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(Technical)

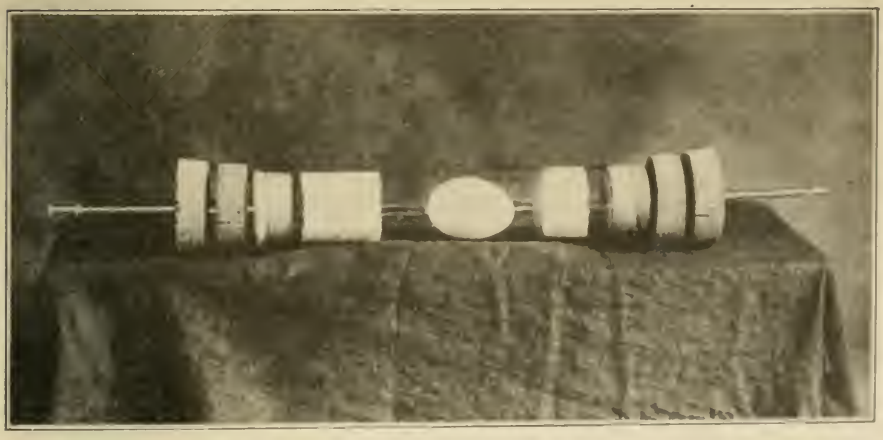

Fig. 1.—An Egg In A Glass Tube Ready for The Incubator.

By
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Amount Of Carbon Dioxide Given Off By Eggs During Incubation

During incubation eggs give off moisture and carbon dioxide. The circulation of air through the incubation chamber is designed especially for the purpose of carrying away this moisture and carbon dioxide and for supplying oxygen to the developing embryos.

The total loss in weight of eggs thus occasioned during incubation has been studied by the senior author and the results published as Bulletin 73, West Virginia Agricultural Experiment Station. The present publication deals only with the amount of carbon dioxide given off. The chemical work of this investigation has been done entirely by the junior author who also designed the apparatus employed.

PREVIOUS WORK

Practically all of the work reported to date has been based upon the amount of carbon dioxide found in the air surrounding eggs hatched either in incubators or under hens. Brigham (1) reported that he found from 13 to 61 parts of carbon dioxide in 10,000 parts of air, in air drawn from the egg chamber of an incubator. Thom (2) determined the amount of carbon dioxide in incubators and under hens and found an average of 31.98 parts per 10,000 parts of air, in air drawn from under hens, and about one-half as much in air from incubators. Dryden (3) found more carbon dioxide in the air taken from under hens incubating fertile eggs than in the air from incubators. Later he found (4) that the hatch was not improved by increasing the amount of carbon dioxide present in the air in the incubator. Edmond (5) determined the carbon dioxide in incubators and under hens, and found that the amount gradually increased as the incubation progressed. Under hens toward the close of the incubating period he found 22 parts in 10,000 parts of air, and in the air from incubators from 4 parts at the beginning of the hatch to 20.5 parts in 10,000 parts of air at the end. Lamson and Edmond (6) found that the amount of carbon dioxide given off increased in proportion to the

(1) Rhode Island Experiment Station, Annual Report, 1901
(2) Ontario Department of Agriculture, Bulletin 163
(3) Utah Experiment Station, Bulletin 92.
(4) Utah Experiment Station, Bulletin, 102.
(5) Storrs Experiment Station, Annual Report, 1907.
(6) Storrs Experiment Station, Bulletin 76

weight of the developing embryo. Under setting hens the amount increased toward the latter part of the hatch to from 50 to 60 parts per 10,000 parts of air, and in commercial incubators to from 30 to 50 parts in 10,000 parts of air. Good results were obtained when the carbon dioxide did not exceed 60 parts in 10,000 parts of air which, according to these authors, approximates the amount found in natural incubation. The effect of carbon dioxide was slight until a maximum of 150 parts was reached; as the amount rose above that point there was a marked decrease in the number of chicks hatched. Atwood and Weakley (7) showed that fertile eggs gave off about 10 grams of carbon dioxide during the 21 days of incubation and that the amount increased rapidly toward the latter part of the period.

In this connection it may be well to observe that the carbon dioxide given off is the result of an oxidation process in which oxygen from the air combines with some of the carbon of the egg, the result being that although we speak of the loss of carbon dioxide yet the actual loss of egg substance is only about 27.3 percent of the total weight of the carbon dioxide measured, the balance of the weight being oxygen.

The amount of carbon dioxide per 10,000 parts of the air in air taken from under hens or from incubators would be influenced by the rapidity of circulation of the air about the eggs, consequently all of the investigations mentioned, except the last, present few definite data as to the amount of this gas given off by the developing embryos.

METHODS OF INVESTIGATION

In the present work it was felt that each egg should be incubated by itself because when several eggs are incubated together the total amount of carbon dioxide given off might be influenced by the lack of fertility of certain eggs or by the weakness or death of embryos. Also it was desired to study the amount of carbon dioxide given off at various periods of incubation as well as various factors affecting the vigor of the embryos, for which purpose the results from mass-incubation would be entirely inapplicable.

Each egg was incubated by itself in a glass tube (see Figure 1 on cover). One end of this tube was connected to an air supply from which all traces of carbon dioxide had been removed, and the other

(7) Journal of the American Association of Instructors and Investigators in Poultry Husbandry. Vol. I, No. 4, pp. 26-28 (1915).

end was connected to a series of calcium chloride and moist soda lime tubes for absorbing the moisture and carbon dioxide.

Removing the Carbon Dioxide From the Air

The ordinary methods of removing carbon dioxide from the air were found to be unsuitable for this work, as the eggs should be under practically atmospheric pressure during incubation. Consequently the air used was first passed over the surface of a caustic soda solution in a porcelain jar[1] closed by a lid of special design so constructed that the air could be led through a series of concentric passages and through an orifice in each partition thus keeping the air from becoming stratified while passing over the solution. These jars held about five gallons and were filled with the solution of caustic soda to within one-half inch of the top when the lid was inserted. Figure 2 shows a jar and lid. The walls of the concentric rings were about 2½ inches deep. At the rate at which air was drawn through the egg tubes the removal of carbon dioxide was complete.

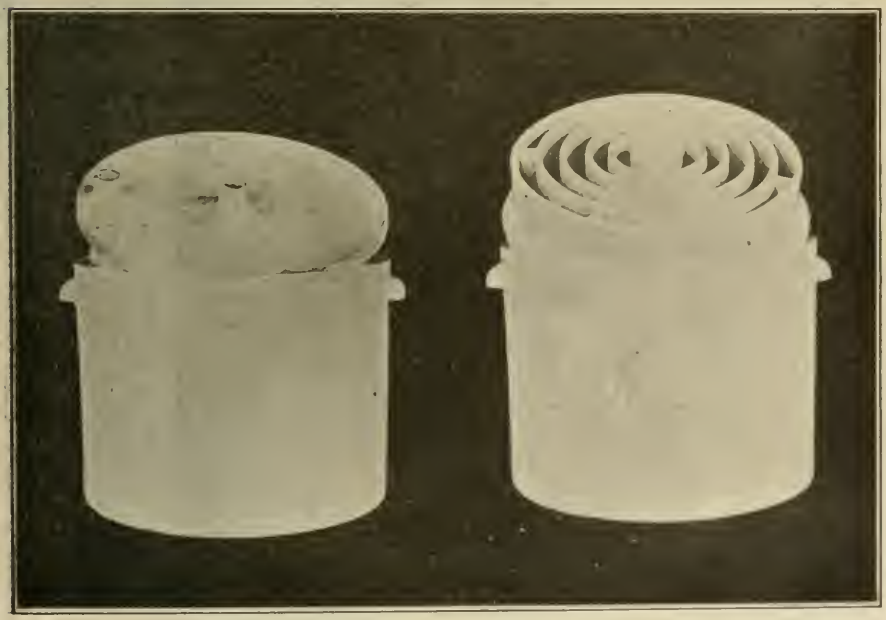

Fig. 2.—Porcelain Jar On Right Shows Concentric Rings of Lid; On Left Shows Openings In Top of Lid.

[1]Made by Abingdon Sanitary Manufacturing Co., Abingdon, Ill.

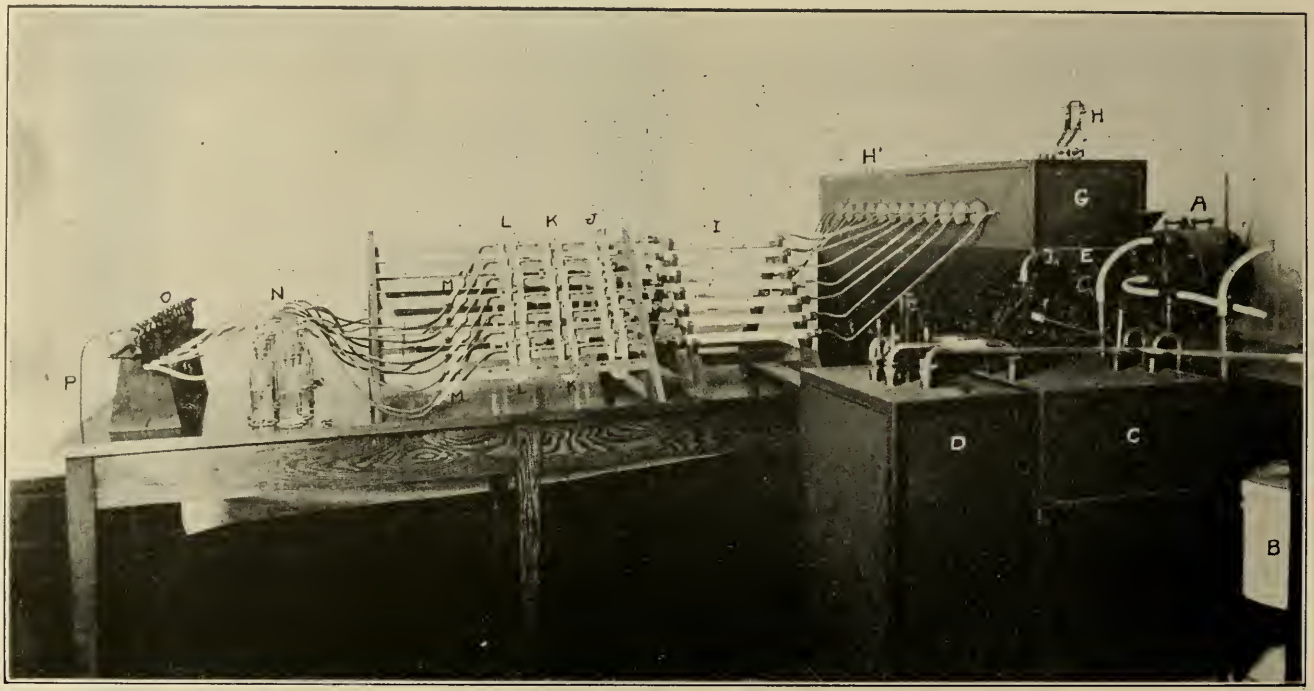

Fig. 3.—A General View of Apparatus Showing Rear of Incubator and Calcium Chloride Train. A, Meter for Measuring the Incoming Air; B, Absorption Jar to Free Air from Carbon Dioxide; C, Humidifier to Moisten Air; D, Condenser to Condense Excess Moisture from Air. (C is Always Held at Higher Temperature Than D, Which Has a Fixed Predetermined Temperature); E, Distributor for Distributing the Incoming Air to the Various Egg Tubes F, in the Incubator G; H and H', the Two Thermostats for Controlling the Temperature of the Upper and Lower Parts of the Incubator; I, Calcium Tubes for Preliminary Drying of the Air After Passing Over Eggs; J, Calcium Chloride Tubes for Final Drying; K, Soda Lime Tube for Absorbing Carbon Dioxide; L, Calcium Chloride Tube Weighed With K; M, Calcium Chloride to Protect L; N and O, Absorption Bottles and Needle Valves for Regulating the Flow of Air; P, Line to Suction Pump. See also Figure 4.

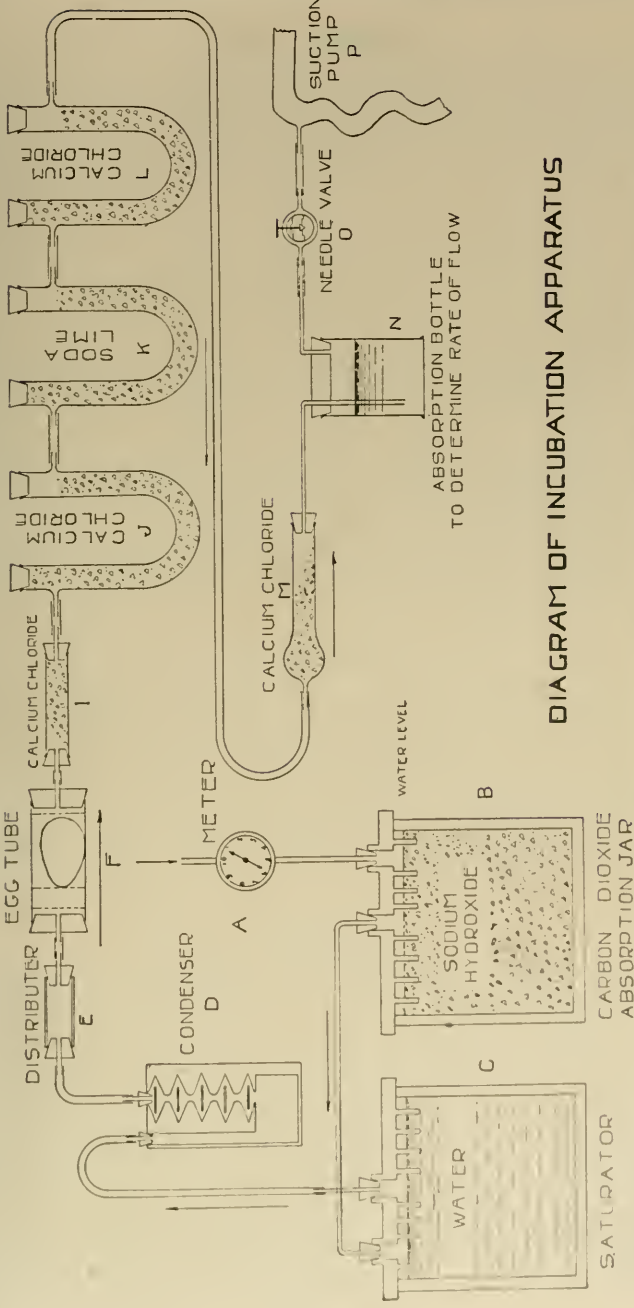

Fig. 4.—Diagram Showing Arrangements of Parts and Air Flow of Incubation Apparatus Shown in Fig. 3.

Incubators Used

Two incubators were used: one a Bausch and Lomb constant-temperature bacteriological incubator modified so as to permit the use of the egg tubes and necessary air distributors; the other a specially constructed incubator in which the top temperature of the egg could be controlled independently of the bottom temperature. The latter was, in effect, two incubators, one superimposed upon the other and provided with independent thermostats.

The egg tubes were about 5 cm. in diameter and 25 cm. long, closed with No. 11 rubber stoppers having glass tubes in the center sufficiently long to extend through the walls of the incubator. Cheese cloth diaphragms were used in each end of the egg tubes to keep the eggs near the center and to break up any direct air currents. The glass tubes extending through the stopper and the sides of the incubator were connected with the air supply and the calcium chloride train by rubber tubes so as to permit the turning of the eggs by merely turning the glass tubing.

Air Circulation

The total amount of air used for each set of eggs was measured by a wet meter and the amount passing through each egg tube was regulated by a needle valve on the suction end, the rapidity of circulation being observed through the use of absorption bottles. The air was sucked through by a vacuum pump which maintained a uniform suction at all times.

Humidity

After the air was freed from carbon dioxide it was passed through a series of jars similar to those used for the removal of the carbon dioxide, but filled with water. This could be held at any given temperature by an electric heater and thermostat. By this means the air could be saturated with moisture at any desired temperature above room temperature. Then it was passed through a constant-temperature condenser of special design which consisted of a series of flattened copper bulbs provided with baffle plates and the whole immersed in water which could be held at a uniform temperature, thus permitting the humidity to be reduced to any desired point.

Measuring the Carbon Dioxide Given Off

After the air, which had been freed from carbon dioxide, had been drawn through the various egg tubes each containing an egg it was passed through two calcium chloride tubes, the first being a horizontal tube about two feet long which removed the greater part of the moisture, and the second a "U" tube which removed the remainder. These tubes before being used were saturated with carbon dioxide. The air then passed through a "U" tube filled with moist soda lime, and a calcium chloride "U" tube to catch the moisture given off by the soda lime. Also, a calcium chloride tube was used as an insurance against any moisture creeping back from the absorption bottles. Figure 3 shows the general arrangement of the tubes.

The soda lime and calcium chloride tube which followed it were weighed together every 24 hours, using a counterpoise of the same sized tubes partially filled. One extra set of calcium chloride and soda lime tubes was provided. When the tubes in Line 1 were removed for weighing, the extra tubes were immediately placed in the line so that there would be as little interruption in the circulation of air through the system as possible. The tubes just removed and weighed were then placed in Line 2 and so on.

Checking the Determinations

Two egg tubes were allowed to remain empty during each hatch and the weighings were made for carbon dioxide as in the other cases. This was done so as to be certain that the apparatus was working satisfactorily.

Management of Eggs

In all instances incubation was started on the day following that on which the eggs were laid. The eggs were turned at least twice each day by turning the glass tubes in which they were contained, and an effort was made to maintain the humidity and temperature as nearly normal as possible. For the purpose of reading the temperature a thermometer was inserted in one of the vacant egg tubes at approximately the height of an egg. The fluctuations in temperature were never greater than ½ degree Fahr. The eggs remained within their respective tubes from the beginning of incubation until the tubes were opened to remove the chicks.

It may be well to mention here that the authors have been engaged for some years in the study of the vigor of chick embryos as measured by the carbon dioxide which they throw off during the period of incubation and the data presented in the following tables have been selected from the material accumulated in that investigation. As a consequence, the eggs studied have been produced under somewhat varying conditions but it is felt that these factors are of negligible importance as affecting the value of these data which are presented for the purpose of affording a basis for the intelligent ventilation of incubators.

Only eggs laid by Single Comb White Leghorn fowls were incubated, and the data represent the results from 63 eggs that hatched normal chicks.

AMOUNT OF CARBON DIOXIDE GIVEN OFF PER EGG

Table I gives the average amount of carbon dioxide, in grams, given off each day during incubation, together with the probable error and the standard deviation.

TABLE I.—Amount of Carbon Dioxide Given Off By Eggs During Incubation.

Day of Incubation	Grams of Carbon Dioxide	Standard Deviation
First	.0128 ± .0003	.0031
Second	.0085 ± .0002	.0028
Third	.0119 ± .0002	.0025
Fourth	.0210 ± .0003	.0034
Fifth	.0312 ± .0002	.0020
Sixth	.0391 ± .0002	.0019
Seventh	.0513 ± .0004	.0050
Eighth	.0681 ± .0005	.0059
Ninth	.0916 ± .0008	.0090
Tenth	.1261 ± .0012	.0147
Eleventh	.1758 ± .0028	.0335
Twelfth	.2488 ± .0026	.0312
Thirteenth	.3377 ± .0036	.0424
Fourteenth	.4580 ± .0051	.0606
Fifteenth	.5739 ± .0057	.0671
Sixteenth	.6706 ± .0047	.0553
Seventeenth	.7277 ± .0057	.0667
Eighteenth	.7563 ± .0041	.0488
Nineteenth	.7801 ± .0065	.0766
Twentieth	1.0036 ± .0171	.2016
Twenty-first	1.4260 ± .0216	.2545

Table I shows that beginning on the second day the amount of carbon dioxide given off increased daily until the end of the period. It seems probable that the amount shown for the first day includes carbon dioxide originally dissolved in the egg substance and which escaped during the first twenty-four hours after the eggs were surrounded by carbon-dioxide-free air due to the lowering of the vapor pressure. In any event the amount so given off is small and of no practical significance.

From the beginning of incubation to the tenth day the daily increase in the amount of carbon dioxide given off was small, but after that half-way period in the development of the embryo was passed, the daily increase was very great. The increase from the nineteenth to the twentieth day is practically equal to the total amount given off during the first eight days, and the increase from the twentieth to the twenty-first day is roughly equal to all the carbon dioxide given off during the first half of the hatch.

Figure 5 shows graphically the average amount of carbon dioxide, in grams, given off per egg per day during incubation.

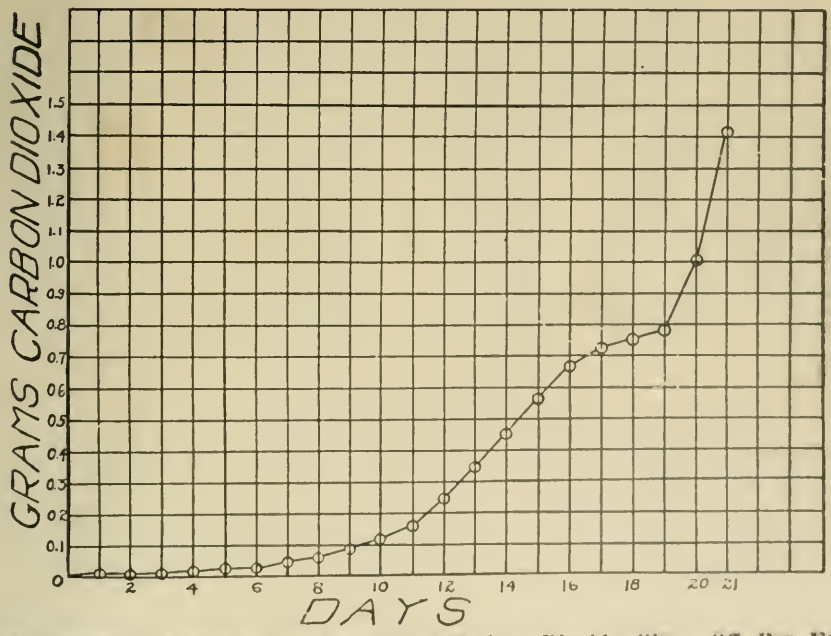

Fig. 5.—Graph Showing the Amount of Carbon Dioxide Given Off Per Egg Per Day During Incubation.

It is apparent from Figure 5 that the circulation of air through the incubator chamber for the purpose of removing the carbon dioxide that is generated may be greatly restricted up to the tenth day. By thus restricting the circulation, the moisture present in the eggs may be conserved during the first part of the hatch. Later, however, the oxidation process is greatly accelerated and the ventilation should be increased correspondingly. Especially during the last two days of incubation should the eggs be given a liberal supply of air.

AMOUNT OF AIR REQUIRED FOR ONE HUNDRED EGGS DURING INCUBATION

As already stated, Lamson and Edmond found that the air under setting hens toward the end of the hatch contained from 50 to 60 parts of carbon dioxide in 10,000 parts of air. Using the smaller value and the weights of carbon dioxide shown in Table I, the number of cubic feet of air required daily per hundred eggs is shown in Table II.

TABLE II.—Number of Cubic Feet of Air Required Daily per One Hundred Eggs to Maintain a Carbon-Dioxide Content of Fifty Parts in 10,000 Parts of Air.

Day of Incubation	Cubic Feet of Air for 100 Eggs Per Day	Day of Incubation	Cubic Feet of Air for 100 Eggs Per Day
First	4.9	Twelfth	94.7
Second	3.2	Thirteenth	128.5
Third	4.5	Fourteenth	174.3
Fourth	8.0	Fifteenth	218.4
Fifth	11.9	Sixteenth	255.1
Sixth	14.9	Seventeenth	276.9
Seventh	19.5	Eighteenth	287.7
Eighth	25.9	Nineteenth	296.8
Ninth	34.9	Twentieth	381.8
Tenth	48.0	Twenty-first	542.5
Eleventh	66.9		

It should be understood that the authors consider Table II largely as suggestive. The comparative values as given are correct, but it may be found that it is not necessary to ventilate so freely as to maintain only 50 parts of carbon dioxide in 10,000 parts of air. It should also be pointed out that the table is based on the assumption of a uniform percentage of carbon dioxide in the air of the incubator from start to finish, but it is possible that the embryos can tolerate a greater percentage of carbon dioxide in the air at one stage of

development than at another. Also in any commercial incubator some of the eggs are certain to be infertile or to have weak germs, which would reduce the amount of air required.

FACTORS AFFECTING THE AMOUNT OF CARBON DIOXIDE GIVEN OFF BY EGGS DURING INCUBATION

Infertility

Infertile eggs give off little carbon dioxide. As an example, the following record is given of an infertile egg weighing 58.939 grams placed in the incubator December 22, 1914.

Day of Incubation	Loss of Carbon Dioxide in Grams	Day of Incubation	Loss of Carbon Dioxide in Grams
First	.0127	Fifth	.0029
Second	.0064	Sixth	.0034
Third	.0058	Seventh	.0029
Fourth	.0040	Eighth	.0008

Weak Germs

Eggs with weak germs may give off nearly a normal amount of carbon dioxide for a few days. The amount then decreases rapidly. As an example the following record may be taken as typical. Weight of egg was 60.976 grams when incubation started May 16, 1914.

Day of Incubation	Loss of Carbon Dioxide in Grams	Day of Incubation	Loss of Carbon Dioxide in Grams
First	.0120	Seventh	.0532
Second	.0080	Eighth	.0758
Third	.0151	Ninth	.1018
Fourth	.0235	Tenth	.0641
Fifth	.0376	Eleventh	.0243
Sixth	.0423	Twelfth	.0230

The embryo died during the tenth day.

Weight of the Eggs

Benjamin[1] has shown that other things being equal the larger the egg, the larger and more vigorous the chick hatched therefrom. Accepting this, one would naturally assume that large eggs would throw off more carbon dioxide than small eggs. To study this problem Table III has been arranged. The same data that were used in the preparation of Table I are used here.

[1]Cornell University Agricultural Experiment Station, Memoir 31.

TABLE III.—Correlation Between the Weight of Eggs And the Amount of Carbon Dioxide Given Off.

Carbon Dioxide Given Off (In Grams).

Weight of Eggs (In Grams).	6.1 to 6.19	6.2 to 6.29	6.3 to 6.39	6.4 to 6.49	6.5 to 6.59	6.6 to 6.69	6.7 to 6.79	6.8 to 6.89	6.9 to 6.99	7.0 to 7.09	7.1 to 7.19	7.2 to 7.29	7.3 to 7.39	7.4 to 7.49	7.5 to 7.59	7.6 to 7.69	7.7 to 7.79	7.8 to 7.89	7.9 to 799	8.0 to 8.09	8.1 to 8.19	8.2 to 8.29	8.3 to 8.39	8.4 to 8.49	8.5 to 8.59	8.6 to 8.69	8.7 to 8.79	8.8 to 8.89	8.9 to 8.99	9.0 to 9.09	9.1 to 9.19	9.2 to 9.29	9.3 to 9.39	TOTAL
45 to 45.99						1																												1
46 to 46.99																																		0
47 to 47.99																																		0
48 to 48.99													1																					1
49 to 49.99												1		1		1																		3
50 to 50.99				1													1																	2
51 to 51.99			2																															2
52 to 52.99	1										1			1			1		1				2											7
53 to 53.99										1		1																						2
54 to 54.99								1				1	1	1	1				1		2													8
55 to 55.99				1												2		1	1				1											6
56 to 56.99									1		1			1		2	2		2	2	1													12
57 to 57.99														2						1	2					1			1					7
58 to 58.99						1								1																			1	3
59 to 59.99												1			1								1											4
60 to 60.99																							1	1									1	3
61 to 61.99																		1																1
62 to 62.99																																		0
63 to 63.99																					1													1
TOTAL	1	0	2	2	0	2	0	1	1	1	2	4	2	7	2	5	4	2	5	3	6	0	5	1	0	1	0	1	1	0	0	0	2	63

The weight of eggs incubated subjective; grams of carbon dioxide given off relative; coefficient of correlation = + .641 ± 050.

The correlation is positive and significant and shows that the larger eggs generally give off more carbon dioxide than do the smaller ones. This is in harmony with Benjamin's results, and it would consequently seem that the practice of selecting the larger eggs for incubation tends toward the maintenance of size and vigor of offspring.

CONCLUSIONS

1.—The amount of carbon dioxide thrown off by eggs during the incubating process is relatively small during the first half of the hatch. Consequently the ventilation may be materially restricted during the first ten days, if this should be desirable in order to conserve the moisture in the eggs.

In the last half of the period of incubation the amount of carbon dioxide given off increases rapidly. Therefore the amount of air passing through the incubating chamber should be increased correspondingly. During the last two days in particular the supply of air should be ample.