FACTORS AFFECTING LENGTH OF INCUBATION[1]

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INTRODUCTION

THE present investigation endeavors to interpret some of the factors affecting the rate and duration of embryonic development in a species of bird whose young hatch out in an altricial state of helplessness and to make some comparisons with species whose young hatch in a precocial condition of partial self-sufficiency. The incubation period of the altricial House Wren, *Troglodytes aëdon,* representative of the order Passeriformes and the chief object of study here, is only thirteen days compared with twenty-one days in the precocial Domestic Fowl and twenty-four days in the precocial Bob-white and pheasant, three species of the order Galliformes. The rate of embryonic development was determined by measurement of the rate of gas exchanges.

Absorption of oxygen or elimination of carbon dioxide should not be considered a criterion of the rate of development unless there is a constant ratio between energy of combustion in the egg and energy transferred into embryonic tissues. Tangl (1903) believed that the energy of combustion in the egg of the Domestic Fowl amounts to about one-third of the total energy exchange. Tangl and later Needham (1928) showed that this ratio is relatively greater early in the developmental period than later. Some attention is here given to changes in rate of metabolism with age of embryo but the chief emphasis is on effect of temperature on embryos of the same age. Temperature may likewise affect the efficiency of energy utilization, for Romanoff and Faber (1933) found this to be greater with older chick embryos at 34° to 36° C. than at either higher or lower temperatures. Therefore, interpretation of rates of gas exchange in terms of development of embryo is justified only with some reservations but nevertheless gives an approximation of major changes and is here used as such.

During the present century extensive measurements of gas exchanges in the eggs of the Domestic Fowl have been made by Bohr and Hasselbalch (1900, 1903), Atwood and Weakley (1924), Murray (1925), Hanan (1928), and Barott (1937). The last worker studied the effect of temperature between 35.6° and 39.7° C., but the others made their measurements at approximately 37.8°. Measurement

of gaseous exchanges in the eggs of an altricial species has not come to my attention.

METHOD

A constant-pressure manometric type of respirometer was utilized for determining the rate of gaseous exchanges of the eggs. This has two detachable rear chambers of the same size (each of 100 cc. capacity) which are connected by a bent capillary glass tube extending down the front of the instrument. This tube contains a small amount of di-methyl-aniline to serve as a manometer to indicate differences in air pressure between the two chambers. Connected to the right side of the manometer is another tube of 5 mm. diameter calibrated up to 4 cc. in 0.1 cc. divisions. At the bottom of this side tube is a mercury well made from rubber tubing and with a coarse and fine screw adjustment for regulating the mercury level.

To measure oxygen intake, suitable absorbents for moisture (calcium chloride) and for carbon dioxide (soda-lime) are placed in each chamber and the egg only in the right one. A balance in air pressure between the two chambers as far as moisture and CO_2 output are concerned is soon reached. However, the manometer is continually being displaced to the right as oxygen is absorbed by the embryo from the enclosed air. The amount of the absorption is measured by balancing the manometer by changing the height of the mercury in the side tube at the beginning and end of the experiment and taking the difference in the two readings of the mercury level. For eggs nearly ready to hatch, one or two hours' time was sufficient to get a value that was constant on repetition, but, when fresh, three eggs were run simultaneously for three to five hours. The relative humidity in the chamber averaged less than 10%. No shaking arrangement was used as the numerous small granules of the absorbents presented a large surface area for absorption.

The instrument was partially submerged in a water bath capable of maintaining the temperature within one-tenth degree of any desired point. Sufficient time, usually a half-hour, was allowed at the beginning of each experiment for the egg temperature to approximate the temperature of the bath as previous studies indicated would be obtained. The carbon-dioxide output was ordinarily measured after the determination of the rate of oxygen absorption. This was done by duplicating the procedure but with only the water absorbent in the chambers. The reading obtained from the manometer then represented the difference between the amount of gases exchanged and had to be added or subtracted from the value for the rate of oxygen absorption.

We wish to acknowledge our indebtedness to Dr. Bertil G. Anderson of Western Reserve University for suggesting the possibilities of this particular respirometer, for aiding us in modifying it to fit our particular needs, and in the loan during two different summers of the water bath.

Another method employed a modified Haldane type of apparatus and is described elsewhere (Kendeigh, 1939). It is sufficient here to say that a current of air was caused to circulate over three to seven eggs at a time and this air was purified of carbon dioxide and moisture before it entered the egg chamber and again after it left so that the loss of these two gases from the eggs was measured. Determination of the oxygen and carbon-dioxide exchange by this method did not prove satisfactory although results obtained in regard to moisture loss are discussed beyond. The relative humidity in the egg chamber varied between 8 and 16 per cent and the ventilation varied between 30 cc. and 120 cc. per minute. Temperature was controlled by placing the egg chamber inside an incubator. The experiment in each case ran for twenty-four hours, after a preliminary half-hour to allow reaching an equilibrium.

A total of 845 eggs was employed in these metabolism experiments. One hundred seventeen experiments were performed with the manometric respirometer and 137 with the open-circuit system. This covered a period from 1933 to 1938 inclusive, but only for the summer months.

TIME OF BREEDING

The House Wren has two breeding periods. The first begins in middle May and lasts to the end of June; the second begins in late June and lasts to the middle of August. Egg laying occurs most regularly during the first two weeks of each period but occasional sets may be deposited at any time. A study was made to determine if any difference in rate of metabolism occurred between eggs laid during these two periods. Seventeen comparisons involving 58 separate experiments using the manometric respirometer are available from eggs with 4, 7, 10, and 12 days' incubation at temperatures of 21.1°, 26.7°, 32.2°, 35.0°, and 37.8° C. The rate of metabolism of first-period eggs compared with second-period eggs was slightly greater in six, less in nine, and equal in two experiments. Combining all experiments for each period into a single average, the oxygen absorption per egg per hour for the first period was 0.575 cc. and for the second 0.570 cc. At no age nor at any temperature were there consistent differences in rate of oxygen absorption by eggs from the two periods.

TEMPERATURE

With rise in temperature from 21.1° to 37.8° C., there is an increase in rate of oxygen absorption at all ages but with a further rise to 40.6° C. there may be a decrease (Table 1). The coefficient $(100 \times \frac{\text{standard deviation}}{\text{mean}})$ representing the degree of variation of individual measurements from the mean is quite high early in the incubation period but then rapidly decreases. The average values for all temperatures at ages 0, 2, 4, 7, 10, and 12 days are 65, 36, 25, 13, 15, and 10, respectively. The coefficients of variation, averaged for all stages of incubation excluding fresh eggs, vary at temperatures of 21.1°, 26.7°, 32.2°, 35.0°, 37.8°, and 40.6° C. as follows: 14, 18, 24, 13, 26, and 20. These values may indicate that the rate of oxygen consumption varies least between different eggs at 35.0° C., is greatest at temperatures slightly above and below, and is less again as temperatures depart farther from this possibly optimum temperature.

The Q_{10} value (amount of increase per 10° C.) for the range of 21.1° to 31.1° C. increases from 1.5 at two days to 2.6 at four and seven days, to 2.9 at ten days, to 3.1 at twelve days, and averages 2.5. At the higher range from 27.8° to 37.8° C., there is no age effect discernible and the average value is 2.6. It would appear from this that for the lower range, temperature affects the rate of metabolism of embryos of greater age somewhat more than at younger stages, but that fluctuations at higher temperatures have the same relative effect at all ages.

Edwards (1902) long ago established the temperature threshold for development in unincubated eggs of the Domestic Fowl at approximately 20° C. This study shows that for eggs with embryonic development started, energy exchanges and probably growth are still relatively high at 21.1° C. and may occur at temperatures several degrees below this point although at reduced rates. The temperature threshold may very well vary for embryos in different stages of development.

RESPIRATORY QUOTIENT

For stages of incubation from two to twelve days inclusive, differences in air temperature cause no consistent variation in the respiratory quotient $\left(\frac{\text{Volume } CO_2}{\text{Volume } O_2}\right)$. An average of these ages gives values of 0.71 to 0.72 at all temperatures except 21.1° and 40.6° C. The average value for 40.6° C. is 0.75, but would be 0.72 if the two-day

TABLE 1

RATE OF GASEOUS EXCHANGE PER EGG PER HOUR AT VARIOUS TEMPERATURES

Oxygen absorption and carbon-dioxide output are given in cubic centimeters, water loss in milligrams

Days of incubation		0		2		4		7		10		12	
Temperature	Gas	No. of records	Average	No. of records	Average	No. of records	Average	No. of records	Average	No. of records	Average	No. of records	Average
21.1° C. = 70° F.	O_2	1	0.0010	2	0.0151	4	0.04	2	0.10	2	0.21	3	0.33
	CO_2	1	0.0031	2	0.0097	4	0.02	2	0.06	2	0.16	3	0.23
	H_2O	2	0.13	4	0.21	5	0.21	5	0.44	3	0.47	3	0.89
26.7° C. = 80° F.	O_2	1	0.0000	1	0.0185	2	0.08	3	0.19	4	0.42	4	0.59
	CO_2	1	0.0038	1	0.0144	2	0.06	3	0.13	4	0.30	4	0.41
	H_2O	2	0.28	4	0.31	2	0.41	2	0.43	1	0.29	1	1.13
32.2° C. = 90° F.	O_2	2	0.0011	2	0.0240	5	0.11	2	0.28	4	0.64	2	1.12
	CO_2	2	0.0064	2	0.0177	4	0.06	2	0.18	4	0.44	2	0.78
	H_2O	4	0.58	1	0.40	2	1.35	1	0.43	1	0.57	1	4.40
35.0° C. = 95° F.	O_2	3	0.0050	2	0.0506	4	0.16	2	0.40	6	1.01	2	1.32
	CO_2	3	0.0096	2	0.0386	4	0.10	2	0.28	6	0.73	2	0.94
	H_2O	3	0.71	4	0.70	2	0.95	2	0.94	2	1.28	2	4.34
37.8° C. = 100° F.	O_2	4	0.0077	5	0.0521	4	0.17	2	0.62	4	1.12	5	1.76
	CO_2	4	0.0157	5	0.0346	4	0.12	2	0.44	4	0.81	5	1.31
	H_2O	7	0.51	7	0.67	5	0.63	4	1.15	6	1.87	4	5.83
40.6° C. = 105° F.	O_2	4	0.0026	3	0.0519	4	0.21	3	0.60	3	1.17	3	1.55
	CO_2	4	0.0055	3	0.0386	4	0.15	3	0.42	3	0.88	3	1.16
	H_2O	4	0.53	3	0.80	3	0.88	3	0.83	2	1.63	3	7.64

embryo were excluded.   At 21.1° C., the average of the 2, 4, 7 days is only 0.61, but for 10 and 12 days it is 0.73.

If the values for all temperatures are averaged together for each age, the following variations are noted: 0 days, 3.4; 2 days, 0.78; 4 days, 0.64; 7 days, 0.69; 10 days, 0.72; 12 days, 0.72.   There is considerable difficulty in using the respiratory quotient as a criterion of the type of food being metabolized, although for the two older ages, one may suppose that fats are involved.   The very high values for unincubated eggs suggest that carbon dioxide is eliminated in excess of that freed by energy exchanges, while for the intermediate stages of incubation the low values indicate that some of the carbon dioxide produced is retained in the egg.   Barott (1937) found in chickens a high value of 1.0 at 2 days, the R. Q. of fresh eggs was not measured, a decrease to the low value of 0.60 at 9 days and then a return to around 0.66 for the rest of the period.   The direction of changes is similar to what they are in the House Wren, although the values and time intervals are different.   Bohr and Hasselbalch (1900) measured carbon-dioxide output in unincubated and newly incubated eggs and found a very high loss the first day, a decreased loss the second day, and a minimum loss during the third day, beyond which it again increased.   The loss the first day was 7.5 times the loss the third day.   Atwood and Weakley (1924) found a slightly greater loss of carbon dioxide the first day over the second but both days were below the third.

HEAT PRODUCTION

Heat production may be calculated from the oxygen absorption if the total energy of combustion is assumed to result in heat (Bohr and Hasselbalch, 1903) and assuming that the respiratory quotient throughout is 0.72.   Most investigators believe that the chief energy for growth is derived from the metabolism of fat (Bohr and Hasselbalch, 1903; Murray, 1925; Romanoff, 1932; Barott, 1937), although carbohydrates and proteins are utilized to some extent during the early part of the incubation period.   Unpublished data on wet weights of embryos freed from surrounding membranes have been obtained by Mr. Theodore Kramer for this species.   The heat production in calories per gram wet weight of embryo per day decreases as follows:

	35.0° C.	*37.8° C.*
2 days	0.8784 cal.	0.9048 cal.
4 days	0.3168 cal.	0.3384 cal.
7 days	0.2136 cal.	0.3312 cal.
10 days	0.2376 cal.	0.2640 cal.
12 days	0.1800 cal.	0.2400 cal.

If the weight of the embryonic membranes were added to the weight of the embryo, the decrease in metabolism with age would be less evident, since in the early stages the membranes, at least in the Domestic Fowl, make up a greater percentage of the total weight (Barott, 1937).

MOISTURE AND HEAT LOSS

Moisture loss increases about three times with increase in age up to ten days at all temperatures, except for some unknown reason at 26.7° and 32.2° C. This increase may be due to increased circulation of the blood in the allantoic membrane beneath the shell and also to possible thinning of the shell and increasing size of the shell pores. At twelve days of age the moisture loss with the hatching of the embryos amounts to about four times the rate at ten days. At all temperatures except 21.7° C. it was usual to have one or more embryos hatch at this stage during the course of the experiment. In the development of Domestic Fowl eggs the increase in moisture loss from the eggs is very slight from the first week until just before hatching, but with hatching the moisture loss increases 12 to 15 times (Barott, 1937).

Moisture output increases with rise in temperature until a maximum is reached, partly because the potential rate of evaporation from the surface of the eggs becomes greater and partly because of the increased metabolism and circulation of blood in the embryo and its membranes. The point at which the maximum loss is attained varies from 32.2° to 40.6° C. but occurs most frequently at the latter temperature and at 37.8° C.

Coefficients of variation in the rate of moisture loss for individual eggs are slightly higher for the latter half of the incubation; the average values for the six stages measured are 21, 26, 26, 32, 41, and 30, respectively. When the values for all ages are averaged for each of the six temperatures, they vary from the lowest to the highest temperatures as follows: 37, 27, 29, 15, 43, 20. There may be significance in that eggs lose moisture at the least variable rate at 35.0° C. and there is a tendency for the greatest variability in moisture output to occur at nearby temperatures rather than at extreme temperatures. The high value for 21.1° C. does not bear this out, but it may be a random variation.

Loss of heat from the eggs is through radiation, conduction, and vaporization of moisture. The importance of heat loss through evaporation may be estimated in terms of heat produced if a value of 0.58 calories is given for each gram of water lost. If values for all

ages from two to twelve days, inclusive, be averaged together, no consistent effect of temperature can be noted. In proportion to heat produced, that lost through evaporation varies from lowest to highest temperatures: 57, 66, 87, 66, 58, and 67. When values for all temperatures at each age are averaged, this proportion at 2, 4, 7, 10, and 12 days incubation varies: 173, 75, 28, 17, and 42. At two days incubation and doubtless also in fresh eggs, more heat is lost than is produced by the embryo. This should depress the temperature of the egg below that of the air and actual measurements of egg temperatures show that this occurs (Baldwin and Kendeigh, 1932, p. 136). As the heat produced by the embryo increases with the development of the embryo, moisture loss eliminates a smaller proportion and the egg temperature remains slightly above the temperature of the air. The increase in proportion of heat loss through evaporation at twelve days is due to eggs frequently hatching during the course of the experiments.

With 29 eggs weighed daily throughout their normal incubation period in the nest the loss in weight before hatching compared with the weight when fresh amounted to 13.7%. The weight of fresh eggs averages 1465 mg. The loss in weight of the eggs during the experiments varied in the same manner as did moisture loss although it tended to average slightly greater at all ages except twelve days.

PERCENTAGE HATCH

After the eggs had been in the open-circuit apparatus for about twenty-four hours, they were returned to the birds' nests and later their success in hatching was noted. The removal of the eggs from the nest had least effect at ten and twelve days, at which stage hatching averaged 80%. The most tender age appeared to be seven days when the average for all temperatures gave a hatch of only 50%. This was especially true for temperatures of 32.2°, 37.8°, and 40.6° C., while at 21.7°, 26.7°, and 35.0° C., the most sensitive ages were at 4, 12, and 0 days, respectively. Romanoff, Smith, and Sullivan (1938) in somewhat comparable experiments with the Domestic Fowl, found that a 24-hours' exposure to 29° C. increased mortality to some extent at four to five days incubation and greatly so at 16 to 17 days. On the other hand exposure to 41° C. had greatest effect during the first four to five days. When the percentages of hatch in the House Wren are averaged for all ages at each temperature, a removal of eggs from the nest and exposure for twenty-four hours at 35.0° and 37.8° C. had the least unfavorable effects, giving hatches of 86% and 85%,

respectively. At 40.6° C. the hatch decreased to 60% and with a drop of temperature to 21.7° C. it decreased to 40%. It must not be inferred from these experiments that these percentages of hatch would be obtained if the eggs were maintained at these various temperatures for the entire period of incubation.

DISCUSSION

Romanoff (1934) found an increased rate of growth and percentage hatch of pheasant and Bob-white embryos up to 38.3° or 38.9° C. but a distinct retardation at 40.6° C. In White Holland turkeys Romanoff (1935) found greatest hatch between 36.5° and 37.5° C., above which there was abrupt retardation. Likewise, in the Domestic Fowl, according to both Barott (1937) and Romanoff, Smith, and Sullivan (1938), greatest hatch comes at about 37.8° C., although the metabolic rate increases up to 38.9° or 39.7° C. According to Romanoff and Faber (1933), if the period from sixteen days of incubation to hatching alone is considered, the most efficient temperature for growth, metabolism, and survival comes at 34° to 36° C. In contrast to this, however, Eycleshymer (1907) found egg temperatures under an incubating hen to be 37.8° C. the first week, 38.3° C. the second week, and 38.9° to 39.4° C. the third week. We have found egg temperatures under incubating Bob-white to be 37.5° to 38.5° C.

From the data presented for the House Wren on oxygen absorption, carbon-dioxide output, moisture loss, and percentage hatch, a rise in temperature speeds up the rate of metabolism and probably growth but there is an upper limit of temperature above which harmful factors enter. This upper limit is approximately 37.8° C. or slightly above but certainly below 40.6° C. It would seem that temperatures close to 37.8° C. would be most favorable for rapid and successful incubation, although 35.0° C. is almost as good. A fall in incubation temperature to 32.2° C. or even to 26.7° C. would do less harm to the embryo than the smaller rise to 40.6° C., if continued for several hours. Apparently the most favorable incubation temperatures in natural nests of these wild birds would be between 35.0° and 37.8° C. and some sacrifice might be made in speed of development to certainty of hatching. The fact that the rate of oxygen loss and moisture output as measured on different eggs is less variable at 35.0° C. and more variable at 37.8° C. than at any other temperature, would indicate that the embryonic development under natural conditions may be best adjusted to the lower of these two temperatures. We have previously found (Baldwin and Kendeigh, 1932) that egg

temperatures in the nest of the House Wren fluctuate between 33.9° and 36.9° C. This would make the average temperature about 35° C.

The total energy of combustion for the development of the embryo to hatching is one of considerable interest. This may be computed from Table 1 in terms of oxygen absorption. At 35.0° C., this total oxygen absorbed amounts to 149 cc. Assuming a respiratory quotient of 0.72, this would be equivalent to 0.701 calories per egg. If 35° C. is considered the temperature at which incubation to hatching is normally accomplished in thirteen days and if a certain total amount of energy transformation is required for the development of the embryo up to hatching, then metabolism and development at 37.8° C. should be 126 per cent as rapid and require only ten days, while at 32.2° C. it should be only 72 per cent as fast and require eighteen days.

Tangl (1903) gives the developmental total for the sparrow (probably *Passer domesticus*) as 0.755 calories. This species has an incubation period of about the same length as the House Wren, but it is a larger bird with larger eggs and it would seem, in ratio to the House Wren, that its developmental total should be larger. With precocial species, on the other hand, such as the Domestic Fowl that requires twenty-one days for incubation and has, of course, very much larger eggs, Tangl and Mituch (1908) computed the total energy in terms of heat as 23 calories and Barott (1937) as 20 calories per egg. Barott states that 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 the product of these two, remained constant. Although Romanoff (1934) did not measure the developmental total for Bob-white and pheasant embryos, there may very well be one, for at 36.7° C. five or six more days were required for hatching than at 38.9° C. Factors determining the variation in the incubation period for different species of birds may well include the relative stage of development which the young embryo attains at hatching and the total energy utilization required to reach this stage.

A third factor that would be involved in determining the length of the incubation period would be the rate at which these energy transformations are taking place or, in other words, the rate at which growth is proceeding. If rate of oxygen consumption be taken as the measure of growth, in embryos of the House Wren of 7, 10, and 12 days of age this averages 1.87 cc. per gram wet weight per hour at a temperature of 35.0° C. In the Domestic Fowl a similar average for the same-aged embryos at a temperature of 35.6° C. is only 0.47 cc.

(Barott, 1937). Similar comparisons at 37.8° C. give 2.47 cc. for the House Wren and 1.4 cc. for the Domestic Fowl. From this it appears that energy exchange is four times faster in the House Wren than in the Domestic Fowl at 35° C. and 1.8 times faster at 37.8° C. Hereditary differences between species in rates of development probably occur and doubtless this will affect the time required to reach the stage for hatching.

Comparing the energy exchanges in these two species at 35° C. and at 37.8° C., as has just been done, demonstrates that the House Wren is better able to maintain a high rate at the lower temperature than is the Domestic Fowl. With a drop from the higher to the lower temperature, the rate of oxygen absorption decreases 66% in the Domestic Fowl and only 24% in the House Wren. This may well mean that different species are adjusted to different incubation temperatures although in general the higher the incubation temperature the more rapidly will energy exchanges take place. Differences between species in incubation temperature may well affect the length of the incubation period even allowing for differences in adjustment to them.

The relative humidity in the nest of this species has not been measured but may be estimated from the data given in this paper. The average hourly loss in weight of eggs in the nest during the first ten days of natural incubation is 0.65 mg. This loss in weight is approximately all moisture. The measured amount of moisture loss per egg per hour at 35.0° C. in the experiments averages 0.85 mg., when the relative humidity averages about 12%. As no moisture is lost from the eggs at a relative humidity of 100% and moisture loss varies directly with relative humidity (Romanoff, 1934; Barott, 1937), a straight line may be drawn between these two extreme humidities. On this line an evaporation rate of 0.65 mg. per hour would represent a relative humidity in natural nests of about 35%. The average relative humidity of the atmosphere in this region during June is between 70 and 80% and the average air temperature about 21° C. If there is no change in the average amount of moisture in the air, and the air temperature is raised from 21° to 35° C., the relative humidity would be decreased to around 35%. This implies that in this species, neither the eggs nor the incubating parent affect the relative humidity in the nest except indirectly through change in the nest temperature. In other species this may vary, depending upon nest material and habits of the incubating parent. In artificial incubation of Bob-white and pheasant eggs, relative humidities varying

between 65 and 75% appear most favorable (Romanoff, 1934); in the Domestic Fowl, a relative humidity of 61% gives the largest hatch (Barott, 1937). However, these most favorable humidities for artificial incubation may not actually be maintained in natural nests. Cadman (1923) measured the relative humidity under a sitting hen and found it to vary between 15 and 50%, or over a range around that estimated for the House Wren. Romanoff (1929) found that in artificial incubation a low humidity of 40% retarded growth but did not increase mortality of the embryos. On the other hand, a humidity of 80% favored growth but increased mortality. Perhaps the low humidity complements the high temperatures in natural nests of the Domestic Fowl so that low mortality and high rate of development are both attained.

In artificial incubation, turning and cooling the eggs is important. In natural nests of the House Wren, the eggs are turned frequently. This turning is incidental to the adult bird's inserting and raising its feet from between the eggs and the bottom of the nest. Cooling the eggs is partially accomplished when the adult bird leaves for its inattentive or feeding periods. These periods last several minutes and come frequently during the day, since they alternate with somewhat longer attentive periods when the bird sits on the eggs. This cooling may average only 2.8° C., but the alternating change of temperature may actually be stimulating to the development of the embryo, thereby compensating for the lower average temperature.

In this study, oxygen consumption per gram weight of embryo appears to decrease with increasing incubation and size of embryo. Another study (Kendeigh, 1939) shows that, after hatching, gaseous exchange per gram weight of bird increases with age and size. There is no marked change on the day of hatching; the newly hatched bird has only a slightly higher rate of gaseous exchange than the twelve-day embryo at all temperatures except possibly 40.6° C. This is contrary to the finding of Lussana (1906) and Mitchell, Card, and Haines (1927) that at hatching in the Domestic Fowl, oxygen absorption increased two or three times. Precocial birds at hatching are, of course, much further developed than altricial ones and their body-temperature regulating mechanism becomes functional at about this time. The decrease in the rate of metabolism per gram weight of embryo may be deceptive. Barott (1937) shows that when all the living tissues of the egg are considered, including the extra-embryonic membranes, the metabolic rate fluctuates but does not decrease throughout the incubation period as it does when figured only on

the basis of the weight of the embryo. In fact, for the latter half of the twenty-one days of incubation in the Domestic Fowl, it seems to be increasing. There may be, then, a gradual increase in metabolic rate through most of the developmental period which is the typical condition and which is conditioned by the increased efficiency of the vital processes concerned with the maturing of the cells and the coordinating of body functions.

SUMMARY AND CONCLUSIONS

1. The rate of oxygen absorption by House Wren embryos is the same throughout the breeding season although there are two breeding periods.

2. The rate of gaseous exchange in the egg is increased when the incubation temperature is raised from 21.1° to 37.8° C. but not to 40.6° C.

3. Carbon-dioxide output from the egg is high compared with oxygen absorption during the early days of incubation and low during the intermediate days, probably because some carbon dioxide is freed or retained independently of combustion processes. The respiratory quotient is 0.72 for the latter days of the incubation period, representing fat as the predominant source of energy.

4. The rate of moisture and weight losses by the egg increases with amount of incubation and becomes considerable on the day of hatching. At all ages a rise in temperature causes an increase in moisture and weight losses until a maximum is reached. The proportion of heat loss from the egg, due to vaporization of moisture, to embryonic heat production decreases as incubation proceeds, so that the temperature of the egg becomes slightly raised.

5. A twenty-four-hour exposure of eggs to temperatures both above and below 35.0° and 37.8° C. decreases the percentage of hatch. In half of the experiments the greatest effect of removal from the nest and exposure to various temperatures occurred with embryos having seven days of incubation.

6. If rate of gaseous exchange is taken as an index of rate of growth, then the most favorable incubation temperatures for rapid development are between 35.0° to 37.8° C. Considering the greater uniformity of response, the high percentage of hatch, the average egg temperature in the nest, and the possible stimulating influence of fluctuating nest temperatures, incubation temperatures varying around 35° C. appear normal in this altricial species, the House Wren.

7. The length of the incubation period may be determined by the stage of development reached by the embryo at hatching, by the total energy exchanges necessary to attain this stage of development, and by the rate at which these energy exchanges are going on. These factors in turn may be affected by the genetic inheritance of the species as well as by conditions in the nest.

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