# CHANGES IN *p*H OF THE WHITE AND YOLK OF FERTILIZED HEN'S EGG DURING INCUBATION IN AIR, CARBON DIOXIDE AND LIQUID PARAFFIN.*

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## INTRODUCTION.

The study of the variation in *p*H is important in elucidating the mechanism of many natural biochemical processes. The *p*H of the constituents of hen's egg incubated in air has been reported by earlier workers (Baird, J. C. and Prentice, J. H., 1930; Gaggermeier, G., 1931; Needham, J., 1942*a*; Romanoff, A. L., 1944; Rubinstein, M., 1932; Sharp, P. F., 1929; Sharp, P. F. and Powell, C. K., 1931).

As means of preserving eggs from deterioration, dipping in oil or enclosing in an atmosphere of carbon dioxide immediately after the egg is laid has been suggested (Tauber, H., 1946). The change in *p*H of egg white and yolk during incubation in air, carbon dioxide and liquid paraffin has been studied and presented in this paper.

## EXPERIMENTAL.

*Fertilized eggs.*

Fertilized eggs (as detected by an examination of the blastoderm) were selected for the study. In eggs incubated in air, the development of the blastoderm was rapid whereas in those incubated in carbon dioxide and liquid paraffin, development was inhibited.

*Incubation of egg in air, carbon dioxide and liquid paraffin.*

The egg, soon after it was laid, was put into an incubator. For incubation in carbon dioxide, the air in the incubator was replaced by carbon dioxide at atmospheric pressure. For incubation in liquid paraffin, the egg was placed in liquid paraffin in a bottle kept in the incubator. The temperature of the incubator was throughout kept at 41° C.

*Glass electrode.*

Sharp, P. F. and Powell, C. K. (1931) used a hydrogen electrode for the measurement of *p*H. On account of the high viscosity of the white and yolk, saturation with hydrogen was not possible. To overcome this difficulty the white and yolk were diluted. The error introduced by dilution was reported to be negligible. A glass electrode (Albert W. Davison, Henry S. van Klooster and Walter H. Bauer, 1941) was employed in the present work, as being most suitable

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from the standpoint of rapidity of work, accuracy and reproducibility. The electrometer circuit of the electrode included a Compton quadrant electrometer and a Tinsley's K-type vernier potentiometer. High tension was applied to the needle (Dole, M., 1941). The unknown potential was applied to one pair of quadrants, the other pair being earthed. In tapping out a known potential, the potentiometer permitted of an accuracy of 0·01 millivolt.

*Measurement of pH.*

At different stages of incubation, the eggs were removed, the white and the yolk were carefully separated by a hypodermic syringe and the *p*H was quickly determined. The attainment of equilibrium potential when the glass electrode was dipped in the test liquid was remarkably quick. Within two minutes steady values were obtained. The results are shown in Tables 1, 2 and 3 and Fig. 1. Acetate-hydrochloric acid (Britton, H. T. S., 1932*a*) and phosphate-borate (Britton, H. T. S., 1932*b*) buffers were employed as standards for purposes of comparison.

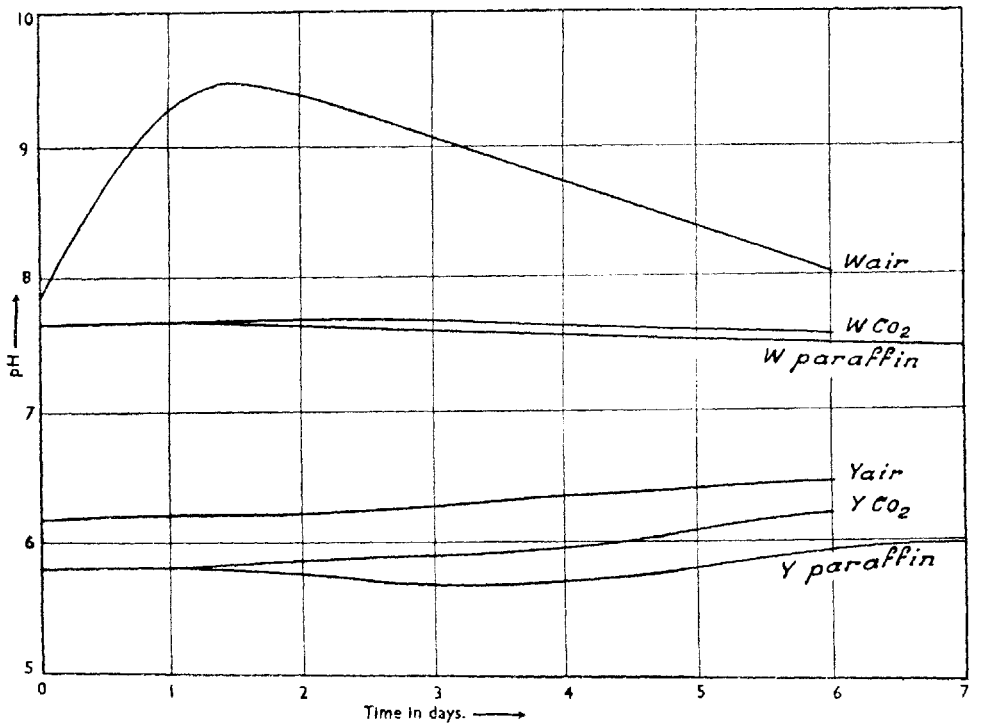


FIG. 1.

*p*H of white (W) and yolk (Y) of fertilized hen's egg incubated at 41° C. in air, carbon dioxide and liquid paraffin.

TABLE 1.

*Variation in pH of white and yolk of fertilized hen's egg during incubation in air.*

| | Time of incubation. | | | | |
|---|---|---|---|---|---|
| | Fresh. | 1 day. | 2 days. | 3 days. | 6 days. |
| 1. White .. | 7·85 | 9·27 | 9·37 | 9·05 | 8·05 |
| 2. Yolk .. | 6·17 | 6·17 | 6·20 | 6·35 | 6·44 |

TABLE 2.

*Variation in pH of white and yolk of fertilized hen's egg during incubation in carbon dioxide.*

| | Fresh. | Time of incubation. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 day. | 2 days. | 3 days. | 4 days. | 5 days. | 6 days. |
| 1. White .. | 7·65 | 7·63 | 7·63 | 7·67 | 7·67 | 7·56 | 7·54 |
| 2. Yolk .. | 5·84 | 5·85 | 5·88 | 5·90 | 5·99 | 6·11 | 6·19 |

TABLE 3.

*Variation in pH of white and yolk of fertilized hen's egg during incubation in liquid paraffin.*

| | Fresh. | Time of incubation. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 day. | 2 days. | 3 days. | 4 days. | 5 days. | 6 days. | 7 days. |
| 1. White .. | 7·68 | 7·61 | 7·60 | 7·55 | 7·55 | 7·52 | 7·50 | 7·44 |
| 2. Yolk .. | 5·85 | 5·84 | 5·79 | 5·70 | 5·74 | 5·82 | 5·91 | 5·93 |

## DISCUSSION.

*pH of white and yolk of egg incubated in air.*

In fresh egg, the white has a *p*H 7·85 and the yolk 6·17. On continued incubation, the *p*H of white increases to a maximum of 9·5 up to 36 hours and decreases continuously thereafter. The *p*H of yolk, however, is practically constant up to the second day and later increases slowly but continuously. The yolk remains in the acid range, tending to become less acidic while the white is in the alkaline range tending to become less alkaline. The characteristic feature of the *p*H-time curve of egg white is the hump. The significance of this hump and the nature of the continuous variation in *p*H with time, of egg white and yolk during incubation, are of interest.

These changes in *p*H of white and yolk are naturally connected with the metabolic processes occurring within the egg during incubation.

The egg is a highly complex system and is known to consist of a variety of substances—carbohydrates, proteins, enzymes, minerals, vitamins and fats in different amounts. Scholl, H. (1893) showed that fresh egg white contained a considerable amount of carbon dioxide and that the major portion of it was present as bicarbonate. These bicarbonates are mainly of potassium and sodium (Healy, D. J. and Peter, A. M., 1925). Brooks, J. and Pace, J. (1938) report that there is little or no carbamino-carbon dioxide.

Before the egg is laid, the constituents of the egg have a partial pressure of carbon dioxide corresponding to that of the body tissue of the hen (Aggazzotti, A., 1914). The partial pressure of carbon dioxide in the atmosphere being very much less, the egg white loses its carbon dioxide through the pores of the shell immediately after the egg is laid. This results in an initial increase in the *p*H. The loss of carbon dioxide is fairly rapid as indicated by the steepness of the curve. The portion of carbon dioxide that escapes, probably exists in the white in free condition, because no carbonic anhydrase activity has been noticed in the early stages of development of the Chick embryo. In 36 hours, the *p*H reached a maximum of 9·6. By this time, the natural metabolic processes will have commenced in the egg under aerobic conditions, at the temperature of incubation. The cells respire, oxygen is taken in and carbon dioxide is produced. The subsequent fall in *p*H is due to the accumulation of carbon dioxide in the white.

In the early stages of development, the diet of the embryo is mostly carbohydrate and relatively small amount of protein. After about the fifth day, fat and protein enter the embryo in large amounts (Needham, J., 1942*b*). The

digestive work borne by the yolk in the early stages of development involves a powerful enzymic equipment.

The respiratory quotient of the blastoderm of the hen's egg is invariably in the neighbourhood of unity during the first week of development and this indicates that the component of the diet combusted is mostly carbohydrate.

The small increase in *p*H of the yolk during incubation may be due to protein catabolism resulting in the production of ammonia (Needham, J., 1942*c*).

*pH of the white and yolk of egg incubated in carbon dioxide and liquid paraffin.*

The *p*H-time curve of white and yolk of eggs incubated in carbon dioxide and liquid paraffin are practically coincident. The *p*H of white suffers a very small but continuous decrease. The conditions are essentially anaerobic. In the absence of oxygen, the metabolic processes of the hen's egg are inhibited. A small increase in the size of the blastoderm indicates the existence of slight metabolic activity. The small increase in the acidity of the white is probably due to anaerobic glucolysis (Needham, J., 1942*c*).

As in air-incubation, the small increase in *p*H, after the third day, of the yolk of egg incubated in carbon dioxide or in liquid paraffin is due to protein catabolism.

*Carbon dioxide and liquid paraffin as preservatives for egg.*

Carbon dioxide and liquid paraffin are good preservatives for eggs against deterioration, because in eggs preserved in these substances, the white and yolk suffer no appreciable change in consistency and appearance and develop no odour. Though in large doses carbon dioxide has an inhibiting effect, it is of interest to note that in moderate doses the gas is found to have a stimulating effect on the growing embryo (Romanoff, A. L. and Romanoff, A. J., 1933).

The foregoing considerations reveal that the change in *p*H of the constituents of the egg is the resultant effect of various metabolic processes in the egg.

## SUMMARY.

By employing a glass electrode, the variation in *p*H of the white and yolk of fertilized hen's egg during incubation in air, carbon dioxide and liquid paraffin has been studied.

In eggs incubated in air, the white shows an initial rise in *p*H due to the escape of carbon dioxide and a subsequent steady fall after 36 hours owing to carbohydrate catabolism. The yolk has a constant *p*H up to the second day and later, this gradually rises owing to protein catabolism.

When incubated in carbon dioxide or in liquid paraffin, the white suffers a small but continuous fall in *p*H, whereas the yolk shows a rise in *p*H. This is due to the inhibition of the normal metabolic processes in the egg.

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