POROSITY OF, AND BACTERIAL INVASION THROUGH, THE SHELL OF THE HEN'S EGG

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(With Plate IX and 4 Figures in the Text)

Although bacterial invasion of the developing ovum is known to occur (Haines, 1939), it is fairly clear that the microbial decomposition of eggs encountered in commercial handling is due, in the main, to the invasion of the egg by spoilage-producing micro-organisms after laying. This in turn depends on (*a*) the inherent porosity of the shell, and (*b*) the treatment the egg receives, e.g. washing. The present work is an attempt to gain information on these two factors.

The porosity of the shell

The porosity of the shell may be studied (1) by histological methods and (2) by measuring the rate of movement of liquids or gases through the shell under a given pressure gradient. Difficulties have been met in applying both these techniques. The first has given a useful picture not, however, amenable to quantitative interpretation, whilst the second has shown that porosity is a relative term, varying at different points on the shell and in successive eggs from the same hen.

(1) *Histology*

Pl. IX, fig. 1 is a transverse section (× 120), normal to the surface, of the egg-shell. Four layers can be distinguished. These are: first, the cuticle, composed mainly of fibres of mucin (Moran & Hale, 1936); secondly, the spongy layer consisting of crystals of calcite more or less normal to the cuticle; thirdly, the mammillary layer, also consisting of crystals of calcite, which however do not appear to be definitely orientated; and fourthly, the inner shell membrane.

Pl. IX, fig. 2 shows a section of a shell, again normal to the surface (× 100), in which pores or V-shaped openings can be seen stretching from the outside (cuticle not present) to the mammillary layer. It is evident that these pores do not pass right through the shell as has been claimed by Marshall & Cruickshank (1938). The diameter of one of these openings was 13 μ at the top, 6 μ at the bottom.

Pl. IX, fig. 3 is a section (× 75), cut parallel with the surface of the shell, in the third or mammillary layer, near the inner shell membrane. The extreme irregularity of the structure is shown, with spaces of various sizes between the calcite crystals which possibly form canals or pores leading to the interior of the egg.

Pl. IX, fig. 4 (×30) is a section of the lower portion of the mammillary layer, below the section shown in Pl. I, fig. 3, and is the first crystal formation laid down directly on the network of fibres forming the inner shell membrane.

The conclusions to be arrived at from these sections may be summed up as follows. The shell, mainly composed of crystals of calcite, consists of an inner mammillary layer in which the crystals of calcite are large and spherical, and an outer or spongy layer in which the crystals are smaller, irregular, and with their principal axes normal to the surface of the shell. The outer covering, or cuticle, consists chiefly of fibres of mucin. Distinct pores or channels, up to 15μ diameter, may occur in the spongy layer, but these disappear in the mammillary layer in which only a network of much finer channels can be observed. The crystals of calcite resting on the fibrous network of the inner shell membrane are irregularly packed and tend to overlap unevenly, so that any passage through the shell must be tortuous. In the fresh shell, material, protein in nature, tends to close up these channels.

From these results it is impossible to do more than postulate that channels occur through which bacterial invasion is likely to take place under appropriate conditions. More precise data were sought, first by studying the flow of water and air through the shell, and secondly by following the actual penetration of bacteria.

(2) *The flow of air and water through the shell*

The method adopted in these experiments was in essence a viscometric one. Eggs from trap-nested birds were received daily from the Animal Research Station, Cambridge, and a third of the shell, at the air-space end, carefully sawn off with a razor blade. The contents of the egg were discarded and the inner membranes removed by peeling off with forceps, care being taken not to strain the shell and so induce cracks, nor to smear albumen over the shell which might block the pore exits. The larger portion of the shell was then cemented in a glass funnel fitted with a tap so that suction could be applied, and known volumes of water or air drawn through at pre-determined pressures. Preliminary experiments showed that the time for filtration of a given volume of water at a given pressure was not constant for the same shell but decreased by a factor of two or three times with repetition. Complete agreement between successive readings was never obtained, but after three washings at 60 cm. mercury the readings became roughly steady, so that the technique was arbitrarily standardized by washing through each shell four times at 60 cm. mercury pressure before taking the experimental readings. The underlying assumption was that by the fourth washing the variable amount of protein material in the pores had been largely removed. The results were calculated back to 100 sq. cm. of shell from measurements of the weight, density and thickness of the portion of shell through which the water passed. When plotted, the data give characteristic curves. It is apparent that a complex relation exists between the pressure applied and the rate of filtration. The

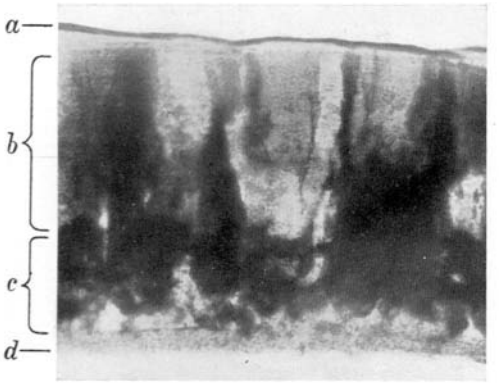

Fig. 1. Transverse section, normal to surface, of egg-shell (× 120). Note four layers: (*a*) cuticle (top); (*b*) spongy layer; (*c*) mammillary layer, commencing about two-thirds of thickness down; (*d*) inner shell membrane.

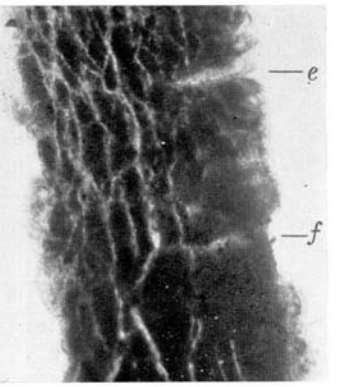

Fig. 2. Transverse section, normal to surface, of egg-she (× 100), showing pores (*e*, *f*) passing from surface t mammillary layer.

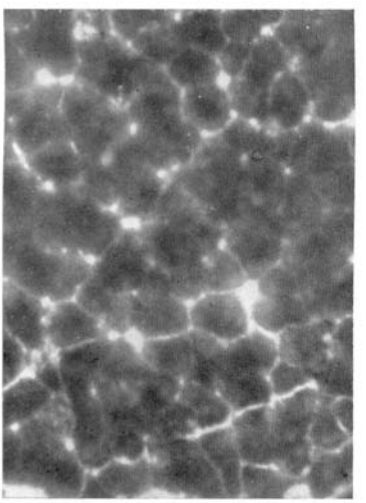

Fig. 3. Section parallel with surface, in mammillary layer (× 75).

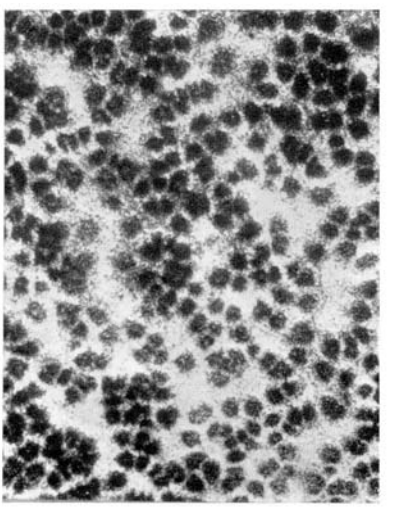

Fig. 4. Section parallel with surface, in lower portion o mammillary layer (× 30). Note isolated crystals restin on network of fibres forming the shell membrane.

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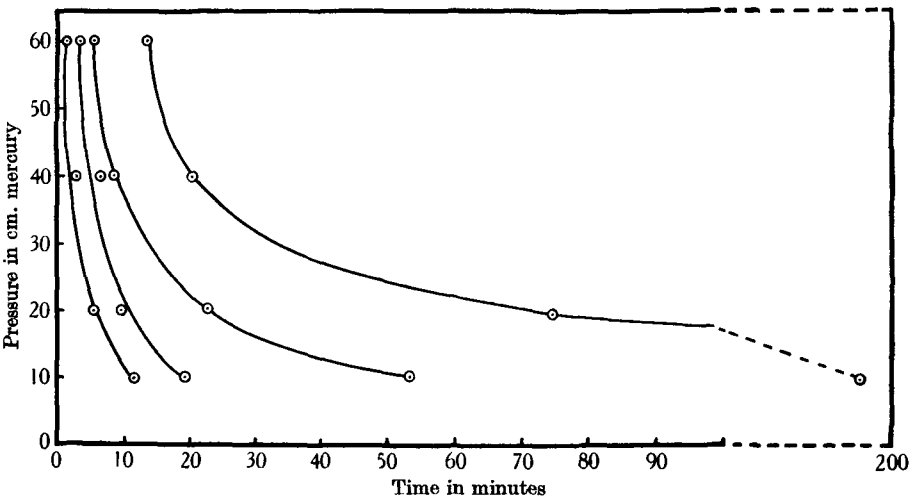
equations to these curves are unknown, but comparative information as between egg and egg can be obtained by determining the filtration curves for a series of eggs of known history from the same hen. For purposes of comparison filtration was also carried out through sintered glass filters, and through a single pore in an egg-shell. In the latter case a large pore was selected, and the surrounding pores blocked up with a suitable cement. Similar types of curves were obtained in both cases (Text-figs. 1 and 2).



Text-fig. 1. The flow of water through the egg-shell. (Time of flow for 25 ml.) Eggs from the same hen over a period of 9 days.

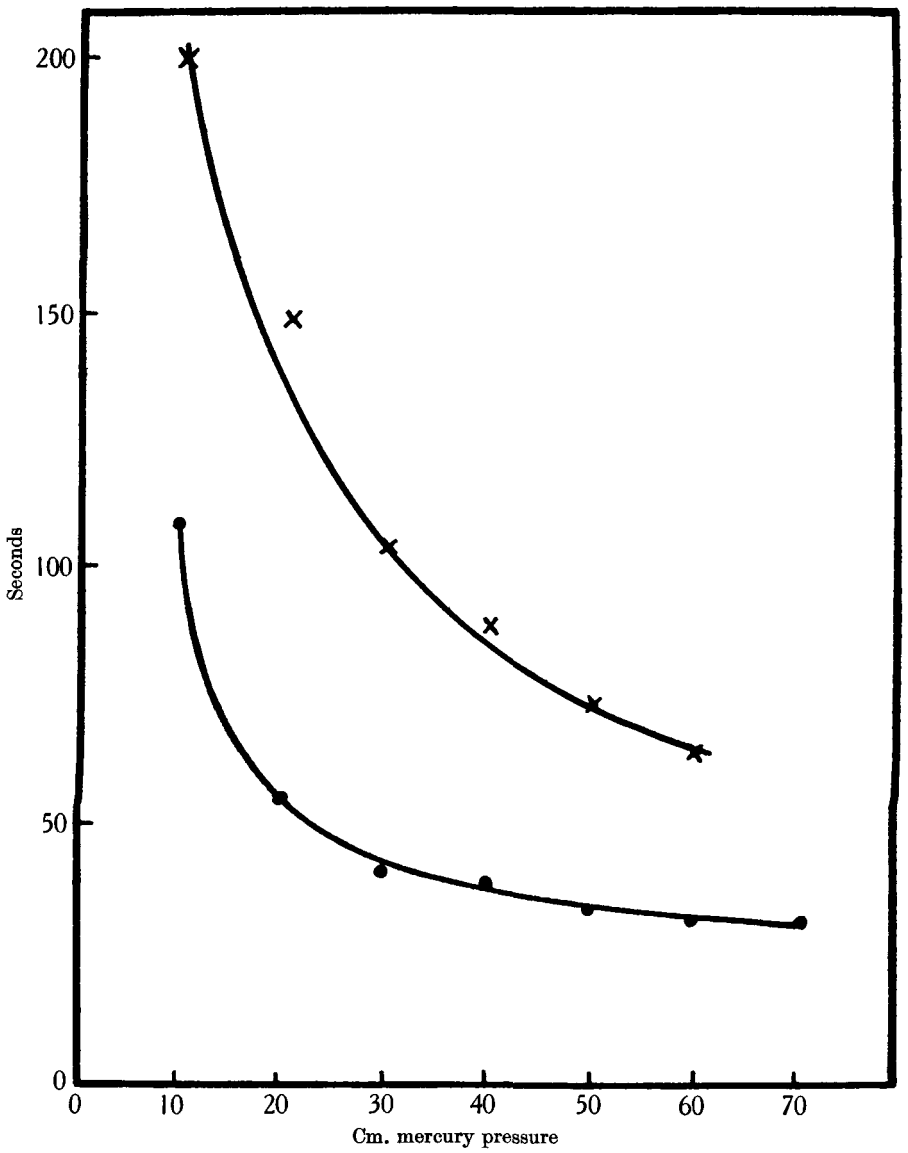
A set of results for eggs from the same hen are given in Text-fig. 1. The data were obtained from four eggs taken over a period of 9 days. It is evident that there are big variations in porosity as determined by the viscometer method in successive eggs laid by the same hen.

In order to see whether there is any correlation between rate of filtration and number of pores, several staining methods were tested for counting the pores. Aqueous methylene blue proved most suitable: alcoholic solutions of dyestuffs were avoided since the lower viscosity of such solutions might render them liable to traverse paths not used in the viscometer experiments. After taking the viscometer readings, 0·5% aqueous methylene blue was pulled through the shell at 60 cm. mercury pressure for 5 sec., excess methylene blue removed, the shell washed and dried, and the number of pores counted, using a low-power objective. In Table 1 is given the number of pores per 25 sq. cm. of shell and the time for standard filtration at 60 cm. mercury also calculated for 25 sq. cm. It is evident that there is no relation between the total number of pores as determined by staining methods and the rate of filtration of water.

The view has been put forward that the pores are definite structures traversing the entire thickness of the shell (Marshall & Cruickshank, 1938).

Table 1. *Relationship between porosity and number of pores*

No. of pores per 25 sq. cm. by staining	1968	1982	2460	2979	2991
Porosity (time of flow for 25 ml. water through 25 sq. cm. in min.)	26·9	39·4	76·1	49·4	5·8



Text-fig. 2. × = Flow of water through a sintered glass filter. ● = Flow of water through a single pore in the egg-shell.

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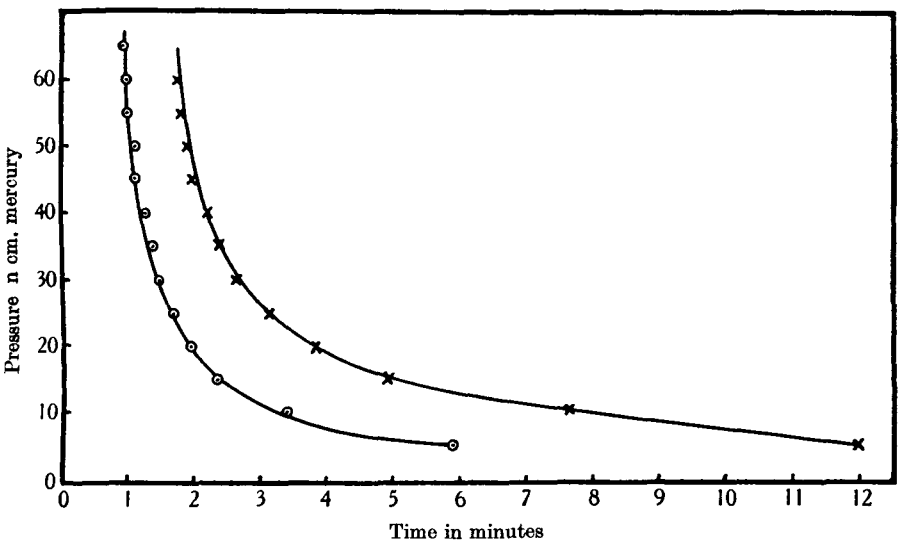
On the other hand the histological evidence quoted previously suggested that the pore as such traversed the spongy layer only, the mammillary layer below being a more compact structure having irrigation paths through which gaseous exchange, and any other entry into the egg, had to take place. If the former view is correct, then the number of pore termini on both sides of the shell should presumably be equal, and possibly also the rate of filtration in both directions (inside → out and outside → in) equal. Evidence on this point was sought by dividing shells longitudinally, drawing through stain in opposite directions with the two halves, and counting the pores. An example of the results is given in Table 2.

Table 2. *Mean number of pores per sq. cm. on the outside and inside halves of the same shell*

	Egg 1	Egg 2
Exterior	114	102
Interior	46	49



Text-fig. 3. The flow of air through the egg-shell. Upper curve = from inside shell outwards; lower curve = from outside shell inwards.

In addition the filtration times were determined in both directions. In each case flow was considerably slower from the inside to the outside than vice versa. Owing to the technical difficulty of mounting the shell for the outside → in determination (comparatively small areas only could be used) these measurements were tedious and not very precise, and the filtration of water was abandoned in favour of filtration of air, which was much quicker. The result of a typical experiment is shown in Text-fig. 3.

The results are in agreement with the histological evidence that the pores do not traverse the shell directly but are more numerous on the outside of the

shell. The fact that the passage of water and air is slowest when the movement is from the inside outwards suggests something of the nature of a "valve action" possibly by the partial clogging of the finer pores on the interior of the shell. A relevant point therefore was to determine whether the rate of evaporation during storage can be correlated with the porosity. Eight eggs immediately after laying were stored at 10° C. and 60% R.H. for 16 days, their loss in weight over this period being accurately determined, after which air-filtration experiments were made with the shells. The mean rate of flow outside → in and inside → out was taken in this case, and is given graphically in Text-fig. 4. It will be observed that the order of the eggs (weight loss) was *G*, *J*, *H*, *B*, *A*, *D*, *F*, *C*, starting from the greatest loss, while the relative order of porosity was *G*, *B*, *H*, *A*, *D*, *C*, *J*, *F*. In other words, with the exception of egg *J* which was badly out of order, and the reversal of eggs *F* and *C* and *B* and *H* adjacent to each other, there did appear to be a rough correlation between weight losses and porosity.

(3) *The passage of micro-organisms through the shell*

A few experiments have been made on the passage of micro-organisms through the shell under the conditions of the previous viscometer experiments. The whole apparatus was sterilized, and the shell cemented to the funnel with as little handling as possible. Concentrated suspensions of *Pseudomonas* in water, from a culture previously isolated from eggs, and of *Saccharomyces ellipsoideus*, were used. (The size of the former organism is about $2\mu \times 1\mu$, and the latter 8–10μ diameter.) Suspensions were drawn through the shell and viable counts made on the liquid remaining on either side of the shell. Some typical results are summarized in Table 3. It is clear that *Pseudomonas* passes readily through the shell in large numbers under the conditions of these experiments, the viable counts in general being the same on both sides of the shell. *Saccharomyces ellipsoideus* can also pass through the shell, but apparently not so readily, since there is usually a difference in the count on the two sides of the shell. Difficulties were encountered in attempting to push this type of work any further, the pores often becoming choked with the organisms, especially in the case of the yeast, and filtration ceasing or cracks developing in the shell.

Table 3. *The passage of* Pseudomonas *and* Saccharomyces ellipsoideus *through the egg-shell at a pressure of* 60 *cm. mercury*

	1	2	3	4	
Count on original suspension organisms/ml.:					
Pseudomonas	2×10^8	$1{\cdot}5 \times 10^8$	2×10^{10}	$6{\cdot}3 \times 10^8$	$6{\cdot}3 \times 10^8$
Saccharomyces	7×10^5	1×10^6	—	—	—
Pulled through the shell:					
Pseudomonas	$1{\cdot}4 \times 10^8$	9×10^7	2×10^{10}	$6{\cdot}6 \times 10^8$	$4{\cdot}3 \times 10^8$
Saccharomyces	9×10^3	3×10^4	—	—	—
	Inside → out	Inside → out	Inside → out	Inside → out	Outside → in

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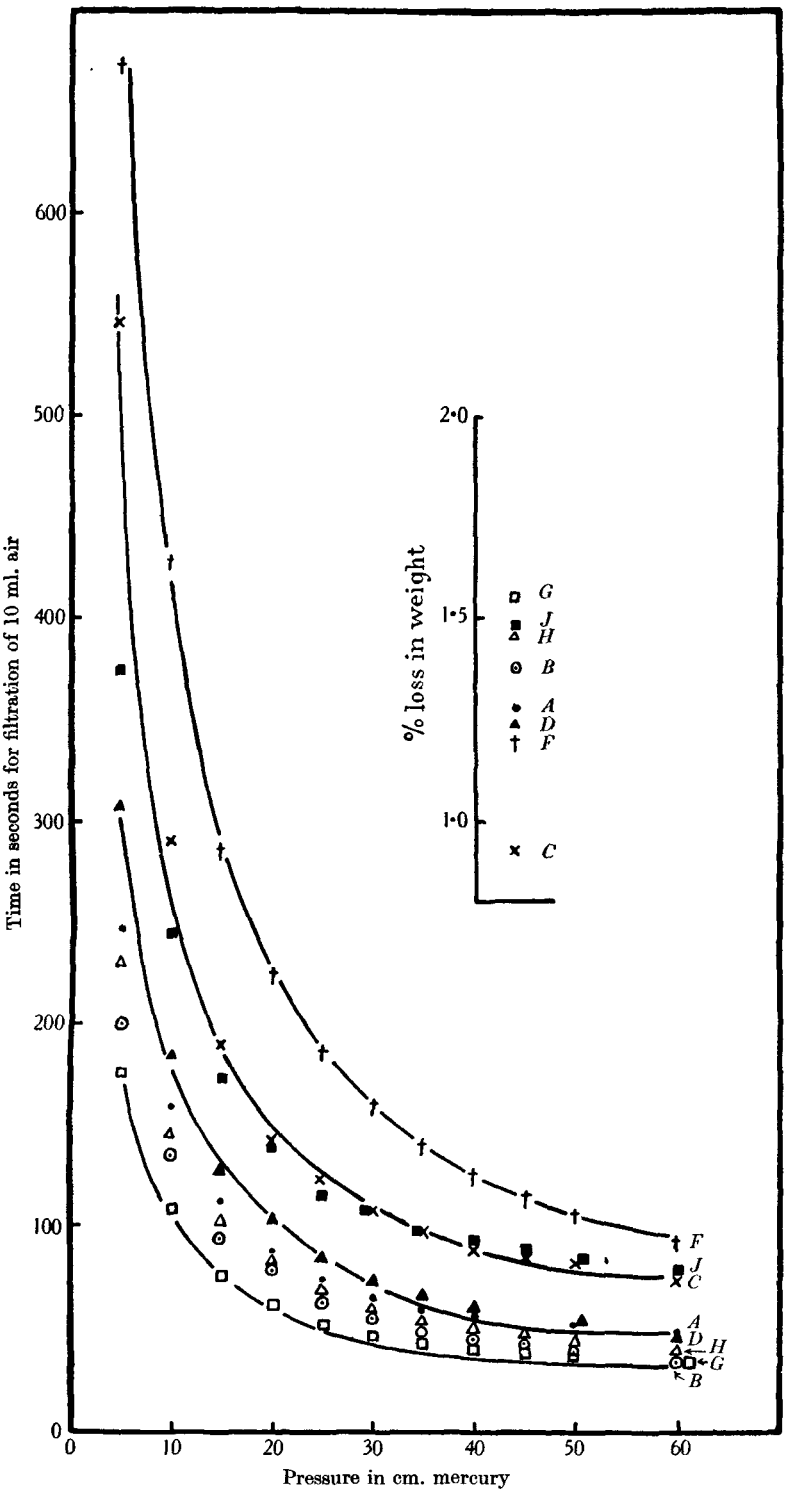

Text-fig. 4. Rate of flow of air through the shell and loss in weight by evaporation. Eggs arranged in order of % loss of weight in inset, same symbols used in porosity curves.

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The effect of the relative temperatures of the egg and its environment on bacterial invasion

The foregoing experiments indicate the presence in the shell of apertures allowing the passage of micro-organisms, and under suction bacteria and yeasts can in fact readily be pulled through the shell. It seems *a priori* probable that such a suction is set up when a warm, newly-laid egg cools down, and the air subsequently to form the air-space is drawn in. In particular it might be expected that a moist film of bacteria present on the shell would thus be sucked into the egg. Experiments have been carried out to test this possibility. In order to bring a fairly large-scale test within the bounds of practicability, conditions were simplified as far as possible by, in general, immersing the eggs completely in a suspension of *Pseudomonas* bacteria in tap-water, some immediately after laying, others at given intervals. All the eggs were dried carefully on removal from the bacterial suspension, incubated for 3 weeks at 25° C., and 90% R.H., cracked open, and the number of green rots ascertained.

Table 4. *Effect of the relative temperatures of the egg and the wetting fluid on the bacterial rotting of eggs*

Treatment	No. of eggs	No. of rots	% rots
Soaked immediately after laying for 1 hr.	27	27	100
Cooled for 1 hr., then soaked for 1 hr.	27	21	78
Cooled for 24 hr., then soaked for 1 hr.*	27	0	0
Soaked immediately after laying for 1 hr.	33	33	100
Cooled for 1 hr., then soaked for 1 hr.	32	20	63
Cooled for 24 hr., then soaked for 1 hr.*	31	5	16
Soaked immediately after laying for 1 hr.	30	30	100
Cooled for 1 hr., then soaked for 1 hr.	30	29	97
Cooled for 24 hr., then soaked for 1 hr.*	49	20	41
Eggs cooled overnight, brought to 98° F. in incubator before soaking, then soaked 1 hr.	50	50	100
Eggs cooled overnight at +10° C., then soaked for 1 hr. at +25° C.	99	7	7
Eggs cooled overnight in laboratory, then soaked 1 hr. in suspension at approximately same temperature as eggs	119	16	13
Eggs dropped immediately on laying into straw sprayed with *Pseudomonas*	45	0	0

* In these three experiments the temperatures of the eggs were not measured before soaking, but the temperatures of the suspensions rose 1·1, 1·3 and 3·2° C. respectively, during soaking, suggesting that the increased rotting in the controls is due to the greater difference between the temperature of the egg (air temperature) and the temperature of the tap-water suspension, as the season advanced.

The error involved in taking rotting as the criterion of bacterial infection is probably not very great in experiments of this kind, and in any event rotting is likely to be a conservative estimate of infection. We are indebted to Captain E. T. Halnan for allowing us facilities to secure eggs at the University Farm immediately on laying (*first quality White Leghorn, Light Sussex, Cambar,* Legbar and cross-bred, mostly from pullets, collected in April and May). The results are summarized in Table 4. The differences are significant and indicate the following points:

(1) Whether or not wetting the shell increases rotting depends very largely on how the wetting is carried out, the respective temperatures of egg and wetting fluid being especially important.

(2) Total immersion of eggs in a fluid containing bacteria at a *lower* temperature than the egg invariably leads to a high percentage of rots.

(3) Total immersion of eggs in a fluid containing bacteria at a higher temperature than, or the same temperature as, the eggs, leads to comparatively little rotting.

(4) Eggs dropped immediately on laying into moist infected straw did not rot, indicating that complete covering of the shell with fluid, under appropriate conditions as regards temperature, is more likely to lead to rotting than partial moistening of the shell.

SUMMARY

1. Histological methods show four layers in the intact shell of the hen's egg, namely, cuticle, spongy layer, mammillary layer, and inner shell membrane. Distinct channels or pores may be found in the spongy layer, but these tend to disappear in the mammillary layer in which only a network of much finer channels can be observed.

2. Viscometric measurements of the passage of water and air through the shell show that

(*a*) shells vary widely in their porosity, even when taken on successive days from the same hen;

(*b*) there is no correlation between porosity so determined and the number of pores counted by staining methods;

(*c*) the rate of flow is slower from the interior to the exterior than vice versa, and the pore count by staining methods also less on the inside than on the outside.

3. A rough correlation exists between porosity as determined viscometrically and the evaporation of water during storage.

4. If the temperature of the egg is higher than a fluid containing bacteria in which it is immersed, the latter are readily drawn through the shell by simple suction as the egg cools down, a point of great significance in the washing of eggs.

The work here described was carried out as part of the programme of the Food Investigation Board, and is published by permission of the Department of Scientific and Industrial Research.

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