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THE AIR SPACE OF THE HEN'S EGG AND ITS CHANGES DURING THE PERIOD OF INCUBATION

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The air space of the hen's egg and that of the bird's egg in general, and its changes during the period of incubation, are insufficiently known in many respects, in spite of the fact that its phenomena might be of interest for the study of foetal respiration in the chick. Even its origin is not fully known. According to Lataste [1925] its appearance is the result of contraction of the soft parts from the rigid egg shell as the egg cools after having left the mother's body. Evaporation of water from the surface of the shell was considered to increase its volume gradually, but no importance should in his opinion be attached to this regarding its appearance. It is not easy to understand why evaporation of water should not contribute to its appearance as well as to its expansion, the egg being exposed to both influences simultaneously as soon as it has been laid. Lataste's argumentation that reptile eggs with flexible walls have no air space does not disprove this view.

There are three properties of the air space which interested us especially: (1) the volume and its changes during the period of incubation, (2) the composition of the gas contained and its changes, and (3) pressure of the gas and its changes.

1. Volume of the air space

Some figures relating to the volume of the air space have been given by Hasselbalch [1902]. In order to obtain greater quantities of gas the eggs had previously been kept in a thermostat at a temperature of 25 and 38° C.

The change of volume which the gas space undergoes during incubation has been a subject for investigation as much as a century ago [Dulk, 1830]. Afterwards Preyer [1885] gave some figures. The field was examined more systematically by Aggazzotti [1914], from whose figures Needham in his great work, *Chemical Embryology* [1931], has constructed a curve.

Method. Hasselbalch [1902] measured the volume of the space by measuring the volume of gas aspirated by a small syringe after the shell over the air space had been removed and the membrane perforated by the needle. During this manipulation the egg was kept upright under distilled water, the gas space upwards. Aggazzotti [1914] collected the gas in a funnel after the egg shell had been perforated, the egg being immersed in a saturated solution of sodium chloride made slightly acid with hydrochloric acid. Measurement of the volume was carried out in a calibrated part of the funnel.

Four series of fresh fertile eggs of White Leghorn hens were used in our experiments. The number in each series varied from twenty-five to fifty. Before being put into the incubator they were kept in a cool room for 24 hr. The incubator had a temperature of nearly 40° C.; it did not vary more than $\frac{1}{2}$° in either direction. By a plate covered with moistened cotton a reasonably constant evaporation of water was ensured. The eggs were cooled every day for 5 min. by opening the incubator, the temperature thus falling to room temperature. At the same time they were turned; this turning was repeated about 7 hr. later. It may be remembered that with the chick the time of hatching is 21 days.

The gas was driven out of the air space by mercury and brought into the burette of a 5 c.c. Haldane gas analysis apparatus for measurement. If its volume was smaller than 3 c.c. it was mixed here with a measured volume of nitrogen. Its volume was thus determined with an accuracy of 0·002 c.c. Afterwards it was analysed in the usual way.

In Fig. 1 the apparatus used is reproduced.

The egg, its blunt end upwards, is pressed softly into a layer of glass beads, with which the glass cup C is filled to about one-quarter. A flat iron ring R about 1 cm. broad is placed over the egg and firmly fixed in a stand. Now the cup C and the funnel F are filled with mercury from the levelling tube L by opening stopcock S_1. By using the levelling tube of the gas analysis apparatus the stopcocks S_2 and S_3, the funnel F, the connecting tube t and the burette of the Haldane apparatus are filled with mercury as is shown in the figure. Then the borer B is brought under the mercury surface, and by moving it in a horizontal direction the egg shell is perforated. A limiting disk prevents the point of the borer from penetrating more than 1 mm. into the egg. After the borer has been removed all the gas is collected in the funnel and may be transported to the gas analysis apparatus for measurement and analysis.

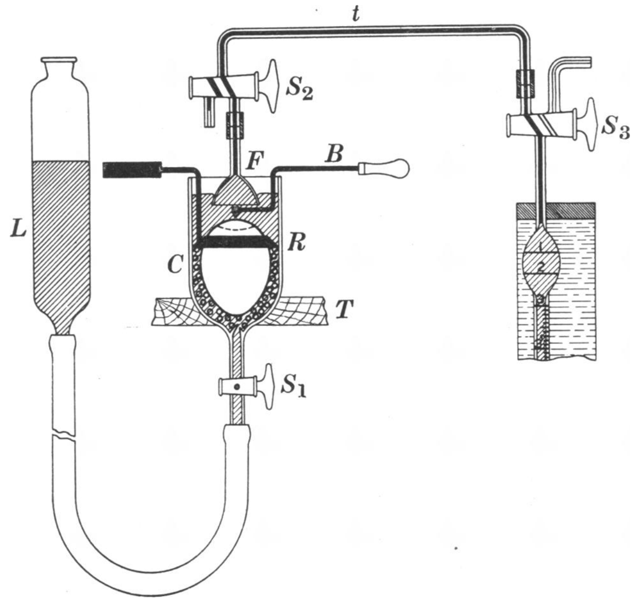

Fig. 1. Apparatus for collecting the gas from the air space, shown just before the egg shell is perforated by the borer (*B*). The gas which will be received by the funnel (*F*) is brought into the Haldane gas analysis apparatus via S_2 and S_3 for measurement and analysis. *L* is the levelling tube filled with mercury. Cup (*C*), funnel (*F*), connecting tube (*t*) and burette are all filled with mercury.

Results. The changes of volume were followed by examining two to eight eggs every day of incubation. The results obtained in the four series agree and will therefore be treated together. Since the eggs varied in weight it seemed desirable to reduce the figures for the gas volume to one and the same weight, viz. 100 g. It was assumed that the pressure in the air space would not differ notably from that of the atmosphere (see under (3)) and accordingly the volumes were reduced to 76 cm. Hg and 40° C. In Fig. 2 a graph is given constructed from the figures obtained in two series.

As Fig. 2 shows, there is a rather wide variation in the volume of air space measured on one and the same day of incubation in various eggs. The volume increases as the period of incubation proceeds, the relation between them being rectilinear. In this respect our curve differs from that constructed by Needham from Aggazzotti's figures, which had an

S-shape, showing the greatest increase of air-space volume between about the 6th and the 13th day. Starting from Aggazzotti's figures we would have preferred to draw a simpler line than Needham did, but this is not of great importance. On account, however, of the rather great divergence found in various eggs on one and the same day Needham's curve, constructed from one or two observations a day, cannot give an accurate picture.

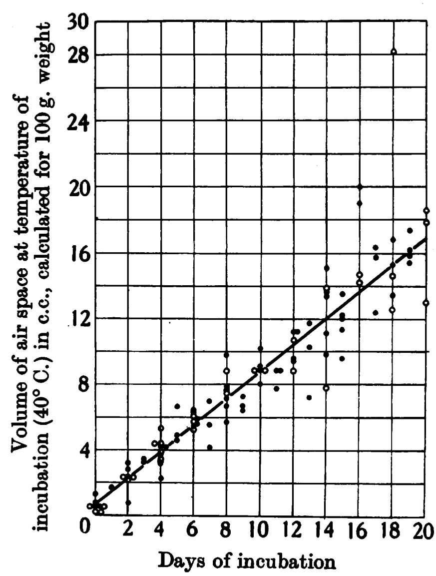

Fig. 2. Increase of the air space during incubation of fertile eggs (•), and unfertilized eggs (o).

A striking difference in slope exists between Aggazzotti's curve and ours. Whereas ours forms an angle of about 40° with the abscissae, the angle of Aggazzotti's curve is less than 30°. Thus the eggs of Aggazzotti's experiments incubated for 18 days had an air space of about 8 c.c., whilst in our experiments the average volume was 11 c.c. (both reduced to 40° C. and 76 cm. Hg). The difference must most probably be attributed to difference in permeability of the shell which varies in animals of different stock. The rather great differences in permeability we found in eggs obtained from one and the same stock, and even from one and the same brood kept under just the same conditions, are doubtless the cause of the wide range within which the volume of air space varies in eggs examined on the same day of incubation.

The question as to how far the increase of volume during hatching is brought about by either the developing foetus or by evaporation of water was examined by bringing into the incubator a series of twenty-eight unimpregnated eggs which for 20 days were treated in exactly the same manner as the impregnated eggs. The changes of volume found here are also given in Fig. 2. The reader will see that the points do not fall far from the curve constructed for the fertilized eggs.

The results show that there is no parallelism between the size of the egg on the one hand and either the loss of weight or the volume of the air space on the other. For example, two larger eggs (72·8 and 75·1 g. respectively), examined on the 4th day, had an air space smaller than two others of 57 and 62 g. respectively. Again, the smallest of the three eggs examined on the 6th day exhibited the greatest loss of weight and an air space, larger than that of an egg which was about 10 % bigger. Moreover, eggs of practically the same size may exhibit notable differences in both loss of weight and volume of air space. The properties of the egg shell rather than the dimensions of the egg determine the progress of volume of the air space. There is no fixed relation, moreover, between loss of weight and volume of the air space.

2. Composition of the gas in the air space

Analyses of the contents of the air space have also been carried out as early as 1830 by Dulk. Preyer [1885] was the first to observe a surprisingly high oxygen content when the eggs had been incubated for some days, no matter whether the eggs were fertile or not. The same observation was made by Hasselbalch [1902]. In incubated fertile eggs he found an average of 21·21 % oxygen, the highest figure being 21·57 %. In infertile eggs about the same maximum was found, whilst the average value corresponded with that of the atmospheric air. The changes of the composition during incubation were investigated by Aggazzotti [1914]. He found a rather high carbon dioxide content (from 1·42 to 2·05 %) in fresh eggs; it decreased during incubation, the oxygen content remaining continually slightly above that of the external air.

We used four series of White Leghorn eggs for our experiments. Since the examination of one egg a day had proved not to give reliable results owing to the wide variation in the figures obtained, forty eggs were taken in every series. Two eggs could thus be examined every day and our conclusions are based on eight eggs examined on the same day of incubation. As the results obtained in the four series corresponded completely, those obtained in one of them (series B) are plotted in the graphs of Fig. 3. The gas analyses were carried out in Haldane's 5 c.c. apparatus. All the foetuses had developed in the normal way and those incubated 18 or 19 days could be reared after having passed another 1–3 days in the incubator.

As Fig. 3 shows, the oxygen percentage falls very slightly (not more than 0·75 %) during the first 10 days of incubation. It then falls rapidly,

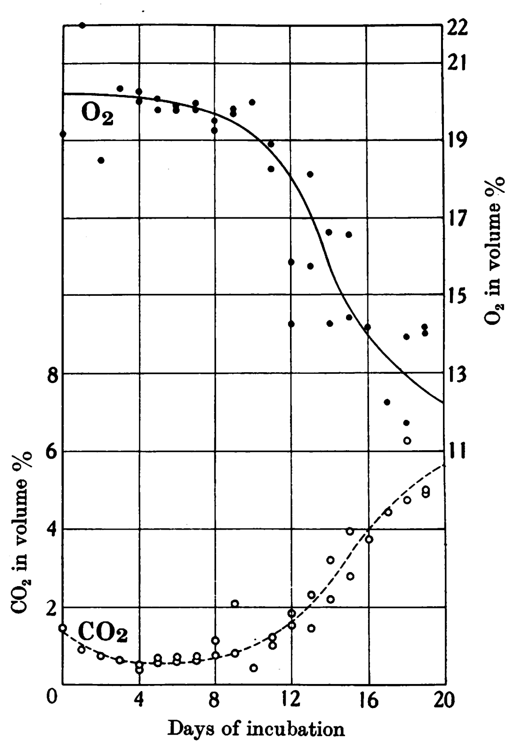

Fig. 3. Composition of the air space of fertile eggs during incubation.

reaching, by the end of the 2nd week of incubation, an average of 16 % (14 % minimally). During the last week the curve falls farther, the minimum being less than 12 %.

The fall of the carbon dioxide curve during the first few days from about 1·5 to 0·6 % may result from the high carbon dioxide tension in the fresh egg which corresponds to that in the oviduct. The carbon dioxide content is then relatively constant up to the 10th or 11th day. During the following days it increases steeply and reaches a maximum of 6 % at the end.

These results agree rather well with those given by Aggazzotti, except that our figures for both carbon dioxide and oxygen at the end of incubation exceed those given by him. The values are collected in Table I. By using an improved method, which will be described in the following pages, these differences could be shown to be still more prominent than they are here.

TABLE I

Author	Beginning of the experiment O_2 %	Beginning of the experiment CO_2 %	End of the experiment O_2 %	End of the experiment CO_2 %	Days of incubation
Aggazzotti					
Series I after 1 day	20·35	1·89	15·28	4·30	18
,, II after 1¾ days	20·65	1·12	13·65	5·43	17¾
Romijn & Roos					
Series I	22·53	0·51	—	6·69	20
,, II	19·17	1·41	12·72	5·58	20
,, III	22·13	1·76	12·96	5·46	20
,, IV	24·33	0·40	12·17	5·86	20

As our figures in Table I show, the gas in the air space before the eggs have been in the incubator has an oxygen content higher than the atmospheric air. After some hours of incubation it had fallen to 19·5–20 %. The difference between the figures at the end of incubation in Aggazzotti's experiments and in ours may be explained, at any rate in part, by the fact that the former are for 18 days of incubation, whereas our measurements were continued to the end of the 20th day.

Moreover, in Aggazzotti's experiments one egg was examined every day of incubation, in each of the two series of eggs used, two eggs being examined occasionally. Now it is clear from Fig. 3 that rather great differences may be met with between two eggs of one series analysed on the same day. For example, two eggs examined on the 14th day exhibited a difference of oxygen content of not less than 2½ % (14·24 and 16·63 % respectively); the difference between the carbon dioxide figures at the same moment was found to be almost 1 % (3·18 and 2·20 % respectively). Even at the beginning of the curve a difference is already manifest (see oxygen curve), and after about the 11th day it increases and becomes permanent (see 18th day). In consequence of this fact examination of one egg can no more give a reliable point for the construction of this curve than it did for the volume of the air space. This difficulty can only be partly solved by examining more eggs on the same day of hatching. It must be remembered, also, that in order to collect gas the egg was immersed in salt solution by Aggazzotti, and under mercury by us. During this time ventilation of the egg will be stopped, and, when the metabolism of the foetus has reached an appreciable intensity, say after the 10th day, errors may thereby be introduced. We were not able to reduce the time required to fill the apparatus with mercury and to withdraw the gas from the air chamber to less than 1 min. Table II shows how the gas concentration

TABLE II

Number of egg	Days of incubation	Kept under mercury during	O_2 %	CO_2 %
C 38	16	0 sec.	16·90	2·97
	16	2 min.	16·68	3·24
C 36	19	0 sec.	16·68	4·05
	19	2 min.	16·11	4·35
C 34	20	0 sec.	13·70	4·90
	20	1½ min.	12·96	5·46
	20	5 min.	6·27	7·85

may be influenced by this procedure. An egg incubated for 16 days or more clearly cannot be kept under mercury or any other liquid for a minute or longer without the composition of the gas in the air space being altered to a noticeable degree.

We therefore devised a method whereby the composition of the air space could be followed in one and the same egg during the whole period of incubation. On the blunt end of the egg, disinfected carefully, an apparatus was fixed by which a sample of air could be withdrawn from the air chamber at any moment, the system being closed afterwards until the next experiment. The manipulations did not impair foetal development in any way. The foetuses exhibited normal increase of weight, and after hatching they were reared in the laboratory as normal chicks.

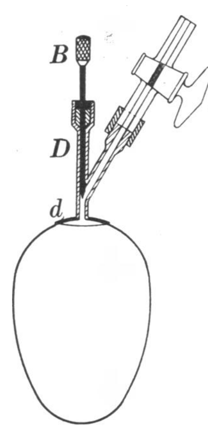

Fig 4. Drilling derrick for collecting gas from the air space. *D*, the derrick; *d*, dish; *B*, borer; *S*, stopcock through which the sample is withdrawn. Magnification about ⅘.

The apparatus is shown in Fig. 4. It consists of a brass dish *d*, 13 mm. in diameter, fixed on the shell. At its centre it supports a narrow brass tube, which we call the "drilling derrick". It contains a borer *B* which emerges from the upper end of the derrick and fits hermetically in a fine rubber stopper covered with a layer of wax. After the egg shell has been perforated and the borer is retracted so as to leave the side tube free, stopcock *S* remaining closed, the layer of wax which covers the stopper is heated a little, thus forming a covering layer in which tube, rubber and borer are involved. Controls showed that closure of the system was complete on both sides, the bottom and the top, at room temperature and also after exposure to the temperature of the incubator. By a vacuum rubber tube a small glass cock *S* was connected with the side tube without leaving any space between. Thus a sample of air could be collected in a sampling tube of 2 c.c. content connected to it. The air sample was analysed in Haldane's 5 c.c. analysis apparatus in the usual way.

The continuous measurement by the drilling derrick method was carried out on six eggs from the 10th day of incubation onwards. Until the 21st day one sample of gas was taken every day. In five of these the chick developed normally until the end. In the 6th the chick was found to be dead on the 20th day of hatching. It had given abnormal figures for the composition of the air space as early as 3 days before, but up to the 16th day it had followed normal development as was also shown by section and by its weight.

The results obtained may be summarized as follows. (*a*) Neither the fall of the oxygen curve nor the rise of the carbon dioxide curve is a continuous one as might be expected. (*b*) In five of the six cases a plateau was exhibited by the oxygen curve as well as by the carbon dioxide curve between the 17th and the 20th day. When, however, the chick perforated the shell before the 20th day the plateau was lacking in the carbon dioxide curve but was observed nevertheless in the oxygen curve. During these days the oxygen and the carbon dioxide content of the air space remained practically unchanged. Subsequently a quick increase of carbon dioxide and a quick fall of oxygen were observed. (*c*) The oxygen curves exhibited a summit at the end of the 12th or the 13th day in four of the six eggs, when the oxygen increased by more than $\frac{1}{2}$ % during one day and continued to fall afterwards. It is not yet possible to explain these observations completely, but it seems plausible to conclude that the points mentioned represent remarkable periods in foetal and placental development in the chick.

In Fig. 5 the results obtained in two eggs are plotted. Here the examination could be continued over a part of the 21st day. In the course of that day four and five samples of gas could be taken respectively. In Fig. 6 we give an example in which the carbon dioxide plateau is lacking in a curve obtained from a chick which opened the egg shell at the beginning of the 20th day. The oxygen plateau is present.

Now it is well known that the chick may be heard squeaking softly mostly on the 20th or on the 21st day, although it has not any direct communication with the atmospheric air, the egg shell being completely intact. This was observed in the eggs (*a*) and (*b*). Thus pulmonary respiration had either appeared beside the allantoic respiration or it had fully replaced it. The samples taken from the air space in this period revealed surprisingly high carbon dioxide figures (the lowest being 5·28 % and the highest 9·11 %), even surpassing the oxygen percentage in one case. The course of the curves during the 21st day is visible in Fig. 5, in which the

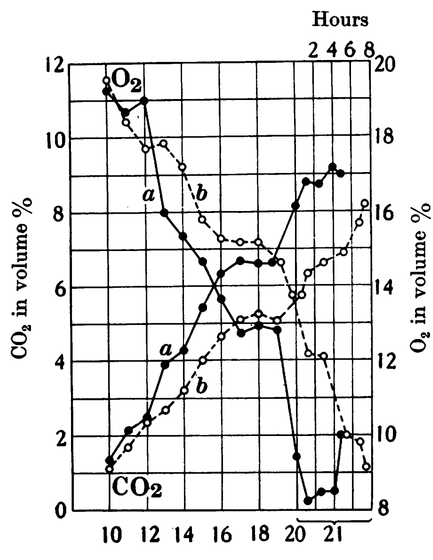

Fig. 5.

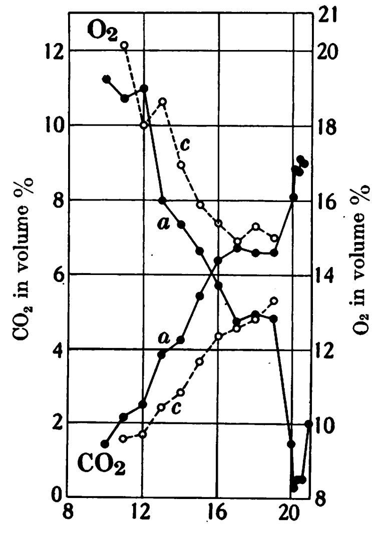

Fig. 6.

Fig 5 Course of the composition of the air space in two eggs (*a*) and (*b*) during the whole period of incubation.

Fig. 6. Course of the composition of the air space in an egg (*c*) in which the shell was perforated by the chick at the beginning of the 20th day; (*a*) is given for comparison.

time scale for that day has been given enlarged, thus indicating hours. Examination of the gas was repeated with intervals of about 1 hr., until the experiment was terminated by the chick's piercing the egg shell.

During the time the chick is squeaking it obviously respires in the air space. No trace of damage to the egg shell could be detected. It is surprising that during this time the animals which had terminated their foetal state respired from an atmosphere which in one case even contained more carbon dioxide than oxygen (9·11 % as against 8·57 %).

Fig. 7 gives a photograph of the air space of an egg which had been incubated 20 days. The chick squeaked. The blunt end of the shell has partly been removed so that the membrane which forms the bottom of the space is visible. Amidst the blood vessels of the allantois which are smaller than they had been before, a tear of the membrane is seen, in which is the beak of the young animal.

Thus it was possible to analyse the atmosphere from which the animal inspired under physiological conditions during the period which apparently lay between the foetal state and the postfoetal, which begins after the egg shell has been left. This interposed period, which may be called the parafoetal period, sets in when the allantoic circulation begins to reduce

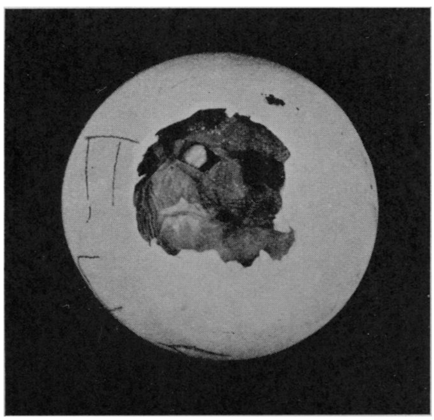

Fig. 7. Egg incubated for 20 days. The chick was squeaking. The blunt end of the shell has been partly removed and the rupture of the membrane which separates the gas space from the rest is visible, together with the beak of the young animal.

its respiratory function, and the air space, separated from the atmospheric air by the egg shell, forms a second atmosphere from which the animal respires directly by its lungs. The permeability of the egg shell, as is well known, increases during incubation. This parafoetal period is terminated when the chick perforates the obtuse end of the egg shell with its beak.

The composition of the atmosphere in which the chick respires in the parafoetal period is surprising, the oxygen pressure having fallen below half the atmospheric oxygen pressure, and the carbon dioxide pressure surpassing that in atmospheric air 200 times and even more. It must therefore be assumed that a condition of anoxaemia and increase of carbon dioxide content is produced during this time.

3. Pressure in the air space

In order to be able to use the figures given in the preceding pages for the problem of respiration in the avian foetus, it is necessary to know the partial pressure of the gases in the gas chamber, and for this it is necessary to know the total pressure. Aggazzotti, in calculating these partial pressures assumed that the total pressure was that of the atmosphere.

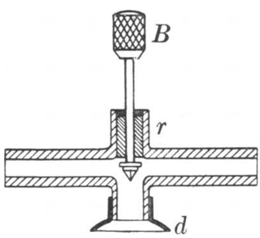

Fig. 8.

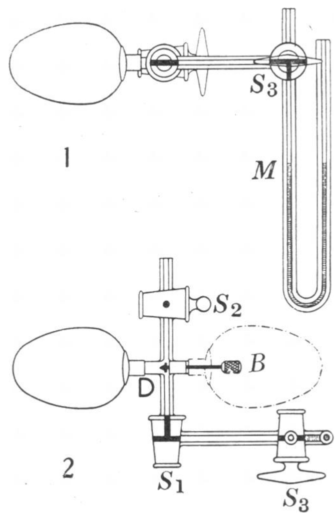

Fig. 9.

Fig. 8. Drilling derrick. *d*, dish; *B*, borer; *r*, rubber stopper covered by a layer of wax. About original size.

Fig. 9. Air space connected to the manometer (*M*) by the drilling derrick (*D*). Pressure can be measured continually. *B*, borer by which the shell is perforated after S_2 is closed. The egg can be turned 180° round the axis S_1–S_2. (1) Seen from the front; (2) seen from above.

The pressure of the gases in the air space has never been determined so far as we know. We used the following apparatus. A drilling derrick, of somewhat different construction than the one described before, was fixed on the blunt end of the egg (Fig. 8). It was connected (see Fig. 9) with a manometer *M* filled with coloured water by capillary glass tube and by a stopcock S_1 which at the same time was the axis round which the egg, together with the drilling derrick, could be rotated 180° without being taken out of the incubator. Thus the egg was turned twice a day without any trouble. The apparatus was fixed on the egg by the brass dish *d*; the borer *B* was made of steel.

After the apparatus had been placed on the egg, stopcock S_2 being opened, the whole was placed in the incubator, and after the temperature had risen to 40°, stopcock S_2 was closed. The shell was then perforated, the borer withdrawn and, by opening S_3, the air space was connected with the manometer. Stopcock S_1 has a T-shaped bore, so that the manometer communicates with the air space in both positions of the egg as shown by Fig. 9 (2). The egg rested on a small table of wire netting which was turned together with the egg.

The apparatus was placed on the egg on the 8th or the 10th day of incubation. The volume of the system by which the volume of the air space was thus increased was 1·526 c.c.

Results. In all ten eggs examined the pressure in the air space was shown to remain relatively constant during the whole time of incubation. It was found to be a little higher than the atmospheric pressure, but the difference was small; it varied from ½ to 3 mm. of water. The figures found for three eggs are given below as representative (Table III).

TABLE III. Pressure in air space (above atmospheric pressure) in mm. water

Days of incubation	Egg 1	Egg 2	Egg 3	Egg C
8	—	—	1½	—
9	—	—	2	—
10	2½	—	—	—
11	2	2	1½	—
12	2	2	1½	—
13	2	1½	1½	—
14	2	—	1½	—
15	1	1½	1½	—
16	2	—	1½	—
17	1	1	1	—
18	2	½	1	2
19	—	—	1½	3
20	—	1½	—	2
21	—	—	—	0 to 2

In the last column figures are given for an egg (C) in which the chick was heard squeaking at the beginning of the 21st day. The water in the manometer moved rather rhythmically to and fro with a frequency of 120 per min., obviously as the result of respiratory movements made by the chick in the air chamber. In another experiment similar rhythmic fluctuations of 2 mm. had a frequency of 100 per min. They could be followed for many hours and came to an end when the chick pierced the shell.

DISCUSSION

When the allantoic vessels develop and cover the shell membrane which forms the base of the air space—they reach full development at the 14th or the 15th day of incubation—the air space becomes important for foetal respiration, its importance increasing with its volume. At the 14th day of incubation it has a volume of 6–7 c.c., which during the last week of incubation increases to 9–11 c.c. The foetus finds here a volume of gas available for allantoic ventilation and separated from its blood by a thin membrane and the capillary walls only. Thereby gas exchange will be relatively intensive between foetus and this atmosphere, from which it is separated by a surface which takes 10–20 % of the respiratory surface of the whole egg shell.

Considering the composition of the gas found in the air space, it may be supposed to be of special importance during the parafoetal period. After the chick tears the shell membrane on the 20th day it respires directly from an atmosphere which contains no more than $8\frac{1}{2}$–15 % of oxygen, carbon dioxide having risen to 5–9 %. Two circumstances are present at this moment by which oxygenation of the foetal blood is deteriorated: (*a*) reduction of the allantoic blood vessels, and (*b*) lung respiration in the atmosphere mentioned.

The composition of the air space during the parafoetal period, and the preceding days, will bring about a state of partial anoxaemia and increased carbon dioxide concentration in the blood. From the observations of Windle & Barcroft [1938] on the effect of various amounts of carbon dioxide and oxygen on the movements of chicks between 13 and 20 days old, we may expect in consequence the following effects: (*a*) increased muscle tonus; (*b*) inhibition of movements which are not a part of the respiratory pattern; (*c*) intensifying of respiratory movements which, within a short time, will have to replace completely ventilation by the allantoic vessels; (*d*) movements of the neck and the trunk by which the egg shell is perforated on the 21st day. Furthermore, Windle & Barcroft observed increased irritability and quickening of all somatic movements as the initial result of carbon dioxide administration or anoxaemia.

These authors state that a respiratory rhythm was never seen before the 17th or 18th day (sometimes not before the 19th), although movements of all the muscles involved in respiration appeared very much earlier. Our observations show that at the 18th day the amount of carbon dioxide in the air space had risen to 5–7 %, and during 2 days, the 18th and 19th of incubation, this concentration was maintained (see Figs. 5 and 6). The plateau formed by the oxygen curve at the same time corresponded with an oxygen content varying from 13 to 15 %. It seems, therefore, that during this constancy in composition of the air space foetal respiration is initiated. The composition, moreover, is such as to induce the respiratory rhythm and the movements by which the air-space membrane is perforated. The air space, therefore, plays an important part in foetal development during that period.

The parafoetal period which follows lasts 24 hr. or more; it is the period in which respiratory rhythm and general muscular activity are further developed. The animal is being prepared for postfoetal life.

Summary

1. The volume of the air space of the hen's egg increases in a rectilinear relation to the time of incubation. At the end of the period of incubation a volume of 11 c.c. may be reached, whilst in the fresh fertile egg it does not exceed some tenths of a c.c.

2. A method has been described by which the composition of the gas found in the air space could be determined in one and the same egg throughout the time of incubation. This procedure is necessary owing to the wide variability between different eggs examined under the same conditions of incubation.

3. The foetus remains under fully physiological conditions during the experiments.

4. A method has been given by which the pressure in the air space was measured in one and the same egg continuously during the time of incubation, development of the foetus remaining normal.

5. When the chick leaves the egg it has terminated its foetal state about 24 hr. previously. During these hours, the parafoetal period, respiration in the air space by pulmonary breathing has been added to allantoic respiration. An exceedingly high carbon dioxide content is present in the air space (up to 9·11%). On the other hand, oxygen percentage has fallen to 9 and less.

6. The importance of the air space for foetal and parafoetal respiration and for development in general is discussed.

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