PHYSICS OF THE HEN'S EGG

I. MEMBRANES IN THE EGG

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(With One Plate.)

This paper is an account of observations made on the composition and behaviour of certain of the membranes in the hen's egg. The composition is based on the examination of stained sections; the results should, however, give pointers in a purely chemical survey. Needham (i) has reviewed the work—chemical and histological—that has been carried out on the nature of the yolk and shell membranes. The common constituent appears to be keratin, but as he points out, this protein can exist in different forms with somewhat different properties.

The fact that the vitelline membrane separates two solutions of very different osmotic pressures has been the subject of much discussion. A few experiments have been carried out in an attempt to measure the osmotic gradient across this membrane.

VITELLINE MEMBRANE.

Egg yolk is not structurally homogeneous; it is made up of rounded masses varying in diameter from 25 μ in the outer portion of the yolk to 100–150 μ towards the centre (4, 3). The membrane surrounding each of these masses, if there is one, is extremely delicate, as they readily break down when simply allowed to flow.

Liebermann (ii) made a chemical examination of the vitelline membrane and concluded that it consists almost exclusively of keratin.

In the present work pieces of membrane were cut away and washed in 0.6 per cent. sodium chloride solution, fixed in saturated mercuric chloride solution and stained by Mallory's fluid. It was found that the membrane consists of three layers which could be teased apart by means of the Chambers micro-manipulator (Fig. 1):

(i) that nearest the yolk, 3–6 μ thick, staining blue;
(ii) a centre layer 6–10 μ thick, staining yellow; and
(iii) an outer layer staining blue, 3–6 μ.

These observations suggested that the centre layer is keratin and that the two outer layers are mucin in character.

Attempts were made to check this using other stains, having in mind also to rule out the connective tissue proteins collagen and elastin. The stains used were
toluidine B, mucicamine, thionin B, orcein, celestin B and van Gieson, and the membrane was compared with the areolar tissue of the rat, collagen fibres from the rat's tail, saliva and the mucin ducts in the earthworm. In every case, with the exception of orcein, the outer layers of the vitelline membrane took on the same stain as the mucin in the earth-worm and saliva. Collagen and elastin behaved differently in several respects. The centre layer of the vitelline membrane is also different from the outer layers; it is unstained by toluidine B, mucicamine and thionin B, whereas the outer layers are stained red. It also stains the same as feather keratin.

Attempts were made to expose the keratin layer by dissolving away the mucin layers. In the paper that follows it is shown that the vitelline membrane weakens under the action of trypsin. Membranes considerably reduced in thickness after 1 hour in a 0·1 per cent. solution of trypsin (pH 9) at 37° C. when fixed and stained with Mallory showed small isolated patches which gave the characteristic yellow stain for keratin. Alkali was more effective. Fresh pieces of membrane immersed in \(N/5\) or \(N/10\) NaOH rapidly dissolved, but in one experiment after 4 hours in \(N/10\) NaOH the membrane, when fixed and stained, showed only a thin and very open network of mucin through which could be seen the keratin. In solutions of hydrochloric acid the mucin was apparently denatured or precipitated and there was little or no solvent action.

The total thickness of the vitelline membrane varied from 12 to 23 \(\mu\). Needham found an average figure of 24 \(\mu\). The thicknesses were measured by the same method: the fine adjustment of the microscope was first calibrated either by a Thoma blood-count cell or by a thin cover-slip, the thickness of which was first determined by an accurate micrometer screw. The thickness of the membrane or layer was then calculated from the number of turns required to focus the upper and under surfaces.

Romanoff (12) has pointed out that there are three different layers of albumin in the white of an egg, an inner layer of dense white covering the yolk from which extend the chalazae, the so-called thick white, and the outer layer of watery or thin white. In a fresh egg he found the relative amounts of these layers to be 3, 57·2 and 39·8 per cent. respectively; the solid contents of each layer vary slightly, his average figures being 14·55, 12·45 and 11·59 per cent. respectively.

The membranous or layer structure in thick white is readily apparent to the naked eye, and, if the structure is ruptured by cutting, a watery fluid flows away. The refractive indices of the various phases suggest distinct differences in chemical composition as the following figures at 20° C. show:

<table>
<thead>
<tr>
<th></th>
<th>Egg &lt; 2 hours old</th>
<th>Egg 5 days old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner layer (white)</td>
<td>1·3582</td>
<td>1·3555</td>
</tr>
<tr>
<td>Fluid from thick white</td>
<td>1·3579</td>
<td>1·3555</td>
</tr>
<tr>
<td>Thick white remaining</td>
<td>1·3553</td>
<td>1·3559</td>
</tr>
<tr>
<td>Thin white</td>
<td>1·3492</td>
<td>1·3546</td>
</tr>
<tr>
<td>Yellow yolk</td>
<td>1·4200</td>
<td>1·4219</td>
</tr>
<tr>
<td>White yolk</td>
<td>1·4140</td>
<td>1·4176</td>
</tr>
<tr>
<td>Water</td>
<td>1·3324</td>
<td>1·3344</td>
</tr>
</tbody>
</table>
The actual values vary slightly from egg to egg, but the differences between fractions and with age persist. The measurements were made with a Zeiss refractometer and could be determined accurately to 0.0002 in the case of the white and 0.0005 with the yolk.

In heat-coagulated thick white the separate layers can readily be peeled apart. According to McNally thick white differs from thin white in having a high content of mucin. It is possible that the "solid" portion of this layer is a weak mucin gel. Heringa and van Kempe Valk concluded that the zones in thick white are due to the presence of actual membranes. They found that the fibrillae in these membranes are weakly doubly refractive, and tentatively suggested that they are composed of a keratinic substance.

Whole thick white was fixed either by heat coagulation or by mercuric chloride solution. It was then embedded in celloidin or paraffin, sectioned and stained. Pl. I, fig. 2, is a photograph of a section and shows the thick white to be composed of bands of a continuous phase bounded by fibres or fibrillae scattered among which are doubly refracting crystals. The bands are approximately 1 mm. wide.

When the section is stained with dilute Mallory's fluid or counter-stained with orange G the continuous bands are blue, the fibrillae and crystals yellow. Similarly after staining with toluidine B, mucicamine and thionin B and counter-staining with orange G the fibrillae and crystals are yellow whilst the continuous bands hold on to their original colour, i.e. blue, red, and blue respectively. The evidence suggests that the fibrillae and crystals differ chemically from the bulk of the thick white and are probably a form of keratin.

SHELL MEMBRANES.

There are two distinct membranes next the shell. Using the same technique the outer membrane (total thickness 30-36μ) is found to consist of three distinct layers. The layer adjoining the shell is made up of a tangle of coarse fibres of keratin (2-5μ diameter). The other two layers contain finer fibres (0.8μ diameter) apparently of mucin (Pl. I, figs. 3 and 4).

The other shell membrane, forming the inner surface of the air space, has much the same structure but is slightly thicker (40-48μ). The layers in this membrane are not distinct and two only could be teased apart; each layer consists of a meshwork of keratin and mucin fibres.

On the outer surface of the shell is a layer composed chiefly of mucin cells. This same substance is present in the lower part of the oviduct.

Osmotic properties of vitelline membrane.

Straub suggested that the difference in osmotic pressure of yolk and white, roughly 1.8 atmospheres (Δ yolk = 0.57, Δ white = 0.45), which exists in a fresh egg and persists for a considerable time after laying, is due to oxidation taking place at the membrane which supplies the energy necessary to reverse the normal flow of water and salts between yolk and white. This now seems unlikely in view of the
work of Hill (5), Needham (6) and A. J. M. Smith (7). Hill showed that the difference persists in an atmosphere of hydrogen, thereby restricting the process to an anaerobic mechanism. Smith confirmed this by observing that infertile eggs showed no measurable consumption of oxygen. Needham examined the possibility that glycolysis in the egg supplies the energy required by the vitelline membrane, but the evidence is against this view unless it be assumed that the energy output of the whole yolk can be made available at the actual membrane. Needham and Smith inclined to the view that the slow attainment of equilibrium is due to hindrance offered by the physical structure of the yolk and to a lesser extent of the white. This also is the conclusion arrived at in the present work.

When yolks are frozen at a temperature above $-6^\circ$ C. the yolk masses are ruptured but the yolk as a whole retains its fluidity (3). This suggested a method of reducing the resistance to the movement of water in the yolk and therefore of promoting more rapid equilibration between yolk and white. It was found that eggs frozen at $-3$ or $-5^\circ$ C. for a few days did in fact show this rapid equilibration. The following are details of one experiment.

Eggs$^1$ frozen and stored at $-3^\circ$ C. for 4 days:

*Portion* thawed rapidly (in rubber bags) in water at ordinary temperatures; yolks removed, rolled on filter paper and membranes examined for punctures:

$\Delta$ whole white = 0.441; water content = 88.3 per cent.
$\Delta$ whole yolk = 0.545; water content = 47.6 per cent.

*Portion* thawed slowly in air at $10^\circ$ C. for 3 days:

$\Delta$ whole white = 0.474; water content = 88.65 per cent.
$\Delta$ whole yolk = 0.462; water content = 50.4 per cent.

Control eggs stored for 4 days at $0^\circ$ C. followed by 3 days at $10^\circ$ C. gave:

$\Delta$ whole white = 0.459; water content = 87.9 per cent.
$\Delta$ whole yolk = 0.573; water content = 46.4 per cent.

It may be suggested that freezing and thawing renders the membrane itself more permeable, and in this connection it should be pointed out that eggs frozen at $-1.1^\circ$ C. for 11 days and thawed at $+10^\circ$ C. for 3 days do not show this rapid equilibration. It is possible that this points to freezing affecting the permeability of the membrane with a critical temperature of freezing between $-1.1$ and $-3^\circ$ C. (such as was found for living muscle (8)), but on the other hand eggs that have been frozen at $-1.1^\circ$ C., on thawing always show a continuous layer of what appears to be concentrated thick white at the surface of the yolk. This layer may retard the equilibration between yolk and white.

Hardy (9) suggested that "there is in the egg no sharp change of pressure at either of the surfaces of the vitelline membrane, but there is a gradient from the centre of the yolk outwards of varying, but always slight, slope which ends at a surface whose position depends upon the rate of evaporation from the shell". This would imply that the yolk and white adjoining the membrane have approximately

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$^1$ All the eggs used in this work were less than 24 hours old (from time of laying).
the same osmotic pressure. Baldes investigated this point using Hill's vapour-pressure technique. He confirmed the existence of the large difference in osmotic pressure between the yolk as a whole and the white, but found that the difference was very much smaller when he compared the white with the thin layer of yolk next the membrane. The actual figures in one experiment were as follows:

Average yolk: \( \Delta = 0.564 \).
Yolk adjoining vitelline membrane: \( \Delta = 0.454 \).
White: \( \Delta = 0.424 \).

Baldes concluded that there is only a small, if any, difference of osmotic pressure across the vitelline membrane.

In our experiments the yolks were first rolled on filter paper and samples of the yolk from just beneath the vitelline membrane to a depth of not more than a millimetre extracted by means of a hypodermic needle. Approximately 0.35 c.c. (total) from nine positions all remote from the germinal spot were collected from each yolk. Care was taken to prevent evaporation by conducting the experiments in a saturated atmosphere in a bacteriological plating chamber. The results are shown in Table I which includes also a number of figures for the white yolk and also the three fractions of the white.

<table>
<thead>
<tr>
<th></th>
<th>Surface of yolk</th>
<th>Residue yolk</th>
<th>Thin white</th>
<th>Thick white</th>
<th>White adjoining yolk</th>
<th>White yolk</th>
</tr>
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<tbody>
<tr>
<td>( \Delta )</td>
<td>Water content</td>
<td>Water content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.565</td>
<td>0.568</td>
<td>0.463</td>
<td>--</td>
<td>0.461</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>47.8</td>
<td>87.8</td>
<td>--</td>
<td>86.9</td>
<td>50.7</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>Water content</td>
<td>Water content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.576</td>
<td>0.575</td>
<td>0.461</td>
<td>87.5</td>
<td></td>
<td>0.601</td>
</tr>
<tr>
<td></td>
<td>47.6</td>
<td>47.35</td>
<td>87.5</td>
<td>50.1</td>
<td></td>
<td>50.1</td>
</tr>
</tbody>
</table>

In two preliminary experiments where presumably the precautions taken to prevent evaporation were not so rigid, the values of \( \Delta \) for the surface yolk were 0.606 and 0.596, and the corresponding values for the residue yolk 0.592 and 0.589. The experiments in Table I were made during a cold spell in February.

Baldes used "fresh" eggs, and it is likely that the yolk adjoining the membrane does come into approximate equilibrium with the white after a few days, particularly at reasonably high temperatures. An experiment carried out as above on eggs stored at 10° C. for 1 week showed that such a drift does take place.

Eggs initially:
\( \Delta = 0.571 \); water content, yolk = 47.6 per cent.
\( \Delta = 0.453 \); water content, white = 87.45 per cent.

After 1 week at 10° C.:
\( \Delta = 0.534 \); water content surface yolk = 49.2 per cent.
\( \Delta = 0.570 \); water content residue yolk = 48.3 per cent.
\( \Delta = 0.440 \); water content whole white = 87.55 per cent.
The inference to be drawn from these experiments is that in the freshly laid egg (1) the gradient of osmotic pressure across the membrane is extremely steep, (2) any gradient within the yolk is slight.

SUMMARY.

An attempt has been made to differentiate between the structural components in the vitelline and shell membranes and also in the thick white of the hen's egg by histological methods. The membranes are made up layers containing two pre-dominating substances, mucin and keratin, in varying amounts.

The osmotic pressure difference between the yolk and white of an egg has been examined by the freezing-point method. The gradient of osmotic pressure across the vitelline membrane of eggs (examined within 24 hours of laying) is very steep; only a discontinuity could be detected by this method.

REFERENCES.


EXPLANATION OF PLATE.

Fig. 1. Three layers in vitelline membrane (×60).
Fig. 2. Thick white showing fibrillae and crystals (×55).
Fig. 3. Three layers in outer shell membrane (×65).
Fig. 4. Layer in shell membrane remote from shell (×270).
MORAN AND HALE—PHYSICS OF THE HEN'S EGG (pp. 35—40).