PHYSICAL PROPERTIES OF THE ALLANTOIC AND AMNIOTIC FLUIDS OF THE CHICK

I. Specific Conductance

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The importance of the composition of the surrounding medium to the development of the organism is well known and may be studied to great advantage in the hen's egg in which the embryo secretes its own external environment, the amniotic fluid. In the later developmental stages, when the allantois has come to surround the other components of the egg, the contents of this membrane are also of significance. The extra-embryonic fluids of the developing chick have received considerable attention in the past, but until recently the investigations have not been sufficiently systematic to afford a complete picture of the changes in the nature of these fluids or of the effects which this changing environment might exert upon the developing embryo. Most of these analytical studies have been limited to investigations of changes in the chemical composition of the fluids at different stages of development. It is equally important to secure information as to the changes in their physical properties. The present series of electrical conductance measurements undertakes to supply some of this information.

Relatively few investigators have concerned themselves with the study of changes in the electrical conductance of the extra-embryonic fluids. Of these, Romanoff and Grover (1936) are the only ones who have examined this question systematically in the chick. The present work, which was completed before the appearance of their paper, has yielded slightly different results and hence is reported here.

Material and Methods

The eggs used in these experiments came from a large flock of Barred Plymouth Rock pullets. Incubation was carried out at 38.5°C. in a regulation Thelco incubator. Samples of the fluids were obtained as follows: The shell and outer shell membrane were removed from the region of the air chamber, and the opaque inner shell membrane rendered transparent by painting with mineral oil. It was thus possible to insert a fine glass pipette into the allantoic cavity without rupturing any of the allantoic blood vessels and withdraw a sample of the contained fluid. This sample was then transferred to a small glass vial, stoppered tightly, and the vial and its contents placed in a wire rack within the temperature bath. In the case of embryos of less than 8 days' incubation, the pipette could not be inserted directly into the allantoic

cavity, since this membrane had not yet become fused with the chorion in the region of the air space. In such instances, more shell had first to be removed to expose the sac.

After the allantoic fluid had been sampled, enough shell was removed so that the contents of the egg could be gently poured out into a finger-bowl. A clean pipette

TABLE I

The Effect on Measured Resistance of Decreasing the Temperature

Material	Resistance in ohms			Increase $\Delta R/R_{38}$
	R_{20}	R_{38}	ΔR	
				per cent
A37 AL.F.	1142	793.9	349.1	43.97
AM.F.	1031.5	719.7	311.8	43.32
A38 AL.F.	1792	1251.5	540.5	43.19
AM.F.	1853.5	1291.7	561.8	43.49
Average per cent increase				43.47

TABLE II

Average Measurements of All Embryos Used

Age	Crown-rump length	Weight	ln W	Specific conductance	
				AL.F.	AM.F.
days	*cm.*	*gm.*		*mhos per cm.*	*mhos per cm.*
7	2.6	0.71	$\bar{1}.66$	0.01157	0.01328
8	3.0	1.02	0.02	0.00994	0.01319
9	3.3	1.41	0.34	0.01026	0.01316
10	3.6	1.95	0.67	0.01021	0.01323
11	4.2	2.76	1.01	0.01145	0.01357
12	4.7	3.87	1.35	0.01042	0.01348
13	5.2	5.10	1.63	0.01088	0.01334
14	5.8	7.21	1.98	0.01066	0.01297
15	6.4	10.26	2.33	0.01031	0.00811
16	7.1	13.13	2.57	0.01047	0.00826
17	7.4	15.00	2.71	0.00865	0.00883
18	7.7	17.27	2.85	0.00904	0.01014
19	8.3	21.00	3.04	0.00762	0.01221

was used in drawing off samples of the amniotic fluid; otherwise the technique was the same as described above. Precautions were taken that all glassware used in handling these samples was thoroughly clean and dry.

In order to obtain enough material in each case to fill the conductance cell (about 0.1 cc.), the range of development studied was limited. No samples were taken from embryos younger than about 7 days or older than about 19.

Since considerable variation was apparent between chicks of the same incubation age, the idea of plotting conductance against incubation time was abandoned, and instead the natural logarithms of the various wet weights (in grams) were used as abscissae. This function of the weight was used merely for convenience, the data being thus spread out in better fashion. On the same graph, however, are to be found vertical lines, more or less arbitrarily drawn, classifying the embryos secondarily according to days' incubation.

The apparatus used in the determination of the electrical conductance was the one described by Jones and Josephs (1928). Since most standard cells for conductance work are designed for relatively large volumes of fluid, it was necessary to design a microcell which could be used with the small volumes of fluid found during certain developmental stages. This cell has been previously described (Walker, 1938 *a*).

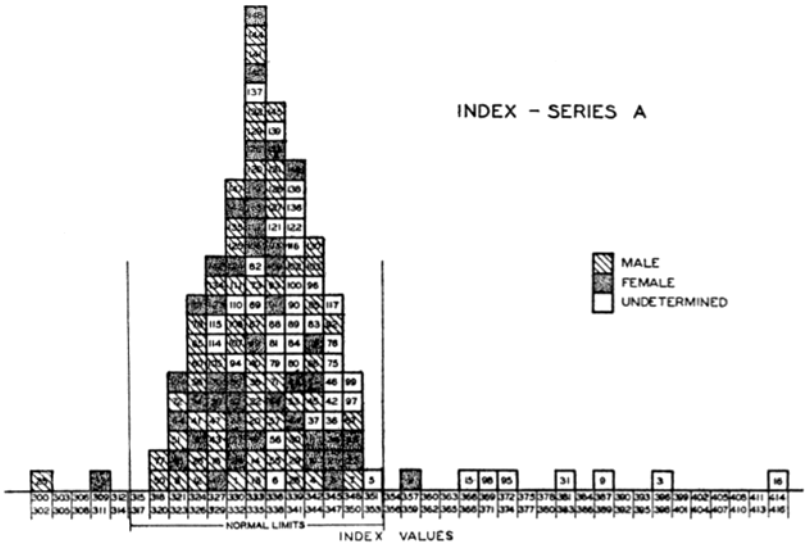

FIG. 1. Histogram resulting from application of index to experimental material. Numbers refer to individual animals used.

Experiment had shown that an interval of about 20 minutes should be allowed before each measurement for the liquid within the cell to come to equilibrium with the bath temperature (20°C.). Readings were then taken at 5 minute intervals until three consecutive readings checked within 1 ohm. This represented an accuracy of at least one part in a thousand, generally in fact an even higher degree. The specific conductance was then computed from the cell constant in the usual manner.

Throughout this series of experiments, the resistances were measured at 20°C., a temperature chosen arbitrarily. It seemed possible that the change produced by cooling the fluids from the incubator temperature to 20° might vary in magnitude with the age of the embryo. Accordingly, the fluids from two chicks, one aged 9 days and the other 16, were measured at both temperatures and the effects of this

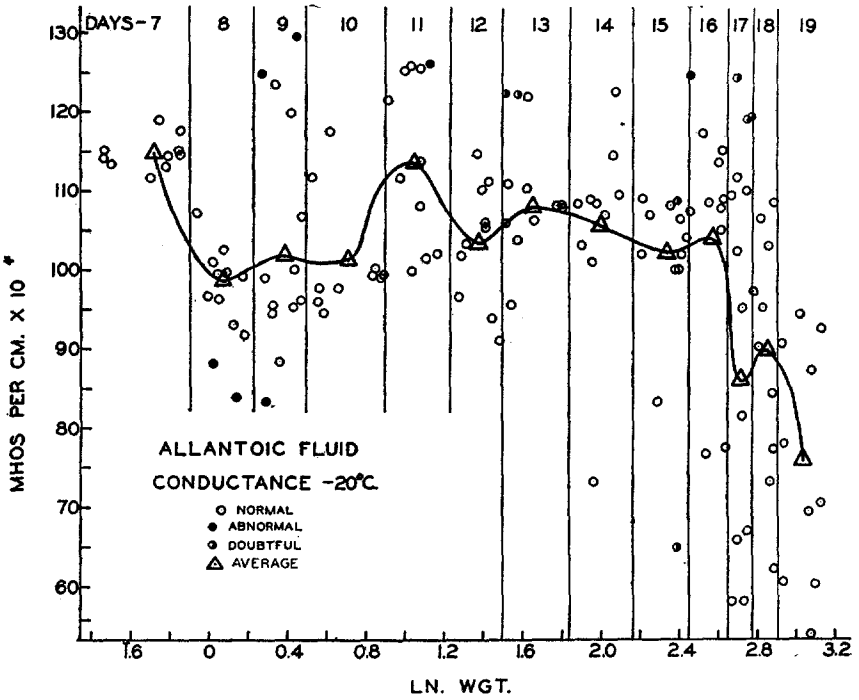

FIG. 2. The specific conductance of the allantoic fluid—approximate incubation age indicated on top scale.

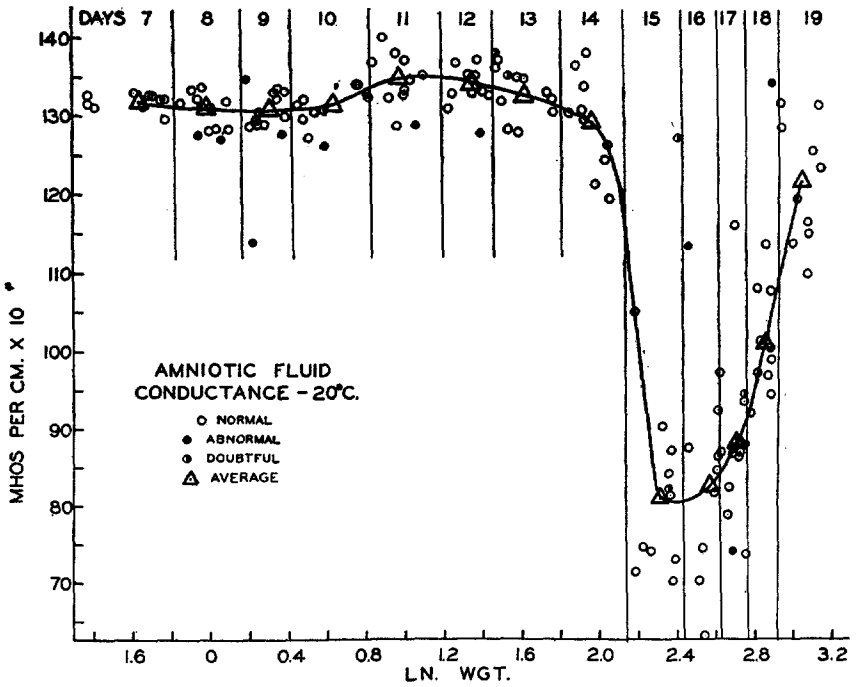

FIG. 3. The specific conductance of the amniotic fluid—approximate incubation age indicated on top scale.

decrease in temperature compared. The results of this experiment are shown in Table I.

The slight variation in the above percentages is insignificant in view of the known variation existing between chicks of the same age. Furthermore, since the ages of these two embryos represent rather widely separated stages, it seems logical to suppose that this increase in resistance is fairly constant throughout the range of development studied. This means, of course, that the shape of the resulting curves has been unaltered, but merely their positions changed by this drop in temperature.

Experimental Data

Specific conductances were determined for each of the two fluids upon ten chicks of each "age" within the range of development studied. Determinations upon chicks whose index value (see Walker, 1938*b*) lay outside the range of normality were ruled out, as were all cases in which either fluid had been accidentally lost or was thought to be contaminated in any way. Fig. 1 shows the histogram obtained after having calculated the indices for the individual chicks used in this series of experiments. The individual conductance values obtained in the case of the allantoic fluid are plotted in Fig. 2; those of the amniotic fluid in Fig. 3. In both cases the average values for each "day" are also shown. These averages are contained in Table II below. Points marked "abnormal" represent determinations on chicks whose index lay outside the range of functional normality (315–353).

DISCUSSION

From an examination of Fig. 2, it is evident that by the 8th day of incubation, a decrease in the specific conductance of the allantoic fluid has occurred. Unfortunately, the true magnitude of this decrease is not determinable, since no measurements were made upon fluids from embryos younger than about 7 days. This decrease agrees with the observations of many workers, among them Needham (1925; 1926*a*; 1926*b*), that there occurs in the chick a succession of end-products in nitrogen metabolism: ammonia ⟶ urea ⟶ uric acid. The period of maximum ammonia production occurs quite early in development (about the 4th day), but the peak in the production of urea is not reached until the 9th day. Accordingly, it is not surprising to find a relatively high specific conductance, indicating a relatively high percentage of these two dissociable compounds present at this time. Between the 7th and 8th days, the concentration of the relatively insoluble uric acid is increasing rapidly, while that of ammonia is decreasing. This is evidenced by the falling conductance values of this period. Little information is available on the important question of the amount of inorganic material present at this stage. Kamei's (1928) data show a high concentration of these substances on the 9th day, but do not tell whether or not this is the case before this time. The

decrease in conductance prior to the 9th day might be thought of as resulting from a decrease in the concentration of these readily ionizable substances.

From the 9th to the 13th days, the conductance of the allantoic fluid shows rather irregular fluctuations, tending to rise slightly. There do not seem to be very significant changes here, although a slight increase in the relative ionic concentration is indicated. However, from the work of many investigators—Fiske and Boyden (1926), Targonski (1927), Fridericia (1912), Needham (1926*a*; 1931), Tomita and Takahashi (1929), Kamei (1928), Yamada (1933), and Needham, Brachet, and Brown (1935)—it is known that the total contained nitrogen (presumably in the form of uric acid) is increasing throughout this period of development. Since conductance values remain relatively stable, this must mean a corresponding increase in dissociable constituents. That these dissociable constituents are not inorganic salts is perhaps indicated by the data of Kamei (1928); however, his examination was not complete, and the results do not agree with those of Iseki (1930) on the chemical constituents at this stage. Hence, too much weight cannot be placed on these findings.

Between the 13th and 16th days the conductance of the allantoic fluid is beginning to diminish somewhat. During this time, the change-over from mesonephric to metanephric excretion is occurring in the embryo, and the changes in conductance may be linked with this phenomenon. Certainly the relative concentration of the non-dissociable uric acid is still increasing at this stage. Furthermore, the absorption of water from the contents of the allantoic sac is now beginning, although it is not nearly as marked as in the last few days of incubation.

The final descent of the conductance curve at the end of the period of development investigated is doubtless explainable on the basis of water absorption. It is known that a marked decrease in the volume of the allantoic liquid occurs at this time, and with this absorption of water would go a decided increase in the relative concentrations of all solutes and substances suspended in the fluid itself. Since uric acid and certain insoluble urates are still the chief constituents here, it is to be expected that the specific conductance of the fluid would show a pronounced decrease.

In the case of the amniotic fluid (Fig. 3), the conductance values recorded over the first period of development studied are decidedly higher than those of the allantoic liquid during this same time. This is to be expected from a consideration of investigations on the chemical constitution of this fluid. Fiske and Boyden (1926), Targonski (1927), Kamei (1928), and others have definitely shown that the amniotic fluid contains decidedly less nitrogen than the allantoic, which is, of course, receiving the end-products of the embryo's nitrogen metabolism. Kamei (1928) has also demonstrated a higher concentration of inorganic substances in this fluid. That so little change is evidenced up to the 13th day of incubation indicates that a fairly constant relationship is being maintained between ionized and un-ionized material. The slight maximum

seen at the 11th day is probably a true one, for it is shortly after this stage, according to Hirota (1894), that the perforation of the sero-amniotic connective occurs, with the attendant passage of the contents of the albumen sac into the cavity of the amnion. These observations are borne out by the subsequent precipitous fall in conductance values recorded during the 14th and 15th days. Apparently, the influx of protein material from the albumen sac takes place gradually at first, then with increasing velocity. Figures on the chemical composition of this fluid at this period (Kamei, 1928) show a maximum in total nitrogen at about this time.

After the 15th day, a rise in conductance occurs. This is coincident with the disappearance of this protein material. Schenck (1932) has pointed out that these proteins which appear in the amniotic fluid (and which can be shown to be identical in nature with those formerly present in the albumen sac) are completely ingested by the embryo, vanishing from the amniotic fluid by the 18th or 19th day of incubation. Kamei's data also show a decrease in total nitrogen during this period.

SUMMARY

1. The specific conductance of the allantoic and amniotic fluids of the developing chick has been determined over the period of incubation between the 7th and 19th days.

2. Changes in this property have been related to changes in the chemical composition of these two fluids.

3. These conductance values are of importance in that they show the relation between ionized and un-ionized materials present in the two fluids.

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