BACTERIAL GROWTH IN ALBUMEN TAKEN FROM THE EGGS OF DOMESTIC HENS AND WATERFOWL

ELIZABETH M. SEVIOUR AND R. G. BOARD

School of Biological Sciences, Bath University of Technology, Bath BA2 7AY, Somerset, England

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SYNOPSIS

Gram-negative bacteria formed small populations in albumen taken from the eggs of domestic hens. Adding yeast extract (final concentration 100 mg/ml) to the albumen did not increase the extent of microbial growth. Large populations were formed in albumen containing FeSO$_4$·7H$_2$O (final concentration 9.95 mg/ml) or this sulphate and yeast extract and the organisms utilised the glucose of the egg white with a concomitant increase in the CO$_2$ released to the atmosphere. The rate of glucose utilisation, as judged by the rate and extent of the change in pH, was slow in albumen of the eggs of domestic hens to which had been added a heavy suspension of a fermentative bacterium. The retardation of glucose fermentation was a feature also of the albumen of eggs of several species of waterfowl. Both the rate and extent of glucose fermentation was increased when yeast extract (final concentration 100 mg/ml) was added to heavily seeded albumen but ferrous sulphate did not have a significant influence on the rate of glucose utilisation.

INTRODUCTION

The inimical properties of albumen taken from the eggs of Gallus domesticus were frequently noted (for references see Haines, 1939) in the 25 years following the first systematic study (Gayon, 1873) of the microbiology of the hen's egg. Subsequent studies of purified proteins from the albumen have indicated that it contains several potentially bactericidal or bacteriostatic agents (Board, 1966) but only some of these have as yet been shown or considered to function in ovo (Board, 1968).

There has been a tendency to account for the defence of the egg solely in terms of the action of lysozyme—an enzyme of the albumen which lyses certain bacteria (Laschtschenko, 1909; Fleming, 1922). Sharp and Whitaker (1927), however, demonstrated that the alkaline (pH 9.6) reaction of the albumen was important in preventing microbial growth in albumen in vitro. Ovotransferrin (conalbumin) was identified by Alderton, Ward and Fevold (1946) as the principal component of the hen's egg's defence. They showed it to be a chelating agent which, through sequestering Fe$^{3+}$, had inhibited microbial growth in the studies of Schade and Caroline (1944). From this information together with that derived from the many studies of the course of microbial infection of eggs which had been intended for human consumption (Brooks and Taylor, 1955), Board (1969) defined the egg as an ecosystem, the infection of which was impeded by the shell and its underlying membranes...
and the colonisation of which was hindered by the antimicrobial properties of the albumen. It was deduced that the latter was the principal component of the hen’s egg defence.

Although the defence provided by albumen has itself been the subject of many studies (e.g. Rettger and Sperry, 1912; Sharp and Whitaker, 1927; Garibaldi, 1960), the literature does not provide a concise picture of the course of bacterial growth in the albumen in vitro. Moreover, it does not provide any clues as to the fate of glucose, the most probable energy source for microbial growth. The present study was undertaken with the objects of establishing the pattern of bacterial growth in and the fate of the glucose of the albumen.

MATERIALS AND METHODS

Eggs of the domestic hens were obtained from a commercial flock of Thornber 404’s. The hens were housed in batteries and fed on a proprietary layers’ ration. The eggs were kept at room temperature for short-term storage but at 4 °C for storage of more than 1 week. The eggs of waterfowl were obtained from the Wildfowl Trust, Slimbridge. Glos. Their age was not known.

Albumen

The shells of eggs were swabbed with 70% (v/v) ethanol and cracked with a flamed scalpel. The contents were collected in a sterile Petri dish and the white harvested with a 10 ml, wide-bore sterile pipette. The albumen of several eggs was collected in a sterile screw-capped bottle and mixed by gentle shaking. Appropriate volumes (20 ml) of the blended albumen were dispensed in sterile Erlenmeyer flasks (150 ml capacity; closed with a cotton wool bung), or sterile 50 ml beakers (closed with aluminium foil).

The bacteria

The bacteria used in the main part of the work (Pseudomonas fluorescens, Pseudomonas maltophilia, Pseudomonas aeruginosa and Escherichia coli C20) had been isolated from rotten eggs (Board and Board, 1968; Seviour, Sykes and Board, 1972). For comparative purposes, bacteria derived from the Department's stock culture collection were used. All the cultures were stored on slopes of Nutrient Agar (Oxoid Ltd) at 4 °C. The bacteria were grown: (a) in bottles filled almost to the top with Nutrient Broth (Oxoid) containing 0·5% (w/v) glucose; (b) on Nutrient Agar (Oxoid) adhering to one of the large faces of 16 oz "medical flats" having a rectangular cross-section, or (c) in vigorously shaken Erlenmeyer flasks containing mineral salts, an ammonium salt and an energy source such as succinate. The actual derivation of the cells used for an inoculum is given in the text. The cells were harvested by centrifugation, washed in sterile distilled water and finally suspended in 5 ml of 0·1 M-phosphate buffer (pH 6·8–7·0). Sterile pipettes were used to inoculate the albumen.

Additives

Analar grade FeSO\(_4\)·7H\(_2\)O and powdered Yeast Extract (YE) (Oxoid Ltd) were dissolved in glass distilled water and sterilised by autoclaving (1·05 kg/cm\(^2\) for 20 min).
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Viable counts

Serial decimal dilutions were prepared by the addition of 1 ml cell suspension to 9 ml quarter-strength Ringer's solution. Known volumes (0.02 ml) of appropriate dilutions were dispensed by the method of Miles and Misra (1938) on to the surface of Nutrient Agar (Oxoid Ltd) the surface of which had been dried by storage at room temperature. The Petri dishes containing the Nutrient Agar were incubated at 27 °C for 24 to 48 h and the colonies counted.

pH

A portable meter (Cambridge Instruments Ltd) was used.

Glucose determination

Two grams of albumen were precipitated with perchloric acid (about 0.33 N) and the precipitate harvested by centrifugation. The supernatant was made up to volume (50 ml) in a volumetric flask and its glucose content determined with the Boehringer kit for determining the glucose concentration in blood.

Collection of CO₂

Round bottomed, three-necked flasks (500 ml; Quickfit Ltd) were sterilised by autoclaving (1.05 kg/cm² for 20 min). One hundred millilitres of albumen were placed in the flask and the latter positioned so that the bottom half was submerged in a water bath maintained at 27 °C. A rubber bung in the centre neck carried two glass tubes. One end of the longer tube was attached via a sterile air filter (cotton wool) and a U-tube containing a carbon dioxide absorbent (Carbosorb; B.D.H.) to a source of compressed air and the other end was positioned about 3 cm above the surface of the albumen. The shorter tube in the central bung was connected by a rubber tube to another glass tube the free end of which was drawn to a capillary. The latter tube was inserted through a rubber bung positioned at one end of a Pettenkofer tube which was inclined and which contained 50 ml of Ba(OH)₂·8H₂O (31.5 g/l). The location of the tip of the capillary in the barium hydroxide and the inclination of the Pettenkofer tube were adjusted so that gas bubbles retained their identity when moving along the Pettenkofer tube. At 24-h intervals, the barium hydroxide was collected, the Pettenkofer tube rinsed with glass distilled water and the liquid made up to 100 ml in a volumetric flask. This was titrated with 0.1 N HCl using bromocresol purple as indicator and the amount of CO₂ trapped by the barium hydroxide calculated. In all experiments, CO₂-free air sweeping over uninoculated albumen was bubbled through barium hydroxide, the latter was treated as above and the quantity of CO₂ subtracted from that obtained with an inoculated albumen. The angled side necks were closed with ground glass stoppers which were held in position with adhesive tape. Samples of albumen (10 ml) were taken by introducing a wide-bore pipette through one of the angled necks.

RESULTS

The extent of microbial growth

The general trends noted in this study are shown in Figures 1 and 2. A ten-fold reduction in the number of viable organisms in the 3 h following the inoculation of the albumen of the hen's egg was a common feature. In native albumen, the
Fig. 1.—The growth of *Escherichia coli* C20 in albumen taken from eggs of domestic hens. The blended albumen (20 ml) of several eggs was held in 150 ml Erlenmeyer flasks at 27 °C and inoculated with a suspension of washed bacterial cells. At the times indicated a flask was randomly selected and changes in the size of the bacterial populations and the glucose and \( H^+ \) concentrations in the albumen determined. The results obtained with native albumen (○) was compared with those for albumen containing yeast extract (△; final concentration 100 mg/ml), \( \text{Fe}^{3+} \) (●; added as \( \text{FeSO}_4 \cdot 7\text{H}_2\text{O} \) in a final concentration of 9·95 mg/ml), and yeast extract and \( \text{Fe}^{3+} \) in the concentrations noted above (●).
Fig. 2.—The growth of *Pseudomonas fluorescens* in the albumen taken from the eggs of domestic hens. Experimental details are summarised in Figure 1. ▲ = growth in uninoculated albumen; ○ = albumen containing FeSO₄·7H₂O (9·95 mg/ml); ● = albumen containing FeSO₄·7H₂O (9·95 mg/ml) and yeast extract (100 mg/ml), × = albumen alone.
Fig. 3.—The populations achieved by *Escherichia* spp (●) and *Pseudomonas* spp. (○) in albumen taken from the eggs of domestic hens compared with those in albumen supplemented with yeast extract (final concentration 100 mg/ml) or FeSO₄·7H₂O (final concentration 9.95 mg/ml). For additional details, see Figure 1.
lag continued for 24 h and, even when microbial growth occurred, the size of the final population was only of the same order as the inocula.

**Yeast extract.** The addition of YE (final concentration 100 mg/ml) to albumen limited the extent and duration of the lag phase of growth in the experiment recorded in Figure 1, but it resulted in a population which was only slightly larger than that obtaining in native albumen by the 6th d of incubation. It was noted in many experiments that YE protected small inocula in the 24 h following the seeding of albumen. On extended incubation, however, the presence of YE in albumen did not give a consistent pattern in the growth of organisms. In only half of the 13 experiments (Figure 3) were the populations formed in albumen containing YE larger than those in albumen alone.

**Iron.** When the chelating potential of the ovotransferrin was saturated with Fe\(^{3+}\), extensive bacterial multiplication occurred (Figure 1) and in 12 out of 14 experiments (Figure 3) the populations in albumen containing additional Fe\(^{3+}\) were larger than those formed in native albumen.

**Yeast extract and iron.** The addition of YE (final concentration 100 mg/ml) and Fe\(^{3+}\) sufficient to saturate the sequestering potential of ovotransferrin resulted in the extensive multiplication of *E. coli* (Figure 1) and *Ps. fluorescens* (Figure 2). Moreover, the populations achieved by *E. coli* were greater than that in albumen containing additional Fe\(^{3+}\) alone (Figure 1). This situation was noted in 13 out of 14 experiments (Figure 4). The growth of coliforms in albumen supplemented with YE and Fe\(^{3+}\) resulted in more viable organisms per ml than in a 0.1% (w/v) solution of YE—e.g. the concentration at which YE was present in supplemented albumen (Figure 5). In contrast, strains of *Ps. maltophilia* and *Ps. aeruginosa* formed populations of the same magnitude in both media.

These data indicate that the albumen of the hen's egg provides a poor medium for microbial growth and that it is not markedly improved by the addition of YE, a material which provides the organisms with combined nitrogen for synthetic purposes as well as energy substrates. The enhancement of growth by the addition of Fe\(^{3+}\) to albumen supports the claim (Schade and Caroline, 1944; Garibaldi, 1960) that ovotransferrin is the major component of the antimicrobial defence of the albumen. Nevertheless, the present study has demonstrated that the saturation of ovotransferrin with Fe\(^{3+}\) does not convert albumen to a completely satisfactory medium for microbial growth. Thus the addition of YE to albumen containing additional Fe\(^{3+}\) increased the size of the population achieved by the organisms used in this study. Although the addition of YE or Fe\(^{3+}\) and YE to albumen led to the extensive growth of bacteria, the rate of multiplication was notably less than that occurring in YE alone. It was found (Figure 6) that, in the majority of experiments, the capacity populations in YE broth were achieved 1 to 2 d following inoculation whereas 6 d were required for supplemented albumen. This evidence suggests that the supplements were neutralising only the major bacteriostatic agents of the albumen.

**Glucose and hydrogen ion concentrations and CO\(_2\) evolution**

There was no demonstrable change in the glucose or pH of native albumen inoculated with *E. coli* (Figure 1). Likewise, *Ps. fluorescens* (Figure 2) did not reduce
Log$_10$ bacteria/ml of albumen containing FeSO$_4$.7H$_2$O and yeast extract

Fig. 4.—The populations achieved by *Escherichia* spp (*) and *Pseudomonas* spp (O) in albumen taken from the eggs of domestic hens and supplemented with FeSO$_4$.7H$_2$O (final concentration 9.95 mg/ml) compared with those in albumen containing FeSO$_4$.7H$_2$O (final concentration 9.95 mg/ml) and yeast extract (final concentration 100 mg/ml). For additional details, see Figure 1.

the glucose content of unsupplemented albumen although it did cause a slight but temporary acid shift in its reaction. The presence of YE in albumen inoculated with the coliform was associated with a 30\% loss of glucose by the 6th d of incubation and a permanent shift of half a pH unit (Figure 1). When iron was the only supplement, the coliform (Figure 1) depleted the albumen of all its glucose whereas the pseudomonad (Figure 2) used only a small part. In both cases, the utilisation of glucose was associated with a drift in the pH, but towards the end of incubation, the pH was reverting to a value similar to that obtaining at the start of the experiment. The addition of Fe$^{3+}$ and YE to albumen resulted in the rapid dissimilation of glucose by the coliform organisms (Figure 1) and the pseudomonads (Figure 2). With the coliforms, the utilisation of glucose was associated with a pronounced fall in the pH (from 9.5 to 7.0). With the pseudomonads (Figure 2), there was a progressive increase in pH in the 3 d of observation.

The total amount of CO$_2$ recovered from the cultures was correlated with the amount of glucose used (Figures 2 and 7). Thus the largest amounts of CO$_2$ were
Fig. 5.—The populations achieved by *Escherichia coli* (●), *Pseudomonas fluorescens* (○), *Pseudomonas maltophilia* (×) and *Pseudomonas aeruginosa* (△) in albumen obtained from the eggs of domestic hens and supplemented with FeSO₄·7H₂O (final concentration 9.95 mg/ml) and yeast extract (final concentration 100 mg/ml) compared with those in 0.1% (w/v) yeast extract broth. For additional details see Figure 1.
Fig. 6.—The time taken for *Escherichia* spp and *Pseudomonas* spp to achieve climax populations in 0·1% (w/v) yeast extract broth (x), in native albumen (O) taken from the eggs of domestic hens and albumen containing yeast extract (Δ; final concentration 100 mg/ml), FeSO₄·7H₂O (▲; final concentration 9·95 mg/ml) or yeast extract and FeSO₄·7H₂O in concentrations noted above (●).

recovered from albumen containing YE and additional Fe³⁺. There tended, however, to be a lag in the recovery of the CO₂, only small amounts being recovered during the 1 to 2 d following inoculation of the albumen. The major recovery of CO₂ occurred when the pH was reverting to a value similar to that obtaining at the time of inoculation. It is possible that the lag in the recovery of CO₂ was a reflection merely of the impediment offered to gaseous exchange by the viscous albumen. Similarly, the drift in the pH may well have been due to CO₂ influencing the H⁺ concentration of the poorly buffered albumen (Figure 9). The growth of coliforms in albumen containing YE and additional Fe³⁺ was always associated with the development of a strong "butter" odour but no success attended attempts to demonstrate diacetyl by the method of Pack, Sandine, Elliker, Day and Lindsay (1964).

This information provides further support for the conclusion that both Fe³⁺ and YE are required to change the albumen of the hen's egg from a medium which supports limited growth of the commonly occurring bacterial contaminants of eggs to one which permits extensive, though retarded (Figure 6), multiplication of these organisms. Moreover, the results obtained from the chemical studies suggest that
Fig. 7.—Changes in the size of the populations, glucose and H+ concentrations in albumen derived from the eggs of domestic hens and inoculated with *Escherichia coli* C20. Uninoculated albumen (×), inoculated albumen (○) and albumen supplemented (●) with FeSO₄·7H₂O (final concentration 9.95 mg/ml) and yeast extract (final concentration 100 mg/ml).
the antimicrobial defence of the albumen tends to have a "sparking effect" on the glucose contained in the white.

Conservation of glucose

To obtain further information on the "sparking" of glucose, attention was given to the changes in the pH of albumen seeded with a dense suspension of the resting cells of a fermentative bacterium. The pH of hen egg white is about 9-6. Since hen albumen has poor buffering capacity (Cotterill, Gardner, Cunningham and Funk, 1958) the acid arising from the fermentation of glucose could be expected to cause the pH to fall towards 6. The results, given in Figures 8 and 9, exemplify those obtained with the albumen of hens' eggs and heavy suspensions of Escherichia coli C20. Preliminary experiments emphasised the need for heavy bacterial suspensions if the observations were to be completed in a short period. The changes in pH in native albumen (Figure 8) gave an S-shaped curve over a range of 1-75 units. The addition of sufficient Fe^{3+} to saturate the chelating potential of ovotransferrin enhanced acid production but not to the same extent as did yeast extract alone. The latter was used in a final concentration of 100 mg/ml because it was noted...
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(Table 1) that this was the concentration which gave the most rapid fall in pH. The addition of Fe$^{3+}$ to a YE-supplemented albumen tended to enhance slightly the rate and extent of the accumulation of H$^+$. It was noted (Figure 9) that these

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$^1$ Recorded after 4.5 h incubation at 27 °C.

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$^1$ Recorded after 5.5 h at 27 °C.

$^2$ Inoculum equivalent to 60 mg protein/ml.

* Final concentrations.

changes in the pH occurred over a range in which the albumen had little buffering capacity. Results similar to those in Figure 8 were obtained in albumen seeded with dense suspensions of the following: *Aeromonas liquefaciens, Proteus vulgaris, Salmonella*
Fig. 9.—The pH drift in albumen containing heavy suspensions of *Escherichia coli* C20 and the titration curve for albumen. • = unsupplemented albumen; O = albumen containing FeSO₄·7H₂O (final concentration 9.95 mg/ml); x = albumen containing yeast extract (final concentration 100 mg/ml), and △ = albumen containing FeSO₄·7H₂O and yeast extract at concentrations noted above. Stippled area, curve obtained from titration of uninoculated albumen with 0.3N lactic acid.
waycross, *Salmonella brandenberg*, *Serratia marcescens* and other strains of *E. coli*. Moreover, the same general pattern was observed (Figure 10) when dense suspensions of

![Graphs showing changes in pH caused by heavy suspensions of specific strains of *E. coli* in albumen from waterfowl.](image)

**Fig. 10.**—Changes in pH caused by heavy suspensions (equivalent to 60 mg protein/ml) of *Escherichia coli* C20 in albumen taken from the eggs of waterfowl. *T*, initial pH of the albumen; stippled area, native albumen; dotted line, albumen containing FeSO₄·7H₂O (final concentration 9.95 mg/ml); broken line, albumen containing yeast extract (final concentration 100 mg/ml), and solid line, albumen containing FeSO₄·7H₂O and yeast extract in concentrations noted above.

*E. coli* C20 were incubated in albumen obtained from the eggs of waterfowl. The results given in Table 2 indicate that the change in H-ion concentrations of the albumen reflected changes in the concentration of glucose and that this substrate
was attacked most rapidly when YE was present in albumen. Thus the results given in Figures 9 and 10 show that the albumen of domestic hens and water fowls retard the fermentation of glucose by facultatively anaerobic Gram-negative bacteria.

In the experiments discussed in this section, the resting cells were harvested from bottles filled almost to the top with glucose broth. Thus anaerobic conditions would have obtained shortly following inoculation and the cells would have been adapted to the fermentation of glucose. It was notable, therefore, that results similar to those given in Figure 9 were obtained with cells harvested from vigorously shaken Erlenmeyer flasks, containing a solution of mineral salts, an ammonium salt and succinate, or from the surface of nutrient agar. Similarly, the choice of containers for the inoculated albumen did not appear to be important. Thus the pH recorded at the 5th h of incubation of 20 ml of inoculated albumen (in a 50 ml beaker) which had been vigorously stirred at 30-min intervals with the electrode of a pH meter was the same as that for a 5 ml sample of the same albumen which had been held (in a water bath at 27 °C) in the barrel of a plastic hypodermic syringe ("Plastipak", Becton, Dickinson, Ireland) the needle boss of which had been sealed with a gentle flame. Similarly, the extent of glucose utilisation (Table 2) was of the same order. Contact between the piston of the hypodermic syringe and the inoculated albumen was not broken during incubation and attempts (a sharp tap, increase in temperature etc.) failed to release gas from the albumen.

DISCUSSION

The results obtained in this study have confirmed earlier reports that the unavailability of iron through chelation by ovotransferrin is the principal impediment to the growth of bacteria in the albumen of the hen's egg (Schade and Caroline, 1944; Brooks 1960; Garibaldi, 1960, 1970). The initial studies of the bacteriostatic activity of ovotransferrin showed that the presence of another chelating agent (Feeney and Nagy, 1952) in a medium containing purified ovotransferrin could overcome bacteriostasis presumably as a result of iron being made available by the competitive inhibition of one chelate by another. It is well known that bacteria and moulds growing in media rendered iron-deficient by precipitation and/or sequestration produce iron transport compounds such as fluorescent hydroxamate, 2,3-dihydroxybenzoylserine, etc. (Garibaldi and Neilands, 1956; Ito and Neilands, 1958; Brot and Goodwin, 1968) and that production can be enhanced by using mutants which have an impaired capacity for the synthesis of iron-containing metabolites (Cox, Gibson, Luke, Newton, O'Brien and Rosenberg, 1970). Moreover, these iron transport compounds need not have a high specificity for a particular organism. Thus Arthrobacter terragens can be used to assay (Reich and Hanks, 1964) the mycobactins for which Mycobacterium paratuberculosis has an obligate requirement (Snow, 1970). In the light of this evidence it has been suggested that, during their initial suspension in egg white, bacteria would be able to synthetise iron transport compounds which would negate the bacteriostatic action of ovotransferrin and thus lead to large populations (Garibaldi, 1960; Garibaldi and Bayne, 1962a). It is noteworthy that large populations are formed by bacteria in egg white supplemented with fluorescent hydroxamate or enterobactin (Garibaldi, 1970) or in serum
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containing 2,3-dihydroxybenzoylsereine (Wilkins and Lankford, 1970)—the addition of iron to serum in vitro removes bacteriostasis due to transferrin (Schade and Caroline, 1946). The evidence obtained in this study would seem to argue against the ability of the commonly occurring contaminants of eggs being able to overcome the bacteriostatic action through the synthesis of iron transport compounds in native egg albumen. Moreover, studies with mixed bacterial infections in the shell membranes in situ (Seviour and Board, 1972) did not indicate that the organisms were ameliorating the unfavourable environment within these structures. It has been established that contamination of the site of infection of the shell membrane with traces of iron (Garibaldi and Bayne, 1962a) leads to extensive microbial growth and gross contamination of the albumen (Board, Hendon and Board, 1968) and rapid rotting of the eggs (Garibaldi and Bayne, 1960, 1962b). A similar response was observed (Board, 1964) when bacteria suspended in extracts of soil or faeces were placed on the shell membranes in situ. In the latter study, treatment of the extracts with 8-hydroxyquinoline reduced but did not eliminate the enhancement of bacterial growth in the membranes. This has been noted repeatedly (F. R. Sykes and R. G. Board, unpublished) with inocula on the shell membranes of fertile and infertile eggs held at 38 °C. Thus factors other than iron appear to play a part in promoting bacterial growth during the initial phases of microbial infection of eggs and it may be in this situation that iron transport compounds contained in soil or hen’s faeces are important.

Recent reports indicate that the transferrins’ rôle in antimicrobial defence systems is not confined to eggs. Thus the injection of iron into experimentally infected animals has been shown to enhance microbial growth and/or reduce the LD50 dosage (Jackson and Burrows, 1956; Martin, Jandl and Finland, 1963; Summers and Hasenclever, 1964; Sword, 1966). There is perhaps an unfortunate tendency to consider (e.g. Wilkins and Lankford, 1970) that transferrins are components of “non-specific defence mechanisms”. If viewed from the standpoint of classical immunology, then perhaps there is not the stereochemical specificity which is the basis of the antigen-antibody reaction. Nevertheless, transferrins by chelating iron could be expected to have a specific effect on the physiology of those organisms which require this ion. Theodore and Schade (1965) have shown that ovotransferrin in an aerated culture of Staphylococcus aureus results in the organism having an enzymic constitution similar to that it has when growing anaerobically on glucose—the enzymes of glycolysis being abundant but those of respiration sparse (Strasters and Winkler, 1963).

Both Haines (1939) and Brooks (1960) considered that the low level of non-protein nitrogen in the albumen of the hen’s egg would hamper microbial growth. The results obtained in the present study indicate that large populations of bacteria are formed in albumen supplemented with Fe³⁺ and that these were only increased slightly when yeast extract was present. Further work is required to establish whether it is the amino acids or the growth factors of yeast extract which are promoting growth under these conditions. It was found that yeast extract leads to the utilisation of glucose by bacteria growing or merely suspended in the albumen. This phenomenon was noted by Ayres (1958), but the rôle of yeast extract in accentuating microbial fermentation of the glucose has yet to be elucidated.
ACKNOWLEDGEMENTS

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