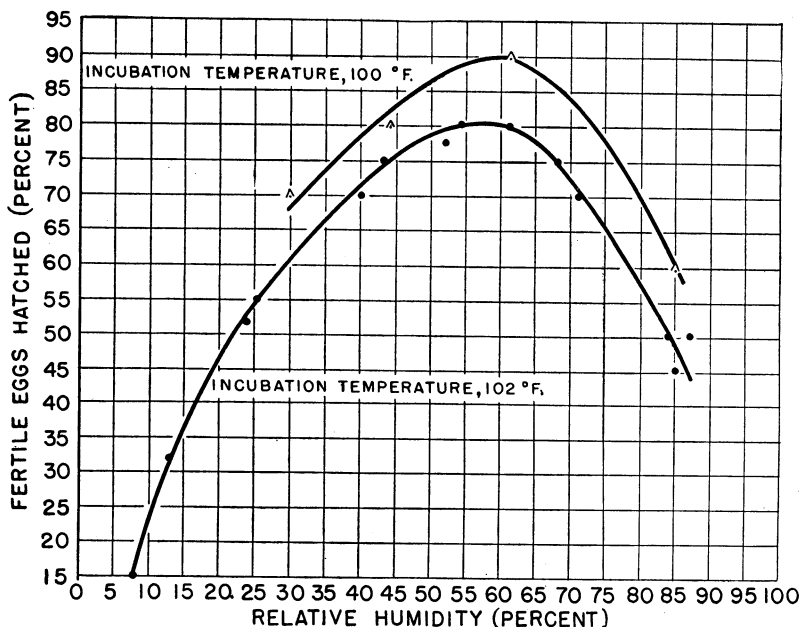

FIGURE 7.—Effect of relative humidity during incubation at two different temperatures on percentage of fertile eggs hatched (oxygen 21 percent, carbon dioxide below 0.5 percent).

temperatures. Figure 7 shows the hatch obtained at the various humidities studied at both temperatures.

The best hatches were obtained at 58 percent humidity at 102° and at 61 percent humidity at 100° F. The effect of variation of humidity was practically the same at both temperatures. The hatch decreased as the humidity varied in either direction from the optimum, at first slowly and then more rapidly as the deviation increased, until at 8 percent humidity, only 15 percent of fertile eggs hatched. Fairly satisfactory hatches may be expected within the range of humidity from 40 to 70 percent, but at higher or lower humidities small hatches of poor-quality chicks are bound to occur.

EFFECT OF CARBON DIOXIDE CONTENT DURING INCUBATION

The results of a series of experiments to determine the effect of varying percentages of carbon dioxide on hatch are shown in figure 8. Curve *a* shows the effect on hatch of varying the carbon dioxide concentration from 0.5 to 4 percent, the percentage in the chamber being kept constant throughout each incubation period. The other factors were: Temperature, 99° F.; relative humidity, 60 percent; oxygen, 21 percent; air movement, 12 cm per minute past the egg.
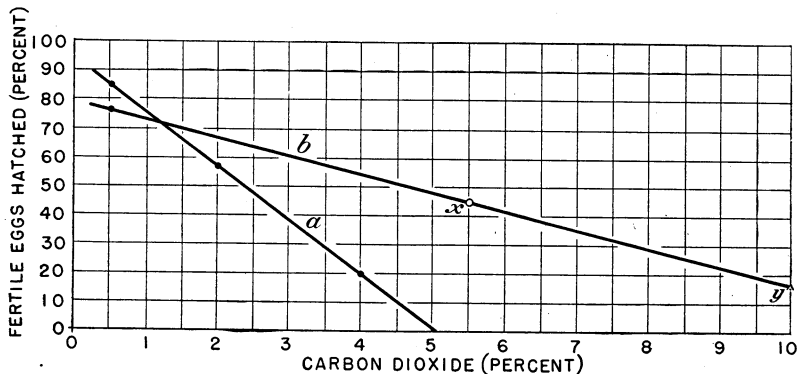

FIGURE 8.—Effect of carbon dioxide during incubation on hatch: *a*, Experiments in which percentage of carbon dioxide at points indicated was kept constant throughout incubation (temperature 99° F.); *b*, experiments in which carbon dioxide for point *x* was as shown in curve *a*, figure 9, and that for point *y* as shown in curve *b*, figure 9 (temperature 102° F.). For both curves relative humidity was 60 percent, oxygen 21 percent.

Curve *b* is drawn through three points, obtained as follows: For the point plotted at 0.5 percent the carbon dioxide was constant throughout the incubation period. For the point *x* plotted at 5.5 percent the carbon dioxide eliminated by the eggs was allowed to accumulate in the chamber. The amount increased, as shown by curve *a*,
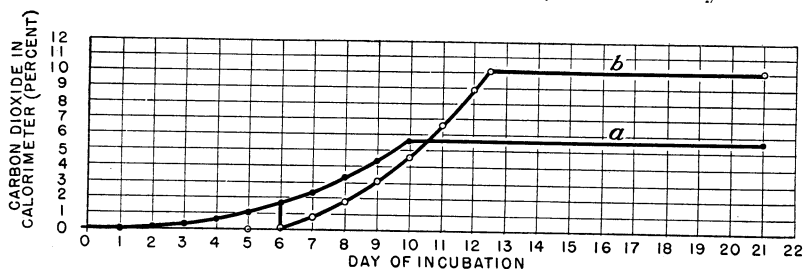

FIGURE 9.—Concentration of carbon dioxide when allowed to accumulate in calorimeter: *a*, 0.5 to 5.5 percent; *b*, 0.5 to 10 percent.

figure 9, until on the tenth day it had reached 5.5 percent; after the tenth day it was held constant at 5.5 percent. For the point *y* plotted at 10 percent the carbon dioxide was allowed to accumulate in the chamber and the amount rose, as shown by curve *b*, figure 9, until on the thirteenth day it had reached 10 percent; after that it was held constant at 10 percent. During these experiments the

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temperature was 102° F.; relative humidity, 60 percent; oxygen, 21 percent; air movement, 75 cm per minute past the egg. Figure 8 shows that the decrease in hatch is proportional to the increase in carbon dioxide. An increase of 1 percent in carbon dioxide reduced the hatch about 15 percent in the experiments in which the carbon dioxide was kept constant. With 4 percent of carbon dioxide, less than one-fourth as many chicks were produced as with 0.5 percent. With 2 percent of carbon dioxide the hatch was over 35 percent less than with 0.5 percent. A gradual increase of carbon dioxide to 5.5 percent was not so harmful as one in which the concentration was 4 percent for the entire time, the hatch for the former being 45 percent as compared with 20 percent for the latter. When there was a gradual increase of 10 percent of carbon dioxide during the first 12 days and a constant percentage of 10 percent after the twelfth day, the hatch—16 percent—was a little lower than when the carbon dioxide was kept at 4 percent during the entire incubation period. The temperature when 10 percent of carbon dioxide was used was not so favorable for incubation as when 4 percent was used.

Results similar to those shown in curve a, figure 8, were also obtained with a relative humidity of 84 percent. With 0.5 percent of carbon dioxide, 51-percent hatch was obtained; with 5.5 percent of carbon dioxide, 33-percent hatch; and with 10 percent of carbon dioxide, 11-percent hatch. The ratio of the hatch at 84 percent relative humidity to that at 60 percent was as follows: With 0.5 percent of carbon dioxide, $\frac{51}{71} = 0.72$; 5.5 percent of carbon dioxide, $\frac{33}{45} = 0.73$; 10 percent of carbon dioxide, $\frac{11}{16} = 0.69$. As the ratio of the hatch is of the same magnitude in each case, it may be concluded that the deleterious effect of high relative humidity is the same at all concentrations of carbon dioxide.

The difference in hatch at 0.5 percent of carbon dioxide in the two curves of figure 8 is due to temperature. The hatch at 99° F. was nearly 10 percent greater than at 102°. Figure 6 also shows the same difference between these two temperatures.

EFFECT OF OXYGEN CONTENT DURING INCUBATION

Eggs were incubated in an atmosphere containing 15, 18, 21, 30, 40, and 50 percent of oxygen. All these experiments were conducted at a temperature of 99° F., relative humidity of 70 percent, carbon dioxide below 0.5 percent, and air movement of 12 cm per minute past the eggs. The effect of the varying percentages of oxygen on the hatch of fertile eggs is shown in figure 10.

The best hatch was obtained with 21 percent of oxygen. It is apparent from the results that it is very important for normal development that the oxygen concentration should not be allowed to decrease below the amount in normal air, and that an excess of oxygen is not nearly so detrimental as a deficiency. A deficiency of 5 percent of oxygen reduced the hatch from 81 to 55 percent, or nearly one-third, yet an excess of 25 percent of oxygen was required to reduce the hatch that much. The hatch decreased approximately 1 percent for each increase of 1 percent in oxygen concentration between 30 and 50 percent, whereas for each decrease of 1 percent in

oxygen below 21 percent the hatch decreased 5 percent. In incubation it is impossible to have an excess of oxygen unless it is artificially supplied. It is very easy, however, to get a deficiency, as the carbon dioxide eliminated is produced at the expense of the oxygen.

EFFECT OF AIR MOVEMENT DURING INCUBATION

A fifth factor which might affect the hatch is the rate of air movement past the egg, although it is difficult to see how this could be a decidedly important factor. With velocities of air movement ranging from 10 to 75 cm per minute past the egg no difference in the hatch could be detected.
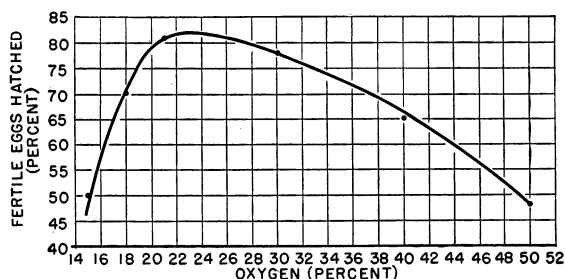

FIGURE 10.—Effect of oxygen during incubation on percentage of fertile eggs hatched (temperature 99° F., relative humidity 70 percent, carbon dioxide below 0.5 percent).

EFFECT OF VARIOUS FACTORS ON ENERGY METABOLISM OF CHICK EMBRYO

FIRST 5 DAYS OF INCUBATION

The carbon dioxide elimination and oxygen consumption of the embryo at the beginning of incubation are so small that it is very difficult to measure them accurately by methods used in this investigation. The results show definite measurable quantities by the end of the second day of incubation.

Heat elimination during the first 3 or 4 days is even more difficult to determine than the gaseous exchange, and much doubt exists as to what occurs during this period. Bohr and Hasselbalch (6) found heat absorption by the embryo for the first 3 days, and Rapkine (33) offers an explanation for the possible theoretical value of such results.

There are many sources of error in measuring the small quantity of heat involved, and the introduction of any one of these errors might easily change the results so that heat absorption would become heat elimination or vice versa. The results of this investigation frequently showed heat absorption during the first 3 days of incubation, but such results were obtained only when the experiment was begun after the calorimeter had stood for several days at room temperatures of from 70° to 80° F. and the temperature was then raised to that for incubation in 4 or 5 hours. When incubation was begun after the calorimeter had been kept slightly above incubation temperature for several days no such results were obtained. Thus it appears that results showing heat absorption were due to the following: Some part of the calorimeter where temperature measurements were not made maintained a temperature lower than that for incubation, and a small amount of heat was conducted to this part.

This heat came from the embryos and was not measured, a fact that made the measured values too low and made it appear that the heat was absorbed by the embryos.

Because of these difficulties in determining heat production, it seemed advisable to treat this portion of the incubation period by itself and obtain as accurately as possible, from all available data, values which will apply to all work subsequently discussed. In this investigation, there were 30 experiments in which the incubation factors were such that the results the first week should have been the same. Some of these were made after the calorimeter had stood at a temperature lower than that for incubation and others after it had stood at a temperature higher than that for incubation. The mean of all the results of these experiments should represent the actual facts very closely and better than the results of any one individual experiment. These results appear in table 1. The data in the last column are used throughout this investigation as representing the heat output for the first 5 days of the incubation period.

TABLE 1.—*Heat elimination of 100 eggs in 8-hour periods for the first 5 days of incubation*

Age of embryo (hours)	Mean results in square calorimeter			Mean results in round calorimeter			Mean of the two calorimeters	Results adjusted[1]
	No preliminary heating	Preliminary heating	Mean	No preliminary heating	Preliminary heating	Mean		
	Calories	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
4	−0.4	0.6	0.1	−2.0	2.3	0.1	0.1	0.00
12	−1.0	.6	−.2	−2.1	2.3	.1	.0	.00
20	−1.0	.7	−.1	−2.6	2.8	.1	.0	.00
28	−1.2	.8	−.2	−2.8	3.3	.3	.0	.00
36	−1.2	.3	−.4	−2.0	2.7	.4	.0	.00
44	−.9	.9	.0	−2.3	2.8	.2	.1	.05
52	−1.0	1.2	.1	−2.5	3.0	.2	.1	.10
60	−.7	.3	−.2	−2.2	3.0	.4	.1	.20
68	−.4	.5	.1	−2.0	3.2	.6	.3	.30
76	.5	.3	.4	−1.6	3.0	.7	.5	.50
84	1.0	.0	.5	−1.9	3.7	.9	.7	.70
92	1.2	.6	.9	−1.0	3.5	1.2	1.0	1.00
100	1.5	1.0	1.2	−.6	3.7	1.5	1.3	1.25
108	1.6	1.2	1.4	−.3	4.0	1.8	1.6	1.55
116	2.0	1.9	2.0	−.2	4.3	2.0	2.0	2.20
124	2.4	2.5	2.4	.3	4.5	2.4	2.4	2.40

[1] The means in the eighth column were plotted on cross-section paper and a smoothed curve drawn through the plotted points. The data in the last column were taken from this curve.

ENERGY AND WATER METABOLISM OF DEVELOPING CHICK EMBRYO AT OPTIMUM CONDITIONS OF INCUBATION

The energy metabolism of the chick embryo gives an accurate measure of its rate of development. Figure 11 shows heat elimination, carbon dioxide elimination, and oxygen consumption for the entire period of incubation when optimum conditions prevail, temperature being 100° F., relative humidity 60 percent, oxygen 21 percent, and carbon dioxide below 0.5 percent. The points plotted for each 8-hour period represent the mean of four experiments.

Energy metabolism was too small to measure at the beginning of incubation but increased in magnitude uniformly for the first week. After the first week the rate was accelerated until the thirteenth day. Between the thirteenth and seventeenth days it again increased uni-

formly. Between the seventeenth and nineteenth days it became practically constant, but after the nineteenth day it increased more
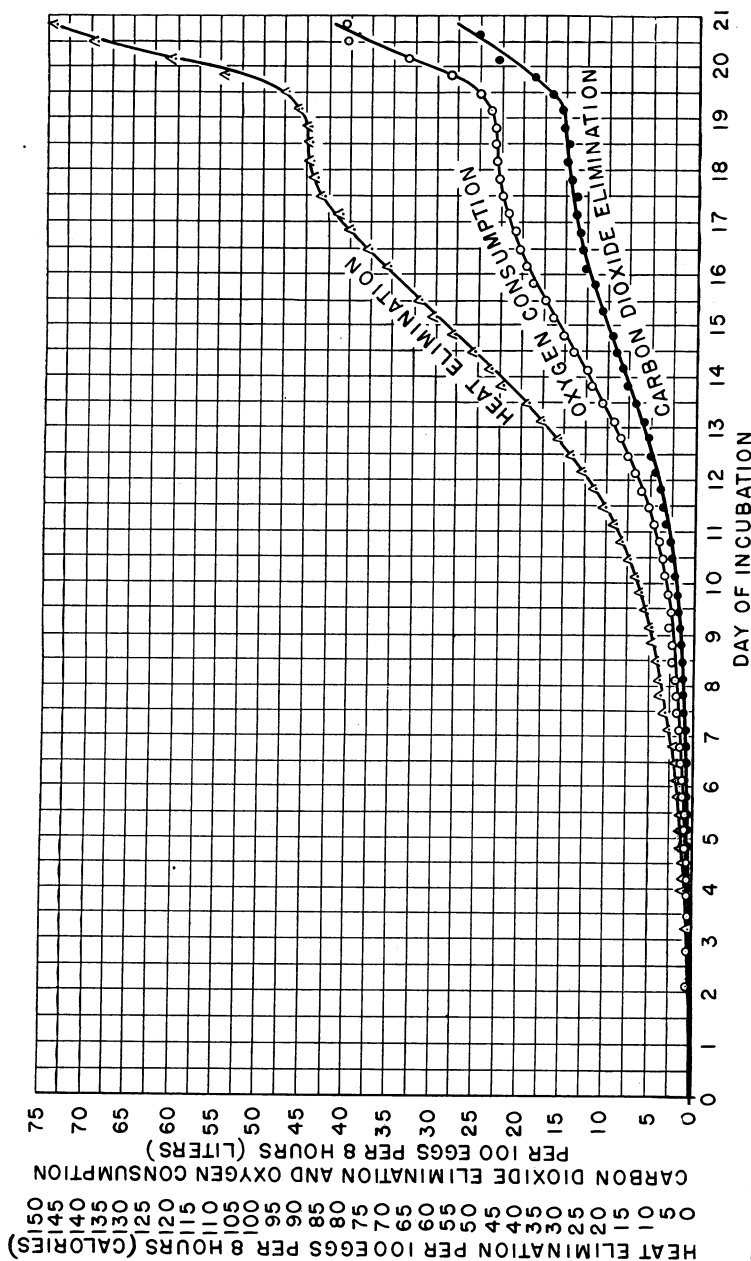

FIGURE 11.—Energy metabolism of the chick embryo at optimum conditions of incubation (temperature 100° F., relative humidity 60 percent, oxygen 21 percent, and carbon dioxide below 0.5 percent).

rapidly than at any previous time. The mean rate for the period between the thirteenth and seventeenth days was approximately 20 times that between the first and seventh days, and the rate between

the twentieth and twenty-first days was 50 times that between the first and seventh days.

Investigation as to the reason that the heat elimination and gaseous exchange increased only slightly on the eighteenth day affords a fertile field for physiologists and embryologists. Very pronounced physiological changes are taking place in the embryo at this time and one is probably closely associated with the other. The respiratory system undergoes a complete change about this time, with initiation of pulmonary respiration of atmospheric air. Romanoff (*35*) in summarizing certain data on growth of embryo finds a marked retardation in growth at this time.

INTENSITY OF ENERGY METABOLISM PER GRAM WEIGHT OF LIVING TISSUE

The relationship between energy metabolism and embryonic growth has been based almost entirely on the wet weight of the chick embryo alone, the weights of the yolk sac and of the allantois being disre-
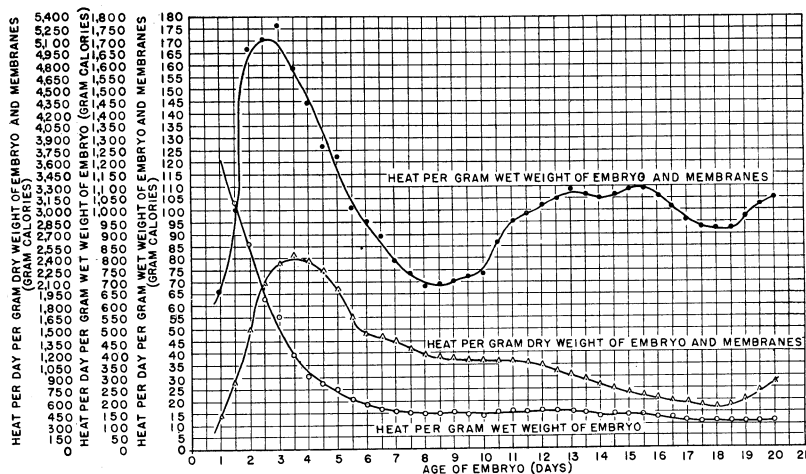

FIGURE 12.—Intensity of energy metabolism of the chick embryo.

garded. Needham (*30*) discusses this question and shows the importance of considering the membranes as well as the embryo. Independent data on wet weights of embryo, of yolk sac, and of allantois obtained by Byerly (*8*), together with the data for heat elimination by the author (table 2), made it possible to compute the ratio of the heat elimination to the wet weight of chick embryo, to the dry weight of embryo and membranes, and to the wet weight of embryo and membranes. The results are shown in figure 12.

The curve involving the wet weight of embryo and membranes starts with low values at the beginning of incubation and rises to a maximum on the third day, then decreases rapidly until the ninth day. The curve involving dry weight is similar in the course followed to that involving wet weight during this period, differing only in absolute value. After the ninth day, however, the curve involving wet weight rises again whereas that involving dry weight

continues to fall. This shows that there is an•increasing percentage of solid matter in the embryo as its age increases. The curve involving the wet weight of embryo alone is very high at the beginning of incubation but decreases rapidly, reaching a constant value about the eighth day. The difference in form of the curves for wet weight of embryo alone and for wet weight of embryo and membranes shows that at the beginning of incubation the embryo is an insignificant part of the total living tissue, the greatest part being the yolk sac which has a weight nearly 10 times that of the embryo.

RESPIRATORY AND THERMAL QUOTIENT

The respiratory quotient is the ratio of the liters of carbon dioxide eliminated to the liters of oxygen consumed. The thermal quotient, as determined in this research, is the ratio of calories of heat elimi-
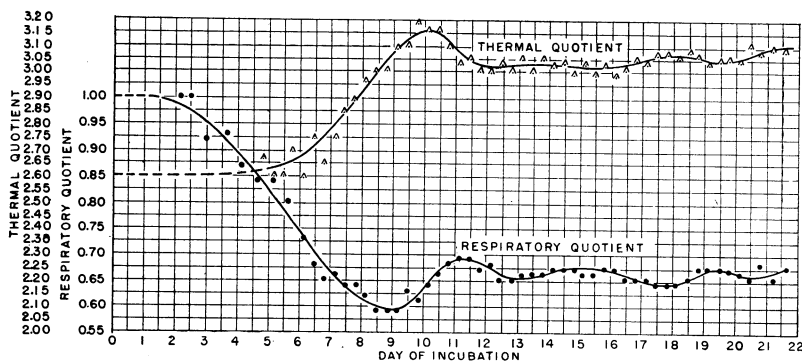

FIGURE 13.—Respiratory quotient and thermal quotient (computed from carbon dioxide).

nated to the grams of carbon dioxide eliminated. These two derived factors are of great importance, according to Needham (29), whenever a study of metabolism is being made. Carbohydrate metabolism alone is represented by a respiratory quotient of 1.00 and a thermal quotient of 2.57. Fat alone gives a respiratory quotient of 0.71 and a thermal quotient of 3.35, and in the chick embryo protein gives practically the same respiratory quotient and thermal quotient as does fat, when the end product is uric acid.

The values for these factors obtained in this investigation are shown in figure 13. The respiratory quotient was 1.00 for the first 2 days dropped rapidly to 0.60 by the ninth day, rose to 0.66 by the eleventh day and remained fairly uniform during the remainder of the investigation. The thermal quotient began at 2.60 and rose rapidly to above 3.00. The results of this investigation indicate a carbohydrate metabolism during the first few days and fat and protein metabolism almost exclusively after about the tenth day.

The values obtained in this investigation—respiratory quotient of 0.67 and thermal quotient of 3.03—after the second week of incubation are both somewhat lower than the theoretical values for fat and protein. These low values can be explained by assuming a transformation of fat or protein to sugar. The low values for respira-

tory quotient and the high values for thermal quotient on the ninth and tenth days of incubation suggest a field of investigation with regard to the transformation of protein or fat to sugar.

EFFECT OF TEMPERATURE ON ENERGY METABOLISM

To determine the effect of variation in temperature of incubation on heat elimination and gaseous exchange, experiments were conducted at 96°, 98°, 99°, 100°, 102°, and 103.5° F. The conditions other than temperature were in all cases as follows: Relative humidity, 60 percent; oxygen concentration, 21 percent; and carbon dioxide concentration, below 0.5 percent. The results obtained appear in table 2. Heat elimination in terms of 100 eggs per day is shown in figure 14 for the entire temperature range studied. The curves for carbon dioxide elimination and oxygen consumption, if plotted, would differ only in absolute value from the curves for heat.

Temperature has a decisive effect on the energy metabolism of the chick embryo during incubation. It is greatly retarded at low temperatures, but as the temperature is raised there is a marked increase as shown by the positions of the curves for figure 14.

TABLE 2.—*Effect of variation of incubator temperature on energy metabolism of chick embryos with relative humidity 60 percent, oxygen 21 percent, and carbon dioxide less than 0.5 percent*

[Results are for 100 eggs per 8 hours]

Age of embryo (hours)	Heat elimination [1] at—						Carbon dioxide elimination at—						Oxygen consumption at—					
	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)
	Calories	Calories	Calories	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters
3							0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11							.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
19							.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
27							.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.1
35							.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.3
43							.0	.0	.0	.0	.0	.1	.0	.0	.0	.0	.0	.3
51							.0	.0	.0	.1	.1	.1	.0	.0	.0	.1	.0	.2
59							.5	.0	.1	.1	.1	.2	.0	.0	.0	.1	.1	
67							.2	.1	.1	.1	.2	.3	.0	.1	.1	.2	.1	.0
75							.0	.1	.2	.1	.2	.4	.1	.2	.1	.2	.2	.5
83							.1	.2	.2	.2	.3	.4	.3	.3	.2	.3	.2	
91							.3	.1	.2	.2	.4	.6		.2	.2	.5	.4	
99							.0	.3	.3	.3	.4	.4	.4	.2	.3	.4	.6	.8
107							.2	.4	.4	.3	.5	.4		.3	.4	.6	.7	
115							.4	.5	.4	.4	.5		.3	.3	.5	.4	.7	.6
123	1.6	2.4	2.0	1.9	2.0	3.4	.4	.5	.5	.4	.6	.3	.3	.3	.5	.6	.7	.6
131	2.2	3.3	----	2.2	2.3	4.4	.5	.9	.5	.5	.6	.6	.3	.3	.6	.6	.6	
139	3.2	2.3	2.5	2.5	2.6	6.3	.5	.9	.5	.5	.7	.6	.4	.4	.7	.7	.8	.8
147	5.2	----	2.8	2.8	2.8	9.7	.4	.9	.5	.6	.8	.6	.4	.5	.7	.8	1.1	
155	----	2.9	3.0	3.2	3.0	8.2		.7	.6	.6	.8	.9	.7	.6	.8	1.0	1.1	.9
163	5.8	3.8	----	3.6	3.5	10.2	.6	.7	.7	.7	.9	.8		.7	1.0	1.2	1.5	.9
171	5.2	4.2	3.7	5.6	4.2	----	.6	.7	.7	.8	1.0	1.2	.4	1.1	1.0	1.4	1.4	1.6
179	5.2	4.5	4.0	6.6	5.6	6.3	.6	.8	.8	.9	1.1	1.1	.8		1.1	1.5	1.6	2.0
187		5.6	4.6	7.3	6.2	7.2	----	.7	.9	1.0	1.3	1.3		.9	1.3	1.6	2.2	1.7
195	3.5	3.6	5.8	7.6	7.1	7.4	.6	.9	1.0	1.1	1.4	1.1		1.2	1.5	1.9	2.3	2.2
203		5.9	6.4	8.0	8.1	7.4	.6	1.0	1.0	1.2	1.5	1.2	1.6	1.4	1.6	2.1	2.6	2.3
211	2.6	5.9	7.1	8.4	9.4	7.9	.6	1.0	1.1	1.3	1.7	1.3	1.1	.9	1.8	2.4	3.0	
219	4.4	6.2	7.6	9.4	10.7	8.5	.7	1.1	1.2	1.5	1.9	1.4	1.2	1.7	2.0	2.6	3.3	2.5
227	3.5	6.6	8.2	10.4	12.4	9.7	.8	1.2	1.4	1.6	2.1	1.4	1.2	2.1	2.3	2.7	3.3	3.3
235	3.7	7.5	9.4	12.1	13.8	9.5	.8	1.5	1.5	1.8	2.3	1.9	1.2	1.8	2.6	2.9	3.5	
243	5.3	8.8	10.8	13.0	14.7	12.1	.9	1.4	1.6	2.0	2.6	1.8	1.5	1.9	2.6	3.0	4.0	3.0
251	5.5	9.3	11.8	14.7	18.5	12.5	.9	1.5	1.8	2.3	2.9	2.3	1.6	2.8	2.7	3.5	4.4	3.4

[1] Because of the minute changes in heat eliminated during the first 5 days and the difficulty of determining them accurately, no results are given for individual experiments. See table 1 and explanation in text.

TABLE 2.—*Effect of variation of incubator temperature on energy metabolism of chick embryos with relative humidity 60 percent, oxygen 21 percent, and carbon dioxide less than 0.5 percent*—Continued

[Results are for 100 eggs per 8 hours]

Age of embryo (hours)	Heat elimination at—						Carbon dioxide elimination at—						Oxygen consumption at—					
	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)	96° F. (1 experiment)	98° F. (1 experiment)	99° F. (3 experiments)	100° F. (6 experiments)	102° F. (4 experiments)	103.5° F. (1 experiment)
	Calories	Calories	Calories	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters
259	5.2	10.9	12.9	16.4	20.2	14.0	1.0	1.6	2.0	2.6	3.3	2.6	----	2.9	2.9	4.0	4.8	4.0
267	7.3	11.3	14.3	18.5	22.3	18.0	1.0	1.8	2.3	3.0	3.8	3.2	1.5	2.8	3.3	4.5	5.5	5.5
275	7.6	11.6	16.2	20.6	26.2	19.7	1.4	2.1	2.7	3.5	4.3	3.5	----	3.4	3.9	5.1	6.3	4.6
283	8.7	14.5	18.5	22.9	29.0	23.1	1.4	2.4	3.1	4.0	5.0	4.1	1.3	3.2	4.5	6.1	7.3	5.5
291	10.5	18.0	20.8	25.5	31.5	26.0	1.5	2.5	3.4	4.5	5.5	4.6	1.7	4.9	5.1	6.9	8.0	
299	9.7	18.5	23.2	28.3	35.8	29.5	1.6	2.5	3.8	5.0	5.8	5.2	2.1	4.1	6.0	7.6	9.1	7.8
307	10.3	24.1	25.6	31.6	39.5	33.2	1.8	3.6	4.3	5.4	6.8	5.6	1.9	5.0	6.8	8.4	10.0	
315	14.0	26.2	28.4	35.6	44.0	40.5	1.9	3.7	4.8	6.0	7.3	6.6	3.2	5.7	7.2	9.1	10.8	10.7
323	12.3	23.5	31.7	39.4	48.1	41.8	2.1	4.3	5.3	6.7	8.2	7.0	2.8	6.2	8.1	10.5	12.1	11.1
331	13.3	28.2	35.7	44.3	52.2	50.0	2.1	4.3	6.0	7.5	8.3	7.8	4.2	6.7	9.2	11.9	12.6	----
339	22.2	----	39.7	47.3	57.1		2.6	5.3	6.6	8.2	9.6	8.5	4.7	8.2	10.2	12.4	13.6	----
347	19.7	35.6	43.6	50.7	61.4	54.4	2.9	5.7	7.1	9.0	11.0	9.4	4.8	9.8	11.3	13.9	14.8	13.3
355	21.6	41.1	47.6	55.9	66.0	61.0	3.3	6.3	7.7	9.7	11.6	10.4	6.2	{10.3	12.5	15.2	16.3	}17.1
363	----	44.3	51.6	60.7	68.3	{69.0	4.2	7.0	8.5	{10.6	11.6	11.0	5.5	(10.3)	13.3	16.3	17.0	(17.1)
371	30.2	47.3	55.9	63.8	71.1	(69.0)	4.4	7.6	9.3	(10.6)	12.4	11.3	6.5	10.2	14.0	17.2	18.3	{18.1
379	33.0	53.4	60.2	----	76.0	71.0	4.9	8.3	10.0	11.6	13.0	11.7	8.3	12.2	14.9	18.8	18.4	(18.1)
387	36.4	55.4	64.0	70.4	78.4	71.0	5.6	8.7	10.5	12.6	14.0	11.7	8.8	13.3	16.0	19.6	19.5	18.7
395	41.0	56.6	67.3	75.2	81.8	71.6	6.4	9.6	11.1	13.0	13.9	12.2	10.1	14.1	17.0	20.2	21.1	18.7
403	45.6	65.5	70.4	79.2	81.9	----	6.8	10.1	11.7	13.2	13.7	----	----	11.3	15.5	17.8	20.7	21.1
411	48.8	65.9	74.1	82.0	83.3	----	7.3	11.0	12.2	13.8	14.2	12.5	10.8	15.8	18.8	21.6	21.5	18.3
419	52.2	69.6	78.1	86.6	84.4	83.7	7.9	11.5	12.6	13.7	14.3	14.6	12.3	17.1	20.0	22.2	22.1	22.9
427	57.2	74.0	81.9	87.4	83.1	89.0	8.7	11.6	12.9	14.4	14.6	15.5	13.1	17.4	21.0	22.7	21.7	22.8
435	59.0	78.5	84.9	89.4	87.6	94.4	10.1	12.7	13.3	14.8	14.4	15.9	13.4	18.1	21.8	22.9	22.7	----
443	62.0	80.0	86.3	89.1	90.2	102.7	9.8	13.3	14.0	14.8	15.7	{18.2	14.4	{20.9	21.9	22.9	23.7	24.7
451	67.6	84.4	87.1	89.1	96.5	108.5	9.5	13.7	14.5	15.4	16.6	(18.2)	16.6	(20.9)	21.5	23.0	24.6	----
459	72.2	87.0	87.9	91.8	102.9	}120.8	11.7	14.0	14.7	15.5	17.3	19.1	17.6	20.7	21.3	23.9	25.0	}33.1
467	75.1	84.2	89.6	94.6	110.0	(120.8)	12.0	13.9	14.9	16.7	18.5	19.6	18.1	}20.4	21.3	24.8	28.8	(33.1)
475	78.7	88.5	93.8	108.8	128.0	128.3	12.6	14.6	15.2	18.6	20.1	21.2		(20.4)	22.1	28.1	29.9	30.3
483	80.4	87.2	100.8	121.2	137.0	(2)	12.3	14.8	16.1	19.2	21.2	(2)	19.4	21.0	24.1	33.2	33.6	(2)
491	78.4	92.7	110.0	139.0	140.0	----	13.1	15.5	17.6	{25.0	22.5	23.2	19.1	22.0	26.8	40.0	35.7	----
499	80.6	102.4	121.8	149.7	146.0	----	13.2	----	20.1	(25.0)	23.2	----		27.0	29.5	40.5	33.8	----
507	80.2	110.7	134.0	155.0	151.0	----	13.7	----	22.5	26.4	23.6	----	20.2	28.3	----	40.4	37.1	----
515	82.9	116.4	145.0	160.0	148.0	----	14.5	20.5	24.5	27.2	23.3	----	20.9	30.6	34.0	41.5		----

523	90.8	133.5	154.7	(²)	(²)	13.9	22.6	26.2	(²)	(²)	22.1	32.2	36.0	(²)	(²)	
531	95.8	159.3	(²)			15.8	24.1	(²)			22.4	38.2	(²)			
539	105.3	169.0				19.1	26.8				27.5	38.2				
547	122.2	157.0				21.6	27.9				29.0	42.3				
555	130.5	(²)				21.2	(²)				31.2	(²)				
563	138.3					23.4					34.2					
571	161.5					26.6					36.6					
579	179.2					26.7					45.6					
587						30.5					49.7					

² Experiment ended.

The higher the temperature of incubation the greater the metabolic rate, and consequently, the more rapid the development of the chick
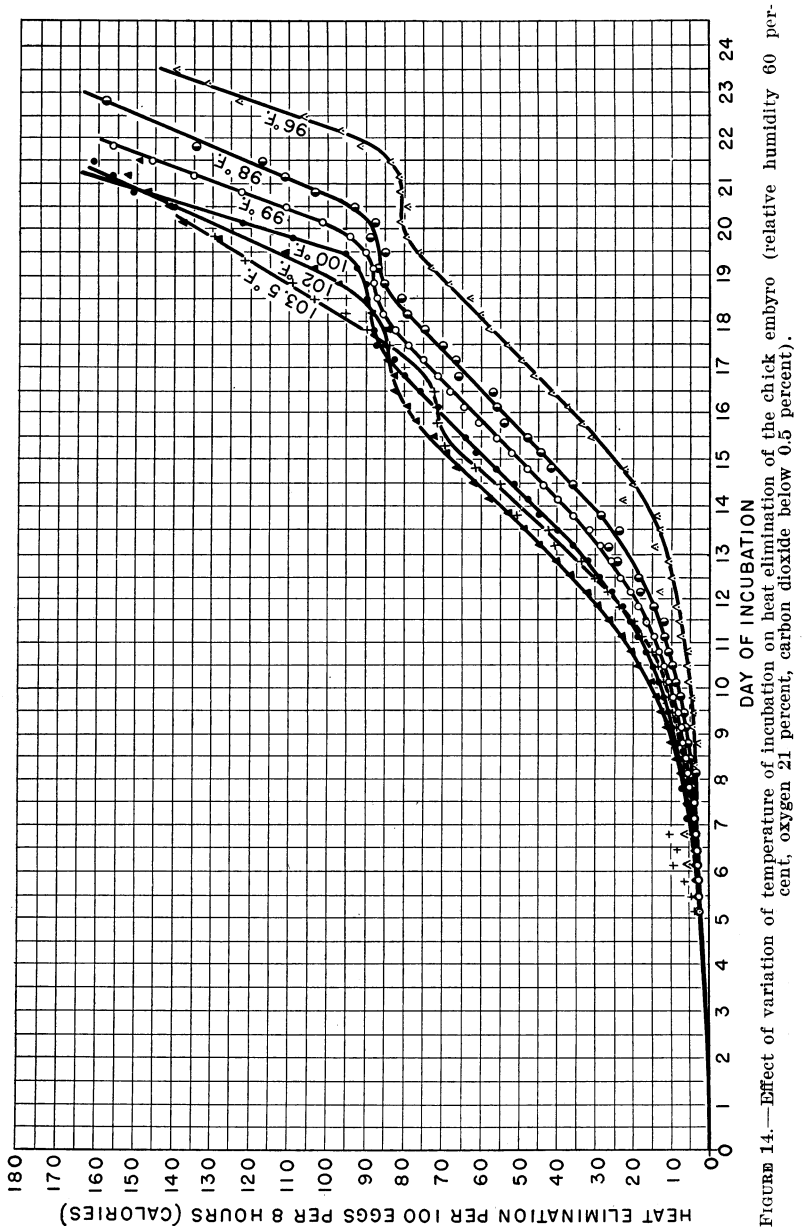

FIGURE 14.—Effect of variation of temperature of incubation on heat elimination of the chick embyro (relative humidity 60 percent, oxygen 21 percent, carbon dioxide below 0.5 percent).

and the earlier the hatch. However, there is an optimum rate of growth, which is produced by a temperature of 100° F., and any deviation from this growth rate produces an inferior chick and a fewer number from a given number of fertile eggs.

The heat elimination for the fourteenth day at various temperatures was as follows: 96° F., 40 cal.; 98°, 80 cal.; 99°, 95 cal.; 100°, 120 cal.; 102°, 145 cal.; 103.5°, 130 cal.

The effect of temperature on the energy metabolism of the chick embryo was greatest during the second week of incubation. After this time growth proceeded at a uniform rate regardless of temperatures, as shown by the fact that most of the curves parallel one another.

The total heat elimination for the entire period of incubation computed from table 2 to time of hatch appeared to be the same regardless of temperature and was approximately 2,000 kg cal. per 100 embryos. As the temperature of incubation was lowered the rate of metabolism decreased, but the time of incubation was increased so that the total, which is a product of these two, remained constant.

EFFECT OF HUMIDITY ON ENERGY METABOLISM

To determine the effect of relative humidity on heat elimination and gaseous exchange of the chick embryo, a series of experiments were made covering the range shown in table 3. The conditions other than humidity were constant, as follows: Temperature, 102° F.; oxygen, 21 percent; carbon dioxide, below 0.5 percent.

TABLE 3.—*Effect of variation in relative humidity on energy metabolism of chick embryos with temperature of 102° F., oxygen 21 percent, and carbon dioxide less than 0.5 percent*

[Results are for 100 eggs per 8 hours]

Age of embryo (hours)	Heat elimination [1] at indicated percentage of relative humidity [2]					Carbon dioxide elimination at indicated percentage of relative humidity							Oxygen consumption at indicated percentage of relative humidity						
	24	42	58	70	84	8	13	24	42	58	70	84	8	13	24	42	58	70	84
	(2 experiments)	(2 experiments)	(6 experiments)	(2 experiments)	(3 experiments)	(1 experiment)	(1 experiment)	(2 experiments)	(2 experiments)	(6 experiments)	(2 experiments)	(3 experiments)	(1 experiment)	(1 experiment)	(2 experiments)	(2 experiments)	(6 experiments)	(2 experiments)	(3 experiments)
	Calories	Calories	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters
3						0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11						.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
19						.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
27						.0	.1	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
35						.0	.0	.0	.0	.0	.0	.0	.2	.0	.0	.0	.0	.0	.0
43						.0	.1	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
51						.1	.2	.1	.1	.1	.1	.1	.0	.0	.1	.1	.0	.1	.0
59						.1	.1	.1	.1	.1	.1	.1	.3	.0	.2	.1	.1	.1	.0
67						.1	.4	.1	.2	.2	.1	.1	.0	.3	.3	.1	.1	.1	.1
75						.3	.5	.2	.2	.2	.2	.3	.3	.0	.3	.1	.2	.1	.1
83						.3	.6	.3	.2	.3	.2	.4	.3	.3	.3	.2	.2	.1	.2
91						.4	.5	.4	.2	.4	.3	.4	.3	.0	.3	.2	.4	.1	.2
99						.4	.5	.6	---	.4	.2	.4	1.6	1.9	.8	.5	.6	.2	.3
107						.6	.9	.9	.3	.5	.4	.6			.8	.6	.7	.2	.4
115						.4	.8	1.0	.4	.5	.4	.7	1.2	1.6		.8	.7	.7	
123	2.9		2.0	2.0	3.4	.7	.4	.9	.5	.6	.5	.5	.8	1.8	.9	.7	.7	1.2	1.1
131	3.3	1.5	2.3	2.4	3.8	.8	1.3	.9	.6	.6	.6	.6			1.0	1.4	.6	1.5	1.1
139	3.9	1.3	2.6	2.4	3.7	1.2	.9	.8	.6	.7	.7	.7		.3	1.0	1.3	.8	.9	.7
147	5.2	1.4	2.8	2.8	4.0	1.3	.9	1.0	.7	.8	.8	.7	1.1	1.3	1.0	1.3	1.1	1.1	.9
155	6.4	2.0	3.0	2.9	5.0	1.0	.9	1.0	.8	.8	.9	.8	2.2		1.4	1.4	1.1	1.0	1.3
163	5.1	4.0	3.5	3.5	5.8	1.3	---	.9	.9	.9	1.0	1.0	4.1		1.5	1.7	1.2		
171	4.5	4.4	4.2	4.7	6.1	1.5	1.0	1.2	1.0	1.0	1.0	1.0	2.8	7.1	1.8	1.7	1.4	1.6	
179	5.0	6.3	5.6	6.0		1.2	1.2	1.0	1.1	1.1	1.1	1.2	6.2	1.3	1.8	1.6	2.2	1.3	
187	6.4	6.1	5.6	5.5	6.3	---	1.7	1.2	1.2	1.3	1.2	1.3	2.3	1.4	2.0	2.2	2.2		
195	6.9	7.9	7.1	7.3	7.3	1.8	2.1	1.5	1.4	1.4	1.2	1.2	4.5	2.4	2.4	1.8	2.3	1.9	1.1
203	8.9	8.4	8.1	9.3	8.4	1.4	2.9	1.4	1.6	1.5	1.5	1.5	4.0	.6	2.7	2.0	2.6	3.5	1.9
211	9.5	11.0	9.4	9.9	10.7	1.7	2.4	1.9	1.7	1.7	1.7	1.5	4.8	2.3	1.8	2.2	3.0	3.0	1.7
219	12.4	13.2	10.7	10.7	6.0	1.6	3.7	1.8	1.7	1.9	1.9	1.9	2.2	3.3	2.8	3.3	3.8	3.0	
227	14.5	14.8	12.4	12.7	9.0	2.5	4.8	2.1	2.0	2.1	2.1	1.9		2.4	2.8	3.6	3.3	3.8	3.0
235	12.9	15.1	13.8	13.0	12.7	1.8	4.7	2.3	2.3	2.3	2.2	1.9	3.9	6.6	3.7	3.5	4.4	3.4	
243	15.6	17.3	14.7	16.0	14.9	2.2	2.4	2.6	2.6	2.6	2.6	2.6	5.0	5.0	5.4	4.0	5.4	2.6	
251	21.1	18.6	18.5	19.2	17.9	2.9	3.3	3.2	3.1	2.9	3.0	3.4	4.3	3.5	5.6	5.4	4.4	5.5	3.2

259	20.5	20.9	20.2	21.4	18.8	3.5	2.4	3.6	4.0	3.3	3.6	3.6	4.9	4.3	4.8	6.1	4.8	6.2	6.0
267	23.4	24.6	22.3	24.9	22.1	2.9	3.1	3.7	4.7	3.8	4.0	4.3	6.3		5.6	6.1	5.5	6.5	5.8
275	26.2	28.1	26.2	28.5	23.3	4.3	4.7	4.3	4.8	4.3	4.5	4.6	6.3	5.9	9.8	7.5	6.3	7.6	6.6
283	29.8	32.0	29.0	30.8	28.7	4.9	4.5	4.4	5.5	4.3	5.0	5.7	7.7	8.2	7.0	8.9	7.3	8.8	6.6
291	33.4	35.3	31.5	34.8	31.8	·3.2	5.1	4.9	6.0	5.5	5.7	5.2	10.0	8.2	7.0	9.2	8.0	9.6	6.3
299	34.6	43.0	35.8	40.5	34.5	5.0	5.8	5.5	6.8	5.5	6.4	6.7	10.0	10.6	8.2	10.2	9.1	10.4	7.7
307	38.7	42.8	39.5	43.9	37.5	5.8	6.4	6.0	7.1	6.8	7.5	7.0	11.5	10.3	10.7	11.1	10.0		7.7
315	43.6	45.5	44.0	49.6	42.7	6.7	7.1	6.3	7.8	7.3	8.2	8.5	12.3	10.8	12.5	12.9	10.8	13.5	10.5
323	47.7	50.8	48.1	51.9	48.3	10.8	7.5	7.7	8.4	8.2	8.6	8.4	12.3	14.0	15.4	13.7	12.1	13.5	12.7
331	51.1		52.2	55.4	50.9	8.6		9.4	9.1	8.3	9.3	8.5	14.9		14.3	13.8	12.6	14.2	13.2
339		57.5	57.1	62.6	52.7	9.0	9.5	9.7	9.9	9.6	9.7	9.3	14.9		14.3	14.2	14.8	15.2	15.4
347	57.6	62.6	61.4	63.4		10.6	10.5	10.2	10.2	11.0	10.5	10.5	18.4	18.5	16.0	16.8	14.8	15.2	15.4
355	59.2	63.4	66.0		58.1		9.9	10.2	11.0	11.6	11.4	10.4	18.4	18.8	16.5	17.4	15.4	17.0	15.3
363	65.2	68.4	68.3	71.1	63.2	10.9	10.5	11.5	11.6	11.6	11.7	11.2	22.7	18.6	16.7	17.6	16.0	18.4	16.0
371	69.9	72.6	71.1	71.2	66.3	11.5	11.5	12.2	12.4	12.1	11.3	11.8	18.5	20.3	19.5	19.8	18.3		17.5
379	71.8	73.6	76.0	76.6	69.0	12.0	11.5	12.6	12.3	13.0	12.8	11.8	20.6	20.3	17.0			19.8	17.5
387			77.0	78.4	71.6	11.8	10.2	13.2	13.4	14.0	12.4	12.1	22.4	20.9	16.5	20.9	18.4	19.8	17.7
395	75.8	77.9	81.8	78.4	75.0	11.8	11.4	12.2	13.5	13.9	13.2	12.2	19.6	21.9	19.8	19.5	21.1	20.1	18.5
403	75.8	79.2	81.9	80.4	74.7	11.8	11.3	11.5	13.4	13.7	13.1	12.4	21.3	29.9	20.3	20.0	21.1	20.8	19.4
411	77.8	79.6	83.3	80.5	75.4	9.7	11.0	12.5	13.8	14.2	13.6	12.3	21.3		20.3	21.7	21.5	21.4	21.8
419	78.2	80.8	84.4	81.6	75.3	11.4	10.4	12.6	13.4	14.3	13.7	11.9	21.3	29.9	20.2	21.5	22.1	21.6	19.9
427	81.4	81.9	83.1	81.9	77.7	11.8	11.1	14.0	14.6	13.8	12.5		19.7		20.6	21.6	21.7	21.0	
435	81.5	82.5	87.6	83.6	77.0	11.7	11.0	13.0	13.9	14.4	13.3	13.0	20.5		20.9	21.5	22.7	23.0	21.8
443	80.6		90.2	85.4	79.3	11.3	11.7	13.3	14.2	15.7	13.6	13.0	19.4	20.1	21.2	22.8	23.7	22.6	19.1
451	86.0	86.7	96.5	90.2	80.8	10.9	10.9	13.3	14.6	16.6	14.2	14.2	23.6	16.6	21.2	22.3	24.6	22.6	19.7
459	92.0	91.4	102.9	96.7	84.4	13.5	11.4	14.5	16.0	17.3	15.4	14.5	23.6	21.0	24.6	25.8	25.0	23.1	21.1
467	96.7	100.5	110.0	106.9	87.9	13.9	12.1	15.5	16.6	18.5	15.8	15.8	23.8		26.2	27.8	28.3	25.7	21.6
475	102.6	104.5	128.0	112.0	95.2	15.0	14.8	17.0	17.4	20.1	17.6	16.6	25.2	29.4	27.2	31.5	29.9	26.8	24.0
483	104.0	110.2	137.0	120.6	104.8	15.9	14.8	18.7	21.2	20.3	17.5	17.5	23.4	31.8	28.7	30.1	33.6	28.8	23.3
491	114.4	116.2	140.0	125.5	111.5	17.6	17.6	18.2	25.0	22.5	20.7	17.5	33.6		32.0	30.6	35.7	29.1	29.5
499	122.0	121.0	146.0	134.5	113.3	19.0	18.5	21.2	22.8	23.2	20.2	18.4	33.6	34.6	31.9	33.8	31.8	28.5	
507	132.5	126.6	151.0		126.0	20.4	18.5	21.3	23.6	23.6	20.6	20.9		35.5		37.1	33.5	31.0	
515	144.2	(3)	148.0	(3)	127.6	20.7	21.1	22.0	(3)	23.3	(3)	20.9	44.3		(3)		(3)		34.7
523			(3)		133.6	(3)	(3)	23.2				23.5		(3)		(3)		(3)	33.3

[1] See footnote 1, table 2.
[2] No heat determinations for 8 and 13 percent relative humidity were made.
[3] Experiment ended.

Heat elimination and gaseous exchange were not appreciably changed by humidity during the first 10 days. During the third week there was a definite deviation. Energy metabolism at 60 percent relative humidity continued at a higher level throughout the incubation period than at either higher or lower humidities, which indicates that the chick embryo develops more satisfactorily. When the humidity was raised to 70 percent or dropped to 42 percent, there was a decrease in energy metabolism, indicating a somewhat less satisfactory growth. The effect of an increase to 84 percent or a decrease to 24 percent was approximately the same in further retarding embryonic growth. When the humidity was further decreased there was an additional drop in energy metabolism, indicating that this condition is very unsatisfactory for proper growth.

The variation in humidity did not have any noticeable effect on the total time required for development of the chick embryo, the time of hatch being practically the same at all humidities, but figure 14 shows that a drop in temperature of 2° from 102° F. caused a retardation in time of hatch of approximately 20 hours.

A series of experiments conducted at a temperature of 100° F. with humidities of 30, 44, 62, and 84 percent showed the same characteristics as the one at 102° F.
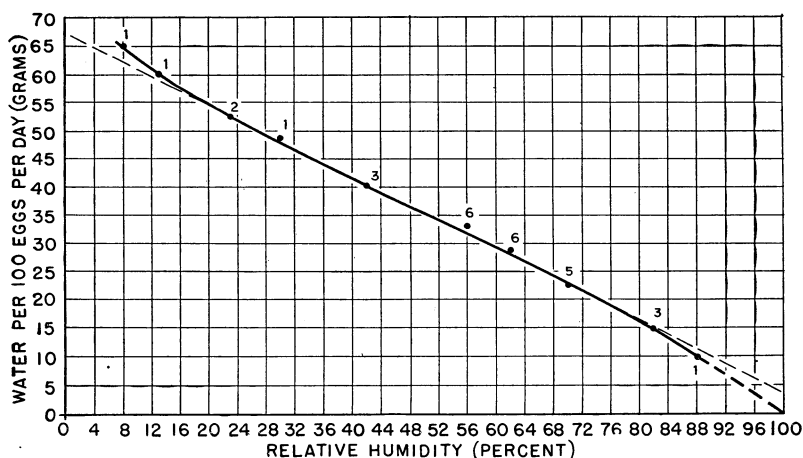

FIGURE 15.—Water elimination of incubating eggs at various humidities during the first week of incubation. The numbers on the curve indicate number of experiments, for each of which there were 21 observations.

WATER METABOLISM OF THE INCUBATING EGG

The amount of water eliminated by the incubating egg depends principally on the humidity of the air surrounding the egg. Figure 15 shows the water elimination for the first week of incubation in grams per day per 100 eggs at from 8 to 88 percent humidity. Water elimination decreased directly with increase in humidity. A change of 1 percent in humidity produced a change of 0.65 gram in water elimination per 100 eggs per day.

The water elimination at 8 and at 13 percent humidity indicated an increase in rate and that the linear relationship no longer applies.

The elimination at 88 percent also deviated from the linear relationship but in the opposite direction from that at 8 and at 13 percent. It is reasonable to assume that at the saturation point (100 percent) there would be no loss of water from the incubating egg. Assuming this is true the curve would pass through the 100-percent point. The data seemed to substantiate this assumption.

During the first week of incubation the water loss at a given humidity remained practically constant, but from about the tenth day to the time of hatch the amount gradually increased. At 60 percent relative humidity, the elimination was 30 g per 100 eggs per day during the first week and by the time of hatch it had become nearly 40 g per 100 eggs per day.

A very accurate determination of the time the hatch occurs can be made from the measurement of water elimination. For this purpose, the water elimination at a given humidity may be considered as constant from the beginning of incubation until hatching begins. When the chick breaks the shell a large quantity of water is liberated, increasing abruptly to 12 to 15 times the original quantity.
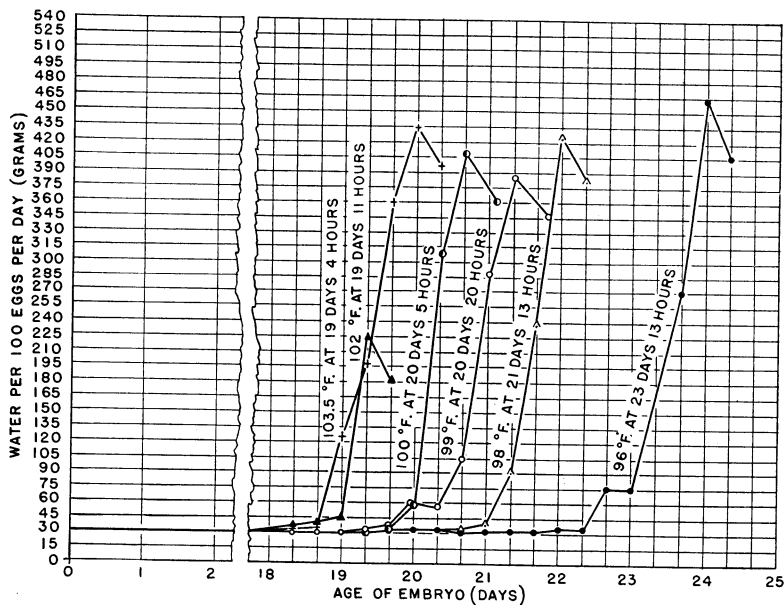

FIGURE 16.—Time of hatch at various temperatures as shown by water elimination (relative humidity 60 percent, oxygen 21 percent, carbon dioxide below 0.5 percent). Steep portion of curves indicates period of hatching.

Figure 16 shows the increase in water elimination at each temperature studied, relative humidity being 60 percent, oxygen concentration 21 percent, and carbon dioxide content kept below 0.5 percent. The portion of each curve where the slope is greatest is the time when the chicks were hatching. The higher the temperature the earlier the hatch. The time of hatch did not vary proportionately to the temperature but more nearly as the logarithm of the temperature.

Figure 17 shows the hatching time for all temperatures between 96° and 103.5° F. as determined from these data. The hatch for optimum temperature (100°) occurred at 20¼ days. It was delayed 3⅓ days at 96° and occurred 1 day earlier at 103.5°.

EFFECT OF CARBON DIOXIDE ON ENERGY METABOLISM

The variation in composition of the gases surrounding the eggs during incubation has been found to have a decided effect on embryonic development. To study the effect of carbon dioxide concentration on energy metabolism, two series of experiments were conducted. One series was made with carbon dioxide concentrations of 0.5, 2, and 4 percent. These concentrations were held as constant as possible throughout the entire incubation period. The factors other than carbon dioxide were as follows: Temperature, 99° F.; relative humidity, 60 percent; and oxygen, 21 percent. Curves
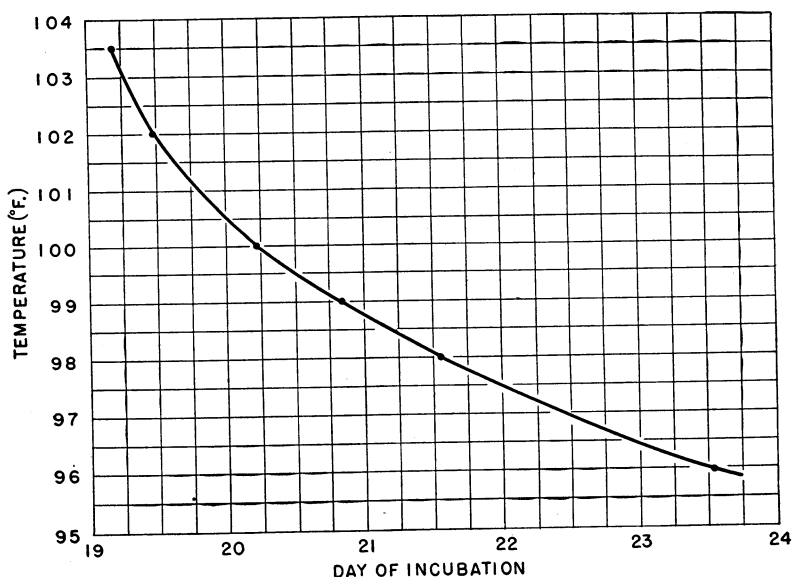

FIGURE 17.—Time of hatch at various temperatures.

a, b, and c of figure 18 give the results obtained for the carbon dioxide concentrations of 0.5, 2, and 4 percent, respectively.

During the other series of experiments the temperature was 102° F., relative humidity was 60 percent, and oxygen 21 percent. At the beginning of the experiment the carbon dioxide concentration was that of normal air. As incubation proceeded the percentage of carbon dioxide gradually increased owing to elimination of this gas by the growing embryos (fig. 9, a), until on the tenth day the concentration in the chamber was 5.5 percent. It was held constant at this percentage for the rest of the incubation period. Other experiments were conducted under similar conditions except that the carbon dioxide was allowed to increase to 10 percent (fig. 9, b), which required 12 days, and was held constant at this percentage for the rest of the incubation period.

The embryo develops more slowly the lower the temperature so that a time-factor correction for temperature must be applied in order to compare the results obtained at 102° F. with those obtained at 99°.

Curves f and a, figure 18, were both plotted from results obtained when there was not more than 0.5 percent of carbon dioxide in the incubation chamber. Curve f is for a temperature of 102° F. and curve a is for 99°. The difference in time between these two curves for any value of ordinate is the time factor which must be added to
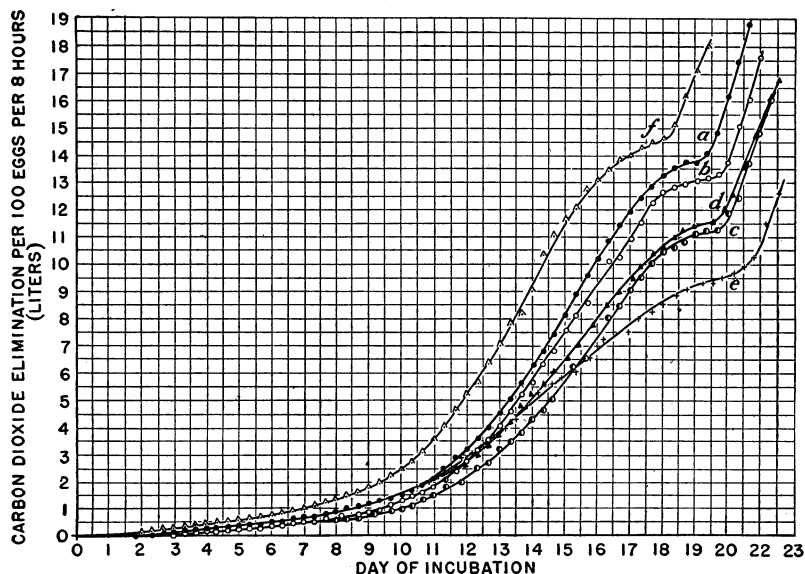

FIGURE 18.—Effect of various percentages of carbon dioxide on energy metabolism. See text for discussion of individual curves.

values obtained at 102° to make the results comparable with those obtained at 99°. This correction for time is given in table 4, last column. To make the results obtained at 102° comparable with those at 99°, each value was plotted at the time given in the first column of table 4, plus the time-correction factor in the last column of this table. The results obtained for the experiment in which the carbon dioxide concentration increased to 5.5 percent are given in curve d of figure 18, and for the one in which the concentration was as high as 10 percent, in curve e. All the curves in figure 18 except curve f are comparable.

Curves for oxygen consumption and heat elimination (table 4), if plotted, would follow the same general trend as those shown for carbon dioxide, differing only in absolute values.

TABLE 4.—*Effect of variation in carbon dioxide concentration on energy metabolism of chick embryos at different temperatures with relative humidity 60 percent and oxygen concentration 21 percent*

[Results expressed per 100 eggs per 8 hours]

Age of embryo (hours)	Results at temperature of 99° F.							Results at temperature of 102° F.									Time factor to make observations at 102° comparable to observations at 99° F.
	Heat elimination[1] at carbon dioxide concentration of 0.5 percent (3 experiments)	Carbon dioxide elimination at concentration of—			Oxygen consumption at carbon dioxide concentration of—			Heat elimination[1] at carbon dioxide concentration of—			Carbon dioxide elimination at concentration of—			Oxygen consumption at carbon dioxide concentration of—			
		0.5 percent (3 experiments)	2 percent (1 experiment)	4 percent (1 experiment)	0.5 percent (3 experiments)	2 percent (1 experiment)	4 percent (1 experiment)	0.5 percent (4 experiments)	5.5 percent (2 experiments)	10 percent (3 experiments)	0.5 percent (4 experiments)	5.5 percent (2 experiments)	10 percent (3 experiments)	0.5 percent (4 experiments)	5.5 percent (2 experiments)	10 percent (3 experiments)	
	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Hours
8		0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0	0.0	0.0	0.0	0
16		.0	.0	.0	.0	.0	.1				.0	.0	.0	.0	.0	.0	0
24		.0	.0	.0	.0	.0	.3				.0	.0	.0	.0	.0	.0	1
32		.0		.0	.0	.2	.0				.0	.0	.0	.0	.0	.0	2
40		.0	.1	.0	.0	.0	.1				.0	.0	.0	.0	.0	.0	3
48		.0		.2	.0		.0				.1	.0	.0	.0	.0	.0	5
56		.0	.1	.2	.0	.0	.1				.1	.1	.1	.1		.1	7
64		.1		.2	.0	.0	.1				.1	.2	.2	.1		.1	9
72		.1	.2	.2	.1	.0	.0				.2	.3	.3	.2	.3	.3	12
80		.2	.3	.1	.1	.3	.0				.3	.4	.4	.2	.4	.4	16
88		.2	.3	.2	.2	.6	.1				.4	.5	.5	.3	.4	.5	21
96		.2	.4	.2	.2	.4	.2				.4	.5	.5	.5		.5	25
104		.3	.3	.2	.3		.2				.5		.6	.6	.4	.5	28
112		.3	.2	.4	.4	.6	.1				.5	.6	.6	.7	.5	.8	30
120		.4	.3	.4	.5	.7	.6				.6	.6	.6	.7	.7	.6	30
128	2.0	.4	.3	.2	.5	.6	.5	2.2			.6	.6	.6	.7	.6	.8	30
136	2.3	.4	.4	.2	.6	.7	.4	2.4	1.8		.6	.6	.7	.7	1.0	.8	32
144	2.6	.5	.4	.4	.7		.2	2.7	1.6		.8	.7	.8	.9		.9	32
152	2.9	.5	.5	.5	.7	.8	.6	2.9	1.9		.9	.9	.9	1.1	.9	.9	32
160	3.3	.6	.6	.3	.8		.4	3.2	2.5	3.5	.9	.9	.9	1.3	.9	1.1	32
168		.7			.9			3.8	4.0	4.4	1.0	1.0	1.0	1.4	1.3	1.2	32
176	3.8	.7	.7	.5	1.0	1.3	1.1	4.9	4.5	4.1	1.1	1.1	1.0	1.5	1.5	1.5	32
184	4.3	.8		.6	1.2	2.0	1.3	5.9	4.9	4.9	1.2	1.2	1.1	1.9	1.8	1.7	32
192	5.2	.9	.7		1.4	1.5	1.2	6.7	7.0	6.7	1.3	1.2	1.2	2.2	2.0	2.0	32
200	6.1	1.0	.8	.6	1.5	1.3	1.5	7.6	8.4	7.5	1.5	1.3	1.2	2.4	2.2	2.8	32
208	6.8	1.1	.8	.7	1.7		1.5	8.3	9.4	8.7	1.6	1.5	1.4	2.8	3.2	2.8	32
216	7.4	1.2	.9	.8	1.9	1.9	.8	10.0	10.2	9.6	1.8	1.6	1.5	3.1		2.8	32
224	7.9	1.3			2.1		1.5	11.5	12.0	10.0	2.0	1.8	1.6	3.3	3.5	2.9	32

232	8.8	1.4	1.1	.8	2.4	2.2	1.5	13.1	13.2	12.7	2.2	2.0	1.8	3.4	3.0	3.3	32
240	10.1	1.5	1.3	1.0	2.6	3.1	1.5	14.2	14.2	17.1	2.4	2.2	2.0	3.8	4.0	3.8	32
248	11.3	1.7	1.5	1.1	2.6	2.8	2.4	16.6	16.0	15.2	2.7	2.4	2.2	4.2	----	4.2	32
256	12.4	1.9	1.6	1.3	2.8	2.9	2.2	19.3	17.4	16.7	3.1	2.6	2.5	4.6	4.7	4.6	32
264	13.6	2.1	1.8	1.5	3.1	4.0		21.2	20.1	17.6	3.5	3.0	2.9	5.2	4.7	4.3	32
272	15.2	2.5	2.1	1.8	3.6	4.2	2.6	24.2	22.7	19.7	4.0	3.3	3.1	5.8	6.1	5.6	32
280	17.4	2.9	2.4	2.0	4.2	4.3	3.5	27.6	26.0	23.1	4.6	3.6	3.4	6.8	6.7	5.4	32
288	19.6	3.2	2.6		4.8	4.9		30.2	28.4	26.0	5.2	4.2	3.7	7.7	7.8	5.7	32
296	22.0	3.6	3.0	2.5	5.5	5.5	5.0	33.6	31.8	29.2	5.6	4.8	3.9	8.5	8.8	6.2	32
304	24.4	4.0	3.5	2.7	6.4	6.9	4.5	37.6	34.9	31.0	6.3	5.3	4.3	9.5	8.9	6.6	32
312	27.0	4.5	4.0	3.3	7.0	7.5	5.6	41.7	37.7	33.7	7.0	5.6	4.8	10.4	9.5	7.2	32
320	30.0	5.0	4.6	3.5	7.6		5.2	46.0	42.5	37.6	7.8	6.0	5.2	11.5	10.7	8.5	32
328	33.7	5.6	5.2	3.8	8.6	8.4	6.4	50.1	45.0	40.2	8.2	6.4	5.6	12.4	11.5	8.2	34
336	37.7	6.3	5.8	4.3	9.7	9.5	7.3	54.6	48.8	42.2	9.0	7.0	5.8	13.1	11.7	8.8	34
344	41.6	6.8	6.4	4.6	10.7	10.3	7.1	59.2	51.7	42.4	10.3	7.8	6.0	14.2	12.2	9.1	36
352	45.6	7.4	6.8	5.1	11.9	11.9	8.3	62.7	54.2	46.9	11.0	8.4	6.5	15.1	11.4	10.1	38
360	49.6	8.1	7.5	6.3	12.9	12.2	9.5	67.2	58.8	49.9	11.6	9.0	7.2	16.2	----	11.3	40
368	53.8	8.9	8.1		13.6	13.4		70.2	62.8	51.1	12.0	9.4	7.3	17.6	15.0	12.8	42
376	58.0	9.6	8.5	6.5	14.4	20.1	10.2	73.5	65.0	53.3	12.7	9.8	7.5	18.3	----		42
384	62.1	10.2	----	----	15.4			77.2	69.8	58.3	13.0	10.3	8.0	19.0	15.9	14.0	42
392	65.6	10.8	10.1	8.0	16.5	15.3	13.0	80.6	72.7	60.3	13.4	10.5	8.2	20.3	14.2	13.9	40
400	68.8	11.4	10.2	8.3	17.4	16.5	11.9	81.8	73.0	60.0	13.8	10.9	8.5	21.1	15.9	14.2	40
408	72.2	11.9	10.9	9.1	18.3	18.1	13.2	82.6	75.6	61.0	13.9	11.3	8.8	21.3	18.0	14.1	39
416	76.1	12.4	11.5	9.4	19.4	17.1	15.5	83.8	75.5	63.3	14.2	11.4	9.1	21.8	19.4	14.0	39
424	80.0	12.8	12.2	10.0	20.5	17.8	14.6	83.7	77.2	65.6	14.4		9.3	21.9	17.9	14.5	38
432	83.4	13.2	12.7	10.5	21.8	19.8	16.0	85.3	78.5	66.9	14.5	11.5	9.2	22.2	18.3	14.6	38
440	85.6	13.5	12.8	10.6	21.8	23.0	16.2	88.9	78.3	67.8	15.0	12.0	9.5	23.2	18.3	15.5	36
448	86.7	13.7	12.9	10.7	21.7	20.7	16.2	93.3	83.0	69.2	16.2	12.5	9.7	24.1	19.3	16.2	36
456	87.5	13.7	13.0	11.2	21.4	21.3	16.4	99.7	88.0	71.7	17.0	13.5	9.9	24.8	23.4	16.8	36
464	88.8	14.0	13.1	11.2	21.3	20.0	16.8	106.4	92.9	75.6	17.9	14.6	10.1	26.6	27.8	19.0	36
472	91.7	14.8	13.1	11.2	23.7	20.9	16.8	119.0	99.0	78.6	19.3	15.5	11.5	29.1	23.8	20.5	36
480	97.3	15.6	13.7	11.8	23.1		17.5	132.5	109.3	87.7	20.6	16.8	12.5	31.2	24.7	23.0	36
488	105.4	16.8	15.0	12.6	28.1	23.7	20.5	138.5	120.1	97.0	21.8	17.8	13.2	34.6	26.3		36
496	115.9	18.8	16.0	13.7		24.9	22.4	143.0	126.9	103.1	22.8	19.5	14.3	34.8	----	24.1	36
504	127.9	21.3	17.5	14.7	----	27.7	18.1	148.5	134.0	112.2	23.4	20.6	15.5	35.5	35.0		36
512	139.5	23.5	20.0	16.1	----	29.8	28.8	(²)	142.0	125.5	24.5	21.6	16.9	(²)	38.5		36
520	149.8	25.3	22.3	18.8	35.0	36.7	27.2	(²)	----	127.1	(²)	24.2	17.2	(²)	----	(²)	36
528	(²)	(²)	24.1	21.8	(²)	38.4	33.3	----	(²)	(²)	----	(²)	(²)	----	(²)	(²)	36
536	----	----	26.0	24.2	----	38.2	35.4	----	----	----	----	----	----	----	----	----	36
544	----	----	(²)	(²)	----	(²)	(²)	----	----	----	----	----	----	----	----	----	36

¹ See footnote 1, table 2.   ² Experiment ended.

The effect of variations in carbon dioxide concentration was perceptible by the third day of incubation and became more and more pronounced as incubation proceeded.   The carbon dioxide elimination was greatest when the carbon dioxide was less than 0.5 percent. As the concentration of carbon dioxide was increased the development of the chick embryo was more and more retarded, as shown by the decreasing amount of carbon dioxide eliminated.   A content of 2 percent for the entire incubation period was not so harmful as one in which the carbon dioxide was that of normal air at the beginning of incubation and gradually increased to 2 percent on the seventh day, to 5.5 percent the tenth day, and held at 5.5 percent for the remainder of the incubation period.   Nor was the latter condition so harmful as 4 percent throughout the entire incubation period.   Most detrimental of all the conditions studied was allowing the carbon dioxide to accumulate from normal air at the beginning of incubation to 10 percent on the twelfth day and then holding it constant for the rest of the incubation period.

Curves d and e (fig. 18) (with carbon dioxide concentrations of 5.5 and 10 percent, respectively, during the latter part of the incubation period) show greater energy metabolism for the first 10 days than when 2 and 4 percent were maintained during the entire period. The reason is that in the case of the first two concentrations the carbon dioxide at the beginning was that of normal air.

EFFECT OF OXYGEN ON ENERGY METABOLISM

The effect of various oxygen concentrations, ranging from 15 to 50 percent, on heat elimination and gaseous exchange was determined. The conditions for incubation other than oxygen were: Temperature, 99° F.; relative humidity, 70 percent; carbon dioxide, below 0.5 percent.

The results are given in table 5.   During the first 2 weeks of incubation, there was no perceptible effect on energy metabolism of an increase or decrease in oxygen concentration over that of normal air. After the first 2 weeks, the energy metabolism with 21 percent oxygen was the greatest.   With 30 and 40 percent the metabolism was slightly less than with 21 percent; with 50 and 18 percent it was approximately 10 percent lower, whereas when 15 percent was used the metabolism was only about four-fifths of that at 21 percent. Therefore, a decrease in oxygen concentration is more detrimental than an increase since a decrease of one-seventh retards the energy metabolism by the same amount as an increase of two and one-half times.

TABLE 5.—*Effect of variation of oxygen concentration on energy metabolism of chick embryos with temperature 99° F., relative humidity 60 percent, and carbon dioxide less than 0.5 percent*

[Results expressed per 100 eggs per 8 hours]

Age of embryo (hours)	Heat elimination [1] at indicated percentages of oxygen						Carbon dioxide elimination at indicated percentages of oxygen						Oxygen consumption at indicated percentages of oxygen					
	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)
	Calories	Calories	Calories	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters
3							0.0	0.0	0.0	0.0		0.0	0.0		0.0	0.0		0.2
11							.0	.0	.0	.0	0.0	.0	.0	0.0	.0	.0		.0
19							.0	.0	.0	.0	.0	.0		.0	.0	.0	0.7	.4
27							.0	.0	.0	.0	.0	.0	.1	.0	.0	.0		.5
35							.0	.0	.0	.0	.0	.0		.0	.0	.0		.6
43							.0	.0	.0	.0	.0	.0	.0	.0	.0	.0		.4
51							.0	.0	.0	.0		.0		.0	.0	.3	1.0	.5
59							.1	.1	.1	.1	.1	.1	.0	.0	.0		.9	
67							.1	.1	.1	.1	.2	.1	.0	.0	.1	.3	1.2	.6
75							.2	.2	.2	.1	.2	.1	.0	.0	.1	.4		.7
83							.2	.2	.2	.1	.3	.2	.0	.1	.2		.6	
91							.3	.3	.2	.1	.3	.2		.1	.2	.7	.1	
99							.3	.3	.3	.2	.4	.3	.2	.3	.3		1.4	
107							.4	.4	.4	.2	.4	.4	.3	.3	.4	.7	1.9	1.5
115							.5	.4	.4	.2	.5	.4	.3	.3	.5	.7	.4	1.1
123		1.8	2.0	2.8	2.2	3.7	.5	.4	.5	.3	.5	.5	.4	.4	.5	.7		1.1
131	0.9	2.8		3.1	2.2	4.0	.3	.5	.5	.4	.5	.5		.4	.6	.7	1.5	
139	1.1	2.1	2.5	3.3		4.5	.6	.5	.5	.4	.7	.5	.7	.5	.7	1.0		
147	2.3	2.6	2.8	3.4	2.8	4.5	.6	.5	.5	.5		.6	.6	.4	.7	1.2		
155	2.5	2.8	3.0		3.4	5.1	.6	.6	.7	.7	.7	.6	.7	.9	.8			1.1
163	2.3	1.1			3.8	5.5		.7	.7	.7	.7	.7	.1	.8	.9			1.1
171			3.7		3.9	5.5	.8	.7	.7	.8	.6	.7	.8	.8	1.0	1.5	1.8	1.5
179	4.8	5.3	4.0	5.6	4.4	6.1	.8	.8	.8	.8	.8	.8	.8	1.3	1.1	1.8	1.6	1.8
187	5.2		4.6	6.8	4.8	6.7	1.0	.9	.9	.9	1.0	.8	.9	1.3	1.3	1.8	1.4	1.8
195	4.8	3.4	5.8	7.1	4.8	6.9	.8	.9	1.0	1.0	.8	.9	1.1	1.5	1.7	3.4	1.9
203	5.6		6.4	7.5	5.9	7.4	1.0	1.0	1.0	1.1	1.1	1.0		1.9	1.6	1.7	2.5	2.3
211	6.0	5.2	7.1	8.4	6.2	8.0	1.3	1.1	1.1	1.2	1.2	1.1	2.0	1.6	1.8	1.9	3.0
219	7.1		7.6	9.1	7.2	8.4	1.3	1.2	1.2	1.3	1.3	1.3	1.7	1.2	2.0	2.3	3.5
227	7.8	5.7	8.2	9.6	7.9	9.2	1.3	1.3	1.4	1.4	1.6	1.5	1.6	1.7	2.3	2.6	3.2	3.7
235	14.1	7.2	9.4	10.6	8.8	9.8	1.6	1.4	1.5	1.6	1.7	1.6	2.2	2.6	2.6	2.8	5.9	3.9
243	8.3	8.8	10.8	10.8	9.9	10.8	1.2	1.6	1.6	1.8	1.9	1.7	1.9	2.8	2.6	3.1		4.2
251	9.5	10.0	11.8	13.4	12.1	12.5	1.9	1.9	1.8	2.1	2.2	2.0	2.6	3.1	2.7	3.4	3.2	4.3
259	11.2	11.5	12.9	14.8	14.6	15.4	2.2	2.0	2.0	2.3	2.3	2.3	2.7	3.2	2.9		7.5	4.3
267	11.1	12.8	14.3	16.6	17.4	15.4	2.3	2.2	2.3	2.7	2.7	2.5	3.8	3.9	3.3	4.1	4.9	4.3
275	15.2	10.9	16.2	19.0	18.6	17.5	2.3	2.7	2.7	3.2	3.3	2.9	4.4	4.8	3.9	4.9	5.9	5.3
283	16.2	13.2	18.5	21.2	20.5	19.4	3.2	3.0	3.1	3.6		3.4	4.1	4.6	4.5	5.4	6.8	6.1

[1] See footnote 1, table 2.

TABLE 5.—*Effect of variation of oxygen concentration on energy metabolism of chick embryos with temperature 99° F., relative humidity 60 percent, and carbon dioxide less than 0.5 percent*—Continued

[Results expressed per 100 eggs per 8 hours]

Age of embryo (hours)	Heat elimination [1] at indicated percentages of oxygen						Carbon dioxide elimination at indicated percentages of oxygen						Oxygen consumption at indicated percentages of oxygen					
	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)	15 (1 experiment)	18 (1 experiment)	21 (3 experiments)	30 (1 experiment)	40 (1 experiment)	50 (1 experiment)
	Calories	Calories	Calories	Calories	Calories	Calories	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters	Liters
291	17.5	19.5	20.8	24.0	24.1	22.8	3.3	3.5	3.4	3.9	4.0	3.6	4.8	5.4	5.1	6.1	7.6	7.7
299	19.9	20.6	23.2	28.2	27.5	25.5	3.6	4.1	3.8	4.3	4.6	4.2	5.5	6.6	6.0	7.1		8.3
307	21.6	22.6	25.6	30.6	29.8	27.2	4.2	4.4	4.3	4.9	5.1	4.6	5.9	6.8	6.8	7.6	10.7	8.6
315	25.1	28.2	28.4	31.8	32.6	29.0	4.6	4.8	4.8	5.5	5.3	5.2	6.2	7.9	7.2	8.3		8.8
323	29.7	32.3	31.7	36.2	35.4	32.0	5.1	5.6	5.3	6.1	6.2	5.8	8.2	8.7	8.1		9.1	9.1
331	31.9	34.4	35.7	38.9	39.6	36.5	5.6	6.2	6.0	----	7.0	6.4		9.0	9.2	----	12.2	10.2
339		39.3	39.7	43.2	43.4	40.0		6.6	6.6	7.5	7.4	7.0		9.8	10.2	11.8	12.6	12.2
347	39.9	46.3	43.6	46.8	47.2	43.6	6.8	7.1	7.1	8.3	8.4	7.8	11.1	11.6	11.3	12.9	12.4	13.6
355	40.9	48.4	47.6	51.2	50.1		7.2	7.8	7.7	10.7	8.5		11.6	12.8	12.5	13.9	15.7	
363	44.0	51.4	51.6	55.5	54.2		7.9	8.5	8.5	11.1	9.6		12.9	13.2	13.3	14.5		
371	46.8	53.3	55.9	59.7	57.9	48.2	8.9	9.3	9.3	10.6	10.3	9.9	13.0	14.6	14.0	15.5	17.1	15.8
379	49.2	56.8	60.2	63.8		56.0	8.0	10.1	10.0	11.0		10.4	13.4	15.7	14.9	16.3	20.7	18.0
387	55.3	61.0	64.0	68.2	59.0	59.0	9.8		10.5	11.4	10.6	11.3	15.1	16.6	16.0	16.5	18.1	19.7
395	57.2	65.8	67.3	71.2	62.5	62.5	10.2	11.7	11.1	12.0	11.8	11.9	15.3	16.0	17.0	17.3		20.8
403	59.0	72.6	70.4	71.8	64.2	64.2	10.9		11.7	12.3	13.1	12.0	16.1	16.5	17.8	18.1	22.1	21.7
411	69.7	76.7	74.1	74.0	66.0	66.0	11.4	12.0	12.2	12.8	13.3	12.3	17.0	18.8	18.8	18.3		22.2
419	66.7	74.0	78.1	74.0	72.2	72.2	11.9	12.6	12.6	13.4	13.5	12.0	17.9	19.7	20.0	18.8	22.6	23.7
427	70.2	72.7	81.9	72.2	75.6	75.6	12.1		12.9	13.7	13.8	12.0	17.6	20.4	21.0	19.3	22.5	24.4
435	69.7	77.4	84.9	72.4	74.5	74.5	11.6	13.2	13.3	13.6	13.6	13.0	17.8	20.8	21.8	19.7	24.7	23.6
443	69.6	81.2	86.3	73.4	75.0	75.0	12.7	13.3	14.0	13.8	13.7	13.5		20.8	21.9	19.9	22.2	23.0
451	72.2	82.0	87.1	79.7	76.3	76.3	12.9	13.6	14.5	14.0	14.0	13.9	18.0	19.8	21.5	20.6	23.6	24.5
459	73.8	81.6	87.9	83.4	76.8	76.8	13.0		14.7	14.1	14.6	14.3	19.6	19.9	21.3	22.0	23.6	24.4
467	78.2	82.6	89.6	84.2	77.4	77.4	13.4	14.1	14.6	14.6	14.7	14.4		20.6	21.3	22.8		23.7
475	80.0	85.0	93.8	86.0	73.9	73.9	14.6		15.2	15.0	15.1	14.3	20.6	20.4	22.1	23.1	23.8	22.2
483	81.4	87.5	100.8	87.8	73.2	73.2	14.4		16.1	16.2	16.9	14.8	21.5	20.8	24.1	23.9		21.7
491	80.7	99.4	110.0	96.8	80.0	80.0	14.5	16.0	17.6	17.2		15.6	23.1	23.0	26.8	24.5	25.2	24.2
499	96.7	127.8	121.8	106.2	94.3	94.3	17.3		20.1	19.6	18.9	16.4	24.9	26.8	29.5	26.6		29.5
507	111.7	133.4	134.0	120.3	103.4	103.4		19.6	22.5	22.2	20.6	17.0	28.7	29.6		30.6	30.0	33.4
515	119.0	134.6	145.0	138.6	106.5	106.5	20.4	20.2	24.5	24.2	22.4	18.5	30.9	31.0	34.0	34.0		35.0
523	110.5	138.2	154.7	151.8	118.5	118.5	20.2	22.1	26.2	26.6	25.3	20.8	32.3	32.3	36.0	37.4		36.9
531	129.7	155.2	(2)	(2)	124.7	124.7	22.3	23.4	(2)	(2)		22.2	34.2	35.7	(2)	(2)		38.7
539	130.5	----	----	----	130.8	130.8	23.3					23.0	35.3	39.4				41.5
547	130.2	(2)	----	(2)	(2)	(2)	24.6		(2)		(2)	(2)	37.1	(2)		(2)	(2)	(2)

[2] Experiment ended.

SUMMARY AND CONCLUSIONS

The eggs used in these experiments were obtained from a selected flock of White Leghorn fowls at the National Agricultural Research Center, Beltsville, Md. Only eggs less than 7 days old were used. They were carefully selected for size and shape, and freedom from cracks and defective shells.

The calorimeters which were used for incubation were specially designed, constant-temperature, continuous-water-flow type, provided with means for preventing interchange of heat between the calorimeter and its boundaries and for measuring the heat given off by the growing embryos. The calorimeter comprised also the respiration chamber, which was of the closed-circuit type, with means for constantly interchanging the gas in the chamber and for removing the products of gaseous exchange and supplying gases necessary to life and growth.

All measurements were made with precision instruments and with high accuracy. All factors except the one under investigation were kept constant throughout the series of experiments.

During the investigations on the effect of temperature on hatch and on energy metabolism, the following were used: 96°, 98°, 99°, 100°, 102°, and 103.5° F. The factors other than temperature were maintained as follows: Relative humidity, 60 percent; carbon dioxide concentration kept below 0.5 percent; oxygen, 21 percent. The hatch was greatest at 100°. The chicks produced at this temperature were also superior in appearance and vitality. The detrimental effect of either higher or lower temperatures became greater as the deviation from the optimum increased.

The effect of temperature on heat elimination and gaseous exchange began to show after the first few days. The higher the temperature the greater was the energy metabolism and, consequently, the more rapid the development of the chick and the earlier the hatch.

The effect of relative humidity on hatch and on energy metabolism was shown by experiments with humidity ranging from 8 to 88 percent. In this series of experiments, the following factors were always held constant: Temperature, 102° F.; carbon dioxide kept below 0.5 percent; and oxygen, 21 percent. The optimum humidity for hatch was found to be 58 percent. In a series of experiments at 100° the optimum was found to be 61 percent. In both series, as the humidity varied either way from the optimum the hatch became less, at first slowly, and then more rapidly as the deviation increased. Little effect on gaseous exchange and heat elimination was noted during the first 10 days. During the last half of the incubation period, the energy metabolism was higher when the humidity was optimum than when it was either higher or lower, indicating that the embryo was stronger and developing more rapidly because of more favorable conditions for growth. The larger the deviation of humidity from the optimum the greater was the detrimental effect.

The water elimination of the embryo was found to depend principally on the humidity of the air around the egg, the water elimination being less in direct proportion to the increase in humidity. The water loss for the first week was practically constant but increased gradually thereafter to the time of hatch.

The time of hatching was determined by the large increase in water elimination when the shell was broken. Temperature was the only factor studied that showed an appreciable effect. The length of the incubation period varied from $19\frac{1}{6}$ days at 103.5° F. to about $23\frac{1}{2}$ days at 96°. Hatching took place at about $20\frac{1}{4}$ days at 100°, which is the optimum temperature.

Experiments were made to determine the effect of 0.5, 2, and 4 percent of carbon dioxide on hatch and energy metabolism. During these experiments the oxygen was held at 21 percent, the relative humidity at 60 percent, and the temperature at 99° F. With 4 percent, less than one-fourth as many chicks were produced as with 0.5 percent. With 2 percent the hatch was over 35 percent less than with 0.5 percent. Other experiments were conducted during which the carbon dioxide eliminated was allowed to accumulate in the calorimeter until the concentration was 5.5 and 10 percent. In these experiments the oxygen was held at 21 percent, the relative humidity at 60 percent, and the temperature at 102°. A concentration of 5.5 percent under these conditions was not so harmful as one in which the concentration was 4 percent for the entire time, the hatch for the former being 45 percent as compared with 20 percent for the latter. The most harmful condition studied was the one in which the carbon dioxide was allowed to accumulate to 10 percent and was held constant for the rest of the incubation period; a 16-percent hatch resulted from this treatment. The data obtained for heat elimination and gaseous exchange show that concentration of carbon dioxide has a decided effect on metabolism. The metabolism was greatest with the lowest concentration and decreased with increase of carbon dioxide.

Experiments with 15, 18, 21, 30, 40, and 50 percent of oxygen were conducted to show the effect of this factor on hatch and on energy metabolism. During these experiments the carbon dioxide was kept below 0.5 percent, the relative humidity at 70 percent, and the temperature at 99° F. The best hatches were obtained with 21 percent of oxygen. The hatch decreased as the concentration varied in either direction from this percentage. An increase in concentration was not nearly so harmful as a decrease in concentration of the same magnitude. Gaseous exchange and heat elimination were practically the same for 21, 30, and 40 percent of oxygen and were highest at these percentages. With 18 and 50 percent, the metabolism was retarded during the last week of incubation, and with 15 percent the effect was very detrimental.

The respiratory and thermal quotients give a very good indication of the kind of food materials being metabolized. The respiratory quotient was 1.00 for the first 2 days and dropped to 0.66 by the eleventh day and was fairly uniform during the remainder of the investigation. The thermal quotient began at 2.60 and rose rapidly to above 3.00. The lowest value for respiratory quotient and the highest for thermal quotient occurred on the ninth and tenth days of incubation. The results of this investigation indicate a carbohydrate metabolism during the first few days and fat and protein metabolism almost exclusively after about the tenth day.

The intensity of metabolism in terms of heat elimination was determined for the chick embryo and also for the embryo plus membranes. The results show that in determining the relationship of energy metabolism to weight of live tissue the membranes must be considered.

Following are the most important results obtained in this investigation:

The optimum conditions for incubation and for growth of the chick embryo, as determined by energy metabolism, so far as the physical factors are concerned are temperature 100° F., relative humidity 61 percent, oxygen 21 percent, and carbon dioxide below 0.5 percent.

The temperature should vary not more than 0.5° F. from the optimum. The relative humidity may vary 10 percent with very little detrimental effect. The carbon dioxide should not be allowed to accumulate to a greater concentration than 1 percent or the oxygen to drop below 21 percent.

It should be emphasized that the results here reported apply only when the conditions of the temperature and of the atmosphere, as related to carbon dioxide and oxygen, are as indicated in this bulletin. The results are not considered applicable to sectional-type and home incubators in which it is not usually possible to control effectively the conditions mentioned.

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