PHYSICAL PROPERTIES OF THE ALLANTOIC AND AMNIOTIC FLUIDS OF THE CHICK

III. SURFACE TENSION

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Up to the present time, little information has been available on the surface tension of the allantoic and amniotic fluids of the developing hen's egg. There seems little doubt but that water is the main constituent of both these fluids, and a study of their respective surface tensions throughout the incubation period of the egg should give some idea of the distribution, within these fluids, of the various dissolved substances. Because of volume considerations, the range studied was limited to that period of incubation between the 7th and 19th days.

The apparatus used was the familiar du Noüy tensiometer. Since the volumes of the liquids to be measured were often extremely small, a special platinum ring, which had a mean circumference of 1.48 (±0.005) cm., was used instead of the standard 4 cm. size. Johlin (1928), in his criticism of the du Noüy method, points out that a considerable error is introduced when ring size is reduced. However, it was felt that this smaller ring would still give relative values which would be comparable, although slightly lower than those which would have been obtained had a standard ring been usable.

The eggs used all came from the same flock of Barred Plymouth Rock hens. They were incubated the desired time at 38.5°C. Samples of the two fluids were obtained in the manner previously described (Walker, 1943a). Immediately after removal from the egg, they were placed in small vials and stoppered. They were allowed to remain in these vials for 20 minutes before the measurements were begun. This afforded ample time for the liquids to cool down to the experimental temperature (25°C.). A measured portion of the sample was then transferred to the small watch crystal used in the tensiometer and left there 5 minutes before making the first determination. In these measurements, the procedure and precautions recommended by du Noüy (1926, 1929) were strictly followed. Three separate determinations were made on each sample tested and the average value taken as the relative surface tension. As du Noüy points out, this procedure is adequate when merely relative readings are desired. The individual values thus obtained are higher than when the "absolute technique" is employed, but they are quite comparable, one with another.

To calibrate the apparatus, the following substances were measured at the
experimental temperature: Distilled water, glycerine, 4M resorcinol, aniline, benzene, chloroform, and methyl alcohol. The tensiometer readings thus obtained were plotted against the surface tension of each liquid as given in the "International Critical Tables," and from the graph thus constructed, the surface tension value for any unknown liquid could be read off directly as soon as the average tensiometer reading had been determined. Determinations on distilled water and benzene (near the limits of the surface tension range studied) were repeated at frequent intervals throughout the course of the investigation. No significant changes were observed.

Since considerable variation was encountered in the weights of chicks of supposedly the same incubation age, incubation time was not used as a primary criterion of the degree of development, but the embryos were classified according to weight. In the accompanying data, an attempt has been made to clas-
Fig. 2. Surface tension of the allantoic fluid (approximate incubation age indicated on top scale).

Fig. 3. Surface tension of the amniotic fluid (approximate incubation age indicated on top scale).
sify the animals roughly by days of incubation, but this is only an approximate, more or less arbitrary classification. The index of normal development previously described (Walker, 1938) was employed, and the histogram thus obtained (Fig. 1) is of the expected configuration, with the mean class represented by indices of 333 to 335. Embryos whose indices lay outside the range of normality (315 to 353) were discarded, although surface tension values for these individuals have been included in Figs. 2 and 3 for comparative purposes. In Table I is presented a brief summary of these experiments; the averages of each set of ten individual points (chicks of a certain weight group), are also plotted in Figs. 2 and 3.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Crown-rump length (cm.)</th>
<th>Weight (g)</th>
<th>In. w.</th>
<th>Surface tension Am. L. (dynes per cm.)</th>
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<td>2.6</td>
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<td>1.70</td>
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<td>8</td>
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<td>1.08</td>
<td>0.08</td>
<td>53.2</td>
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<td>1.41</td>
<td>0.34</td>
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<td>3.8</td>
<td>2.08</td>
<td>0.73</td>
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<td>8.3</td>
<td>20.17</td>
<td>3.00</td>
<td>54.2</td>
</tr>
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</table>

**DISCUSSION**

The decrease in surface tension seen in the case of the allantoic fluid (Fig. 2) between the 7th and 13th days doubtless results in part from the general increase in nitrogenous compounds, such as proteins and uric acid, found during this period of Fiske and Boyden (1926). It may also indicate an increase in concentration of enzymes, for Berczeller (1913) has shown that the surface tension of various protein solutions decreases in the presence of ferment. The work of Przylecki and Rogalski (1927) has demonstrated the presence of uricase in the allantoic liquid of chick embryos from the 2nd to the 10th days of incubation, and it is quite possible that other enzymes may be present.

Following this period of decreasing surface tension values, there occurs a rise from definitely minimal values on the 13th day to as definite a maximum
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on the 16th. This rise is quite puzzling in the light of present information on the composition of this fluid. Since the lowering of surface tension is a property of proteins in general, one might expect either that a decrease in the concentration of these substances is occurring here, or else that the relative proportions of water and inorganic materials increase. Although analyses of the chemical composition of this fluid are admittedly incomplete, several workers, among them Fiske and Boyden (1926), Targonski (1927), and Kamei (1928), have shown that there is a decided increase in the concentration of nitrogenous materials during this time. Also, since both Kamei (1928) and Yamada (1933a) have found that the specific gravity of the allantoic liquid is increasing throughout this period, this observed rise in surface tension cannot be explained in terms of any increase in the proportion of water present. As for the inorganic constituents, conductivity measurements previously reported (Walker, 1943a) indicate that the relative concentration of these substances is slightly on the decrease from the 13th to the 16th days of incubation.

The relation between the hydrogen ion concentration of a solution and its surface tension has received considerable attention in the past. Johlin (1930) has pointed out that the surface tension of gelatine solutions is decreased by the addition of small amounts of acid and increased in the presence of small amounts of base. Sugino (1929) working with solutions of amylamine, Yusawa (1935) studying the surface tension of human urine, and Gardner and Semb (1935) using various anesthetics have found a similar relationship to hold. However, Yamada (1933a), using the hydrogen electrode, and Walker (1943a), using the glass electrode technique, have both found diminishing pH values during this period of incubation, with the maximal decrease (Walker) occurring at precisely this period. This is exactly the opposite of what would be expected from the present results on the investigation of surface tension. The explanation of this pronounced rise in surface tension values must hence be sought elsewhere, possibly linked in some way to the fact that the change from mesonephric to metanephric excretion is occurring at about this stage of development.

The last few days of incubation studied reveal a decrease in surface tension values for the allantoic fluid which does agree with the investigations of hydrogen ion concentration referred to above. This might also be explained simply on the basis of the diminution in the water content of the allantois which is known to take place at this time. This would result in a marked increase in the relative amount of nitrogenous excretory residues. This is also borne out by the sharp decline in conductance values reported by Walker (1943a).

With the exception, therefore, of this period between the 13th and 16th days of incubation, analysis of changes in the surface tension of the allantoic fluid yields results which are essentially in accord with what has previously been found from investigations of chemical and other physical properties. During this brief period, however, the surface tension of the allantoic fluid behaves in a
manner which is exactly the opposite of what one would expect and which still challenges explanation.

On the 7th day, in the case of the amniotic fluid (Fig. 3), the low surface tension values obtained contradict certain current theories as to the origin of the amniotic fluid; this has frequently been described as a sort of embryonic sweat, composed for the most part of water and inorganic substances. Such a mixture should show a much higher surface tension. The presence of substances which would materially lower surface tension is certainly not predictable from results of chemical analyses. Indeed, Fiske and Boyden (1926) reported only 2.9 mg. per cent total nitrogen on the 7th day, and Kamei (1928) but 11.9 mg. per cent on the 9th day. A suggestion as to the type of substance which might be responsible for these low initial values is perhaps afforded by the findings of Yamada (1933a, 1933b) that sugars are present in the amniotic fluid early in development; furthermore, Urbitch (1924), in demonstrating that the amniotic fluid can be recovered from the gut of the embryo as early in development as the 9th day, also suggests that nutritive substances (which would presumably tend to lower surface tension) may be present during these early stages as well as after the rupture of the sero-amniotic raphe. The marked increase in surface tension values between the 7th and 9th days is doubtless correlated with the increase in the volume of this fluid found by Yamada.

Between the 9th and about the 15th days, the relative constancy of the surface tension values for this fluid is interesting in view of the fact that the influx of protein material from the albumen sac begins on the 12th day (Hirota, 1894). That no marked changes in surface tension take place at this time must mean a rapid absorption of this albuminous material by the embryo. As a matter of fact, the change in surface tension, when it does occur (abruptly between the 15th and 16th days), is not of the nature of a decrease, as might be expected from the marked increase in protein material occurring at this time, but is, on the contrary, a rise to a maximal value. It will be remembered that a similar, but relatively greater, increase in surface tension takes place at this same stage in the case of the allantoic liquid. Inasmuch as no reliable chemical analyses of the amniotic fluid have been carried out during this period of development, it is impossible to say what rôle the inorganic constituents may be playing here. From the conductance values reported by Walker (1943a), it is evident that the number of freely ionizing substances approaches a minimum at this time. Furthermore, since the total volume of the amniotic fluid is diminishing, no explanation may be made in terms of an increase in the relative amount of water present. The fact that the curves for both these extra-embryonic fluids rise to the same maximal value on the 16th day is interesting, but impossible of interpretation on the basis of existing information.

During the last few days of incubation studied, the surface tension of the amniotic fluid is decreasing, as would be expected in view of the large decrease
in volume occurring at this time. Another factor here may be the fact that excretory products may now be entering the cavity of the amnion by way of the cloacal opening. The presence of digestive enzymes during this period is quite possible, and these would also tend to reduce existing surface tension values.

The most striking general feature of the present results is that over the entire range of development studied, the values for the surface tension of these extra-embryonic fluids are considerably below 72 dynes per cm., the value for distilled water. Thus, any solutes would become concentrated in the peripheral portions of the liquid mass, or at the boundaries between these fluids and their respective enveloping membranes. In the case of the amniotic fluid, this means that dissolved substances will also become more concentrated at the surface of the developing embryo. Even inorganic salts will be found here, despite the fact that had they been present alone they would have behaved in just the opposite fashion. Their presence at the interphase boundaries together with the organic solutes (proteins, etc.) will further the precipitation and coagulation of these latter substances, a remark which readily admits of experimental verification, since at the end of incubation, the walls of both the allantois and amnion are lined with a copious cheesy precipitate. The close juxtaposition of these inorganic and organic solutes to the highly vascular allantoic membrane would unquestionably favor any processes of selective absorption which might be going on. In this connection, Needham (1931) has postulated a circulation of free basic radicals and ions in addition to the known circulation of water. In the amniotic fluid, this concentration of rich protein and inorganic material at the surface of the embryo may play a significant part in various morphogenetic and histogenetic processes.

The observed day-to-day variations in the surface tension may perhaps be interpreted as symptoms of variations in the rate at which such processes as selective absorption and deposition of protein precipitates are going on. According to this view, the increase in the surface tension of the allantoic liquid noted between the 13th and 16th days might indicate a temporary slowing down of these processes such as might well be occasioned by the transfer of excretory function from the mesonephros to the metanephros and attendant changes in the embryo's metabolism. Similarly, the rise in surface tension seen at approximately the same time in the case of the amniotic fluid might be explainable on the grounds of changed metabolic requirements. That these are not complete explanations in either instance must readily be admitted; they are put forward simply as statements provocative of further experimentation.

CONCLUSION

The study of the changes occurring during incubation in the surface tension of the two extra-embryonic fluids of the hen's egg has yielded results which in part harmonize with previous studies of the chemical and physical nature of
these liquids, and which in part are quite inexplicable in the light of these investigations. The necessity for a more thorough chemical analysis of these fluids is clearly indicated before any exact evaluation of their significance to the developing embryo can be made.

BIBLIOGRAPHY


