The waterproofing process in eggs of *Rhodnius prolixus* Stähl

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The seven layers of the chorion covering the egg of the bug, *Rhodnius prolixus*, are all freely permeable to water. The egg has no active physiological mechanism preventing desiccation, and is waterproofed by a layer of wax, less than 0.5 μ thick, which covers the inside of the chorion. The wax is similar to that which waterproofs the cuticles of most adult forms of insect, and shows a typical transition point in its water loss/temperature curve at 42.5°C. The waterproofing wax layer is secreted by the maturing oocyte, and is securely attached to the innermost layer of the chorion. The secretion of the wax takes place in the ovary, either just before or after the egg is released from its follicle, but a wax layer can be obtained by incubation of eggs with incomplete chorions. The layer of wax is complete across the inner openings of the micropylar tubes; it is supported at these points on the vitelline membrane before fertilization, and on the fertilization membrane after this has been formed.

**Introduction**

In recent studies on the egg of the bug, *Rhodnius prolixus*, Beament (1946b, 1946c) has shown that the chorion consists of seven layers of proteinaceous material. Each of these layers showed impermeability to specific substances, but no part of the chorion was at all impermeable to water. Beament (1946a, 1946b) has indicated that other material may be deposited on the inside of the egg-shell, and defined the term ‘chorion’ as ‘that part of the extra-ovocytic material which is secreted by the follicle cells’. Since the egg, when laid, shows considerable resistance to desiccation, it is apparent that a waterproofing mechanism exists, and that this is distinct from the chorion.

Previous papers on the physiological characteristics of insect eggs, with special reference to the relation of water loss to temperature and humidity (Johnson 1934, 1940; Clarke 1935; etc.), have produced no evidence of the nature of waterproofing. Evans (1934) indicated that the ‘shell’ of the egg of *Lucilia sericata* was responsible for most of the waterproofing of the egg, but that some impermeability was retained when the shell was removed, providing that the vitelline membrane was left intact.

However, the observation of Ongaro (1933) is important. Ongaro found that a waxy material, containing approximately 80% paraffins and making up 5.71% of the shell weight, could be removed from the vacated shells of *Bombyx mori* by extracting them with a lipoid solvent. Since Beament (1946b) has shown that the chorion layers do not contain free lipoid material, it is possible that the waterproofing mechanism of insect egg-shells may be similar to that of most insect cuticles, i.e. a very thin layer of wax (Wigglesworth 1945; Beament 1945).

It will be the object of this paper to investigate the origin and nature of waterproofing in the *Rhodnius* egg.

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Methods

The rate of water loss of eggs was obtained from their weight before and after desiccation. Eggs were placed on watch-glasses in desiccators with phosphorus pentoxide as the drying agent, and all experiments were carried out with the specimens in the same relative position to the desiccating surface. Weighings were performed in as standardized a manner as possible, after known intervals of time at a standard temperature. Single eggs were weighed on a torsion balance (10 mg./0·02 mg.) and groups of five or more eggs on a chemical balance (200 g./0·1 mg.). In order to check the amount of water adsorbed on to the surface of the egg during weighing, and the disturbance of the atmosphere of the desiccator by frequent removal of specimens, the apparent rate of loss after intervals of 1, 2, 4, 12 and 24 hr. was obtained, using ‘waterproofed’ eggs, and extrapolated, thus giving the correction necessary for short intervals of desiccation. The rate of water loss is expressed as milligrams of water passing through 1 sq.cm. of the shell area per hour (mg./sq.cm./hr.), and unless stated specifically, the experimental temperature was 25°C. This convention was adopted in order that direct comparisons might be made with similar experiments on the passage of water through insect cuticle which were carried out by Wigglesworth (1945) and Beament (1945). The surface area of the egg was calculated from camera lucida drawings of flattened pieces of shell; no account of the irregular nature of the shell surface has been taken, since the primary water barrier is shown below to be on the smooth inside surface of the shell. Where eggs were freely permeable to water, the figure given expresses the average rate of water loss over the first 15 min. only, since the water content diminishes rapidly.

The formation of the chorion

The paired ovaries of Rhodnius are telotrophic, and when each oocyte has accumulated its full content of yolk, it is completely surrounded by a single layer of binucleate follicle cells (Beament 1946b, 1946c). The follicle cells then secrete the chorion layers, consisting of an inner endochorion, and an outer exochorion. The endochorion is composed of five layers:

A discontinuous inner polyphenol layer, made up of islands of tanned protein;
A resistant protein layer, readily penetrated by most water-soluble stains;
A discontinuous outer polyphenol layer;
An amber layer, impermeable to large water-soluble molecules;
A thick soft protein layer.

The exochorion consists of an inner soft layer of lipoprotein (‘chorionin’) and an outer resistant layer of a similar material.

All these layers are continuous around the egg, except at the rim of the shell (Beament 1946c), where the pseudomicropylar tubes penetrate from the resistant exochorion layer to the resistant protein layer of the endochorion and the true micropyles form complete open paths through the shell (figure 1).
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With the completion of these layers, the egg is released from its follicle and is stored in the lower region of the ovary. The two ovaries may contain up to twenty completed and unfertilized eggs. These are usually laid together, each spending only a few moments in the lower genital region during which time they are fertilized.

Figure 1. Longitudinal section through the rims of the shell and cap, passing through a true micropyle and showing the inner opening of the micropyle (i.o.m.) closed by the wax layer resting on the vitelline membrane. cav.m. cavity of the micropyle; i.pl.l. inner polyphenol layer; i.pr.m. inner protein lining of micropyle; i.rc.sl. inner recess of the seal; o.li.m. outer lipoprotein lining of the micropyle; o.rc.sl. outer recess of the seal; r.end. resistant endochorion protein layer; s.exo.cp. soft exochorion layer of the cap; s.exo.sh. soft exochorion layer of the rim of the shell; t.amb. termination of the amber layer; t.s.end. termination of the soft endochorion protein layer; vit.mem. vitelline membrane; wx. the waterproofing wax layer. Amber material is shown in black.

Physical and physiological characteristics of eggs

Groups of ten newly waterproofed eggs from the lateral oviducts were desiccated at short intervals of time over phosphorus pentoxide. The desiccator was kept in a thermostatically controlled oven at various temperatures. The change in rate of water loss with increasing temperature is shown in figure 2, where it can be seen that the rate of loss increases very slowly with the initial rise of temperature, but that at a temperature of 42.5°C the permeability of the egg changes abruptly and
Further increases in temperature raise the rate of water loss enormously. (It is obvious from the amounts of water lost per hour at higher temperatures that fresh batches of eggs must be used for each new temperature reading. In such experiments the material was kept in desiccators at 25° C for about 4 hr. before use, to ensure that water held in the shell surface, after removal from the ovary, had evaporated.) The shape of the permeability/temperature curve is in every way similar to those obtained by Wigglesworth (1945) in comparable experiments on insects in all stages other than the egg, and by Beament (1945) for the permeability of various membranes covered with waxes extracted from insect cuticles. The waterproofing mechanism of the Rhodnius egg may, therefore, take the form of a wax layer with similar physical and chemical characteristics to those which waterproof most insect cuticles; this wax would have a ‘transition point’ at 42·5° C.

![Graph showing the relationship of water loss to temperature for freshly water-proofed eggs in a dry atmosphere.](http://rspb.royalsocietypublishing.org/)

Further details of the relationship between water permeation through the egg and temperature are identical with those for insect cuticle. Newly waterproofed eggs, kept for comparatively long intervals of time at temperatures below the transition point (42·5° C), have normal rates of water loss when transferred to a dry atmosphere at 25° C. The higher the temperature to which eggs are exposed above 42·5° C the greater the rate of loss at 25° C, though this rate is always much lower than the rate of loss above the transition point. Finally, the longer the time interval of exposure at a higher temperature, the greater the water loss at 25° C. For example, eggs exposed to 55° C (by immersion in distilled water) for 2 min. have normal desiccation rates at 25° C, whereas those exposed for 1 hr. are completely dried by subsequent desiccation at 25° C for 24 hr. These results are directly comparable with similar measurements on the permeability of Rhodnius nymphs and adults which were made by Wigglesworth (1945).
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Eggs within the ovary

Eggs of various stages were removed from the ovaries; their rates of water loss in a dry atmosphere at 25°C were obtained. Eggs with completed chorions which had been released from their follicles were of two distinct kinds. Approximately 80% of these eggs were 'waterproof' (average rate of loss 0.09 mg./sq.cm./hr.); the remainder were very permeable to water, showing an average rate of water loss of 1.9 mg./sq.cm./hr. over the first 15 min. of desiccation.

Eggs enclosed in follicles again showed two categories of permeability to water. All eggs which had received only endochorion layers were very permeable to water (initial rate of loss 2.3 mg./sq.cm./hr.). These eggs show little difference to the complete but non-waterproof eggs. The majority of eggs with varying amounts of exochorion were very permeable to water (average rate of loss 2.0 mg./sq.cm./hr.), but about 5% of these had the same order of impermeability as the completed 'waterproof' eggs. Therefore, waterproofing occurs during the deposition of the exochorion, or soon after the egg has been released from its follicle.

Batches of eggs with incomplete shells were carefully removed from their follicles and incubated at 25°C for 3 days in osmotically balanced Ringer's solution. About 90% of these eggs became normally waterproof, even though they had incomplete shells. Thus, waterproofing would seem to be independent of the activity or presence of follicle cells, and to be carried out by the oocyte without co-ordination with the process of chorion formation.

Sterile, or fertile, waterproof eggs, killed by cyanide fumigation, showed no change in water loss for several days; the waterproofing process is not an active physiological device.

Eggs in the calyx, lateral oviducts and ovarioles proper are sterile (Beament 1946d). If removed from these regions and placed in ripe spermatozoa, it is probable that successful fertilization would not take place, as the oocyte is still maturing and cannot be regarded as an ovum. The waterproofing process is not, therefore, a part of the fertilization mechanism; it must be considered as a phase in the maturation of the oocyte. Hence waterproofing must involve changes in the surface region of the oocyte, i.e. the vitelline membrane, the inner surface of the chorion, or the innermost layers of the chorion.

The vitelline membrane

After the egg had been waterproofed, the vitelline membrane appeared to have properties identical with the freely permeable membranes found in the earliest stages of chorion formation (and see Beament 1946b), and it could not play any part in waterproofing the egg.

The primary wax layer on the unspecialized shell

Before waterproofing, the inner surface of the egg-shell is composed of tanned protein (the resistant endochorion protein layer), partially covered by the granules of the inner polyphenol layer. Dried shells from which the yolk has been removed
have a hydrophobic inner surface owing to the tanned nature of this material (Beament 1946b). Water droplets placed on the inside of the shell do not spread at first, but if the shell is immersed in water for some minutes, the inner surface becomes quite hydrophobic, due, presumably, to the adsorption of water molecules. On the other hand, when freshly waterproofed eggs were taken from the ovaries and treated similarly, the inner surface was extremely hydrofuge, both when dry, and after immersion in cold water for 3 days. No material additional to the inner polyphenol layer could be detected when sections of the shell (cut by a freezing microtome) were observed under the highest magnification of the microscope. If waterproofing is carried out by the deposition of material, it must form an extremely thin layer on the inside of the shell, or else take the form of an impregnation of the inner layers of the shell.

Further evidence was given by injecting water-soluble stains into the shell cavity. These experiments were first carried out on the rear regions of the shell to avoid any anomalies caused by the presence of the inner openings of the micropyles. Freshly waterproofed eggs from the ovaries were placed in Ringer's solution and the caps removed. The yolk contents were washed out at once and the vacated shells placed in a desiccator before filling the shell cavity. This procedure was carried out immediately after removing the caps to avoid the possibility of an artificial fertilization membrane being formed on the inner surface of the shell (Beament 1946d). The dried shells were half filled with stain solution, using a fine glass pipette with micrometer control, and returned to a dry atmosphere. After the stain had dried, the shells were cut longitudinally into halves and immersed in xylene (in which all the stains used were insoluble). It was found that the deposit of stain crumbled off the inside of the shell (see later). The halves were mounted in medicinal paraffin in cavity slides, so that the cut edge of the shell could be observed under the highest power of the microscope.

When freshly waterproofed egg-shells were examined after injection with watersoluble stains in aqueous solutions, there was no colouration whatsoever in the endochorion layers. Solutions used in these experiments included ammoniacal silver nitrate, basic and acid fuchsins, borax carmine, neutral red and iodine, all of which stain the resistant endochorion protein layer when injected into the rear ends of non-waterproofed egg-shells (Beament 1946b). Very great care was exercised during injection, for it was discovered that the slightest abrasion of the inside of the shell by the fine jet of the pipette would allow stain to pass through to the endochorion at that point (although microscopical examination might not reveal any visible damage to the inner polyphenol layer). Where such abrasion took place, the staining properties of the resistant endochorion layers, including the inner polyphenol layer, were identical with those of eggs freely permeable to water. It is, therefore, most unlikely that the waterproofing process involves any impregnation of the endochorion layers.

The effect of abrasion on the inside surface of the shell was identical with the effect of similar abrasion on insect cuticle (Wigglesworth 1944, 1945). Inert dusts
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(such as fine-grade alumina) rubbed gently over the inside of the shell rendered the inner surface permeable to all the above staining materials; however, dusts placed on the inside of the shell, and left stationary for 24 hr., had no effect on permeability. The material which waterproofs the shell is, therefore, readily abraded, but not adsorbed, by fine dusts (see Wigglesworth 1945; Beament 1945; the cuticular waxes are not affected by static dusts, but are readily disrupted by abrasion).

The yolk contents were removed from freshly waterproofed shells which were then immersed for 1 hr. in cold lipoid solvents such as chloroform, benzene, carbon tetrachloride, etc., making sure that the cavity of each shell was filled with solvent. The shells were then dried and filled with stains as before. After such treatment, the amount of staining in the resistant endochorion was very small; the colour produced, and the depth of penetration, did not compare with that of similarly treated non-waterproofed egg-shells. Lipoid extraction at room temperature does not, therefore, remove all the waterproofing material. On the other hand, extraction of the newly waterproofed shell in boiling chloroform, etc., for 6 hr., restored the shell to its non-waterproofed state, and the penetration of stains was identical to their effect on such shells. This extraction does not alter any of the staining properties of the resistant endochorion layers; the waterproofing material must, therefore, lie entirely on the inner surface.

Similarly, waterproofed shells were injected with stains in an aqueous solution containing 5% of a wax emulsifier known to remove waxes from insect cuticles in a very efficient manner (I.C.I. emulsifier C. 09993; Wigglesworth 1945; Beament 1945). The stain penetrated the endochorion as though the shell had not been waterproofed.

During the development of the embyro, membranes are deposited on the inside of the chorion by the developing egg (Beament 1946d). These are left behind when the larva vacates the shell, and form a fragile lining which is completely adherent to the inner surface of the shell. When such vacated shells were cut longitudinally into halves, and immersed in cold chloroform for a few moments, these membranes separated as an almost transparent sheet. This is due, presumably, to the solution of the waterproofing material which appears to act as a cement between the inner polyphenol layer and the membranes. Such a theory is supported by the action of other solvents. Carbon tetrachloride, benzene, ethyl chloride, and xylene acted in a similar manner; absolute alcohol removed the membranes rather more slowly, while acetone required about 5 min. to effect a separation. On the other hand, while 90% alcohol removed the membranes in about 2 hr., 70% alcohol loosened them sufficiently to scrape them from the inside only after soaking a shell fragment in the solution for 24 hr. They could not be removed mechanically after several days' immersion in 50% alcohol, or in water, though shells used in these experiments yielded the membranes readily when dried and transferred to cold chloroform. These experiments are explained by the known solubilities of insect cuticular waxes in various solvents (Beament 1945, 1946d).
The thickness of the wax layer

In insects, the thickness of wax on the cuticles so far investigated varies between 0·1 and 0·4 μ (Beament 1945). This figure was obtained by extracting the cast skins of insects and calculating the thickness of wax which would be obtained if the lipoid extract of the exuviae were spread as an even layer over the surface of the insect. However, although it has been shown (Beament 1946b) that no chloroform-soluble material is contained in the chorion, chloroform-soluble material is deposited on the inside of the membranes which are formed during development (Beament 1946d). There is some evidence for supposing that this material forms a second wax layer; hence, the term Primary Wax Layer is proposed for the waterproofing material at present under discussion. The chloroform extract of hatched shells consists, therefore, of the lipoid material of the primary wax layer, contaminated by this secondary waxy material. It was impractical to obtain a sufficient number of freshly waterproofed eggs, and to prepare their shells, completely free of all their contents, for extraction with chloroform. (It was estimated that at least 500 shells would be necessary.)

The total wax from 1000 vacated shells was obtained by repeated extraction of clean shells in boiling chloroform under a reflux condenser. This was weighed, and its volume obtained on a basis of its relative density (Beament 1945). It was then calculated that this wax, spread in a continuous even film over the inside of the shell, would form a layer 0·46 μ thick. This figure, therefore, represents the maximum thickness of the primary wax layer. If the wax is distributed evenly between two wax layers, then each would be approximately 0·23 μ thick, a figure which is similar to the average thickness of waterproofing wax layers on insect cuticles (Beament 1945). If the thickness were very much greater than this second value, then the wax would certainly be visible as a clear layer in sections prepared so that the material does not come into contact with wax solvents, and with stained layers on either side of it.

The extracted wax was a white material; it was spread on lipoid-free butterfly-wing membranes, and the permeability of these membranes at various temperatures was obtained by Beament's (1945) method. This membrane did not show any definite transition point, which supports the suggestion that the extract is a mixture of two waxes.

The closure of the micropyles

Up to this stage it has been established that the primary wax layer lines the whole of the rear portion of the chorion. Similar experiments on the penetration of solvents and stains injected into the front ends of shells showed that wax is also deposited over the inner surface of the neck and cap. It is, however, most important to establish if the wax layer covers the inner openings of the micropylar tubes, which, until waterproofing takes place, give complete access to the surface of the vitelline membrane of the oocyte (Beament 1946c).
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Over all other parts of the shell, the wax has been deposited on to the rigid inside of the endochorion; it remains on the shell and there is no indication of waxy material attached to the vitelline membrane when the latter is removed. But if wax is also deposited over the inner micropylar orifices, it must exist either as an unsupported film (and less than 0.46 μ thick) or by being supported on the vitelline membrane of the oocyte. In this second case it would, presumably, be removed with the vitelline membrane when the shell is prepared for injection.

Freshly waterproofed eggs were taken from the ovaries and the rear ends punctured under Ringer’s solution. The yolk contents were washed out, taking great care to remove all the vitelline membrane without abrading the micropylar region. These shells were dried, and, after filling with staining solutions, were again dried. The rim of each shell (containing the micropylar ring) was dissected off and the penetration of the staining materials along the micropyles and pseudomicroptyles was observed. For this purpose the rims were mounted in media in which the particular stain used was insoluble, and without allowing the rim to come into contact with a stain solvent before mounting. Thus, for example, rims treated with oil-soluble materials were mounted direct into glycerol, while those stained with water-soluble stains were placed in medicinal paraffin.

When such shells were filled with basic fuchsin in water, only the true micropyles contained stain; similar results were obtained with ammoniacal silver nitrate and other water-soluble stains. Thus after the removal of the vitelline membrane, the true micropyles are sufficiently open at the inner surface of the shell to allow large water-soluble molecules to pass outwards. When such shells were injected with water-soluble materials after they had been extracted in chloroform, or when stains were applied in water containing 5% of the wax emulsifier C. 09993, both pseudomicroptyles and true micropyles were stained. The wax layer must, therefore, cover the whole of the inside of the rim, with the possible exception of the inner openings of the true micropyles.

However, as soon as the egg is laid and fertilized, a further layer is deposited by the zygote on the inside of the wax. This layer is a thin rigid fertilization membrane (Beament 1946d). If the wax layer has been supported on the vitelline membrane up to this time, it would now have this fertilization membrane as its support. It was found that if the injection of water-soluble stains was carried out soon after fertilization, no material passed into the true micropyles from the inside of the shell. The fertilization membrane is permeable to such stains, and it can be concluded that the primary waterproofing layer of wax is complete over the inner openings of the micropyles after fertilization.

Further evidence was obtained by immersing whole eggs of various ages in solutions of stains. Waterproof eggs (unfertilized), freshly removed from the ovaries, were immersed for 24 hr. in a solution of basic fuchsin in absolute alcohol. They were then dried rapidly on filter paper and transferred to medicinal paraffin, in which they were burst open, under the binocular microscope. It could be seen at once that the staining solution had penetrated to the yolk which was deep red.
in colour. Sections of the shells of these eggs were examined and micropylar rims were dissected from others and mounted in medicinal paraffin. In every egg the only paths that were stained throughout the thickness of the shell were the true micropyles. Thus, after waterproofing, but before fertilization, material capable of dissolving or disrupting a wax layer may pass down the micropyles and into the yolk.

On the other hand, when similar eggs were immersed in aqueous solutions of basic fuchsin, neutral red, etc., no colouration of the yolk ensued. Further examination showed that the linings of the micropyles in these preparations were not stained. Thus lack of penetration to the yolk must be attributed to the inability of the staining solution to wet the linings of the micropyles, rather than to the impermeability of any layer at the bottoms of such tubes. Eggs were therefore immersed in solutions of water-soluble stains made up in 50% alcohol. It is known (Beament 1946c) that this solution will wet the chorionin and the protein linings of the micropyles, and examination of the shells revealed that the stain had, in fact, penetrated to, and stained the resistant endochorion layer via the bases of the micropyles. It is also indicated above that 50% alcohol will not soften the primary wax layer sufficiently to release the fertilization membrane, so that unless the wax layer is not complete across the inner openings of the micropyles, no stain will pass into the yolk. It was found that none of the above-listed stains, made up in 50% alcohol, would penetrate to the yolk, though the solutions must have been in contact with the bottoms of the micropyles for several hours.

When waterproofing wax is deposited by the maturing oocyte, it is present as a thin film across the inner openings of the micropylar tubes, and is supported by the vitelline membrane. After fertilization, the fertilization membrane becomes the supporting substrate for the wax at these points.

**Discussion**

These experiments leave no doubt that the initial waterproofing mechanism of the *Rhodnius* egg-shell consists of a very thin layer of wax which covers the inside of the chorion. It is, in fact, essentially similar in nature to the mechanism resisting the permeation of water in the cuticle of most insects, including all other life stages of *Rhodnius*. The biological properties of such waxes have been described by Wigglesworth (1945). In the insect cuticle the waxes adhere to the polyphenol layer of the epicuticle (Wigglesworth 1945, 1946); in the egg they are attached to the inner polyphenol layer of the resistant endochorion. Their effect on the permeation of water varies with temperature in a like manner, while the degree of waterproofing conferred by the waxes (at 25°C) is of the same order in both cases (0.08 mg./sq.cm./hr. for *Rhodnius* fifth-stage nymphs; 0.09 mg./sq.cm./hr. for newly waterproofed eggs). Beament (1945) has described the physical and chemical characteristics of cuticular waxes from insects, and the molecular structure which accounts for the extreme water permeability of such thin layers of wax. This is due
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to the organization and close packing of the innermost monolayer of wax molecules (cf. Alexander, Kitchener & Briscoe 1944) which are orientated under the influence of the polyphenol substrate on the insect epicuticle.

The waxes probably consist of mixtures of long-chain aliphatic compounds and are similar in composition to beeswax, which contains paraffins, acids and esters (Chibnall, Latner, Williams & Ayre 1934; Chibnall, Piper, Pollard, Williams & Sahai 1934). At the transition temperature (42.5°C for the initial wax layer of Rhodnius egg-shells) the mobility of the wax molecules becomes so great that the orientation breaks down, and water passes through much more freely (Beament 1945).

The transition temperatures of these wax mixtures are diagnostic of their physical properties, for Wigglesworth (1945) and Beament (1945) have shown that the higher the transition point, the harder is the wax, and the more waterproof and resistant is the insect. A transition point at 42.5°C is characteristic of a wax which is about midway between those of the least and most resistant insects so far investigated, and is very similar to that of the waterproofing wax of Pieris brassicae larvae. The transition point of the cuticular wax of nymphal stages and of adults of Rhodnius is 57.5°C. The wax deposited by the oocyte to waterproof the egg is, therefore, quite different from the cuticular wax of this species; it cannot be looked upon as a form of cuticle laid down in the embryonic stages or suppressed into the developmental period. This means that the term cuticle cannot be applied to it.

If the primary waterproofing layer of wax had not been placed across the inner openings of the micropyles, it is certain that water could pass out through the micropyles and would be absorbed by the protein layer at the inner ends of the tubes. If the egg were in a dry atmosphere, the whole of the endochorion layers would be dry, so that the resistant endochorion protein layer would take up any water and distribute it throughout the whole of the chorion. Thus, the rate of water loss would be increased to a value considerably above that which might be expected if evaporation were to take place only from the bottoms of some fifteen narrow micropylar tubes. The necessity of having the primary wax layer over these minute openings is now apparent.

It must also be pointed out that shortly after the completion of the wax layer within the ovary, the egg is fertilized in the lower genital duct, and the spermatozoa must, therefore, be able to pass through this wax film.

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References

Separation of the ‘blue’ and ‘green’ mechanisms of foveal vision by measurements of increment thresholds

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The curve relating the smallest perceptible intensity of a blue test stimulus with the intensity of an orange conditioning field against which it is viewed shows a characteristic division into low- and high-intensity components, indicating the operation of two mechanisms of cone vision at the fovea. The justification for calling these ‘blue’ and ‘green’ mechanisms is taken from an earlier investigation (Stiles 1939). While most subjects show this division clearly, for some the low-intensity component is masked by the intrusion of rod vision. The correctness of this view is established by measurements made while the eye is recovering from an intense light adaptation. The individual variations of the sensitivities of the ‘green’ and ‘blue’ mechanisms in twenty subjects are assessed. Further evidence is obtained of an anomalously low threshold for the ‘blue’ mechanisms at very high conditioning fields of orange light.

INTRODUCTION

The operation of three receptor mechanisms in rod-free foveal vision can be demonstrated, and some of their properties, e.g. their spectral sensitivity curves, can be determined to a first approximation by measurements of the liminal brightness increment (l.b.i.) under suitable conditions (Stiles 1939).* This conclusion rests mainly on measurements for one eye. A key feature of the results was the form of the curve relating the l.b.i. to the intensity of the conditioning stimulation for a test stimulus of short wave-length (below about 510 mμ) and a conditioning stimulation of long wave-length (above about 530 mμ). All such curves were found to show a ‘change of law’ enabling them to be represented as the resultant of two component curves, associated respectively with the ‘green’ and ‘blue’ cone

* This paper is referred to as I throughout.