An Egg-waxing Organ in Ticks

BY

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With one Plate

CONTENTS

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>291</td>
</tr>
<tr>
<td>OVIPOSITION MOVEMENTS</td>
<td>293</td>
</tr>
<tr>
<td>VISUAL DEMONSTRATION OF THE SECRETION OF WAX BY GÉNÉ’S ORGAN</td>
<td>295</td>
</tr>
<tr>
<td>THE FATE OF EGGS LAID OUT OF CONTACT WITH GÉNÉ’S ORGAN</td>
<td>296</td>
</tr>
<tr>
<td>DEMONSTRATION OF THE WATERPROOFING LAYER ON THE EGG</td>
<td>297</td>
</tr>
<tr>
<td>THE EFFECT OF TEMPERATURE ON WATER LOSS FROM THE EGG</td>
<td>300</td>
</tr>
<tr>
<td>THE MORPHOLOGY OF GÉNÉ’S ORGAN IN ORNITHODORUS MOUBATA</td>
<td>303</td>
</tr>
<tr>
<td>THE SITE OF WAX SECRETION ON GÉNÉ’S ORGAN</td>
<td>305</td>
</tr>
<tr>
<td>PROPERTIES OF THE ORNITHODORUS WAXES</td>
<td>308</td>
</tr>
<tr>
<td>Properties of the Natural Waxes</td>
<td>308</td>
</tr>
<tr>
<td>Properties of the Extracted Lipoids</td>
<td>309</td>
</tr>
<tr>
<td>The Spreading Properties of the Egg Wax</td>
<td>310</td>
</tr>
<tr>
<td>The Amount of Wax present on the Egg</td>
<td>313</td>
</tr>
<tr>
<td>THE NATURE OF THE CONTENTS OF GÉNÉ’S ORGAN</td>
<td>313</td>
</tr>
<tr>
<td>THE MORPHOLOGY OF THE FEMALE GENITAL SYSTEM IN TICKS</td>
<td>316</td>
</tr>
<tr>
<td>The Genital Tract and Ovary in O. moubata</td>
<td>316</td>
</tr>
<tr>
<td>The Genital Tract in Ixodes ricinus</td>
<td>319</td>
</tr>
<tr>
<td>THE STRUCTURE AND CHEMISTRY OF THE EGG-SHELL IN O. MOUBATA</td>
<td>322</td>
</tr>
<tr>
<td>The Granular Layer</td>
<td>323</td>
</tr>
<tr>
<td>The Inner Membrane</td>
<td>323</td>
</tr>
<tr>
<td>PERMEABILITY OF THE EGG-SHELL</td>
<td>323</td>
</tr>
<tr>
<td>Permeability of the Shell Layer</td>
<td>325</td>
</tr>
<tr>
<td>Permeability of the Inner Membrane</td>
<td>326</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>327</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>330</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>332</td>
</tr>
</tbody>
</table>

INTRODUCTION

Most general accounts of the natural history of ticks include a brief description of the remarkable oviposition movements encountered in this group. As is well known, each egg, on leaving the vagina of the egg-laying female, is received for a few moments by a glandular organ—the organ of Géné—which is everted shortly before oviposition. The close connexion of this structure with oviposition is emphasized by the fact that it is absent

both from the male and from the immature stages. Its presence, however, is common to both families of ticks: in the Ixodidae it is everted from the space between the basis capituli and the scutum; in the Argasidae it appears from the camerostomal depression. No other groups of Acarina are known to possess the organ.

Nevertheless, despite the fact that this interesting structure was first described just one century ago, our knowledge of its morphology, and more particularly of its function, remains meagre. In one of the earliest accounts of oviposition in ticks, Géné (1848) had noted that when the organ was pricked with a needle the eggs deposited near the vagina soon shrivelled. But he believed that the organ itself served as a receptaculum seminis. In a later experiment by Bertkau (1881), eggs were prevented from coming into contact with Géné's organ by touching the latter with a glass rod and so causing it to retract at the critical moment. Such eggs, he found, subsequently shrivelled much more rapidly than eggs laid in the normal manner. These descriptions both refer to unidentified ticks, possibly *Ixodes ricinus*.

More recent work has been entirely descriptive. Lounsbury (1900) described how in *Amblyomma hebraeum* the organ 'gradually unfolds its glistening arms . . . grasps the egg and apparently envelopes it in slime', and Wheeler (1906) in a brief account of oviposition in *I. ricinus* also referred to the 'glutinous surface' of the organ. Nuttall (1908) mentioned that in *Haemophysalis punctata* the two vesicles of Géné's organ contain a hyaline secretion but did not suggest a function. The internal glandular part of the organ has been variously referred to by other authors as the 'ovipositing gland', the 'cephalic gland' (Christophers, 1906), or as the 'subscutal gland' (Samson, 1909).

These descriptions suggested to us that the function of Géné's organ is to provide the eggs with a waterproof covering—a function proposed indeed by Bertkau (loc. cit.) but never confirmed. Our studies, which are described in this paper, have shown that a waterproofing agent is undoubtedly transferred from Géné's organ to the egg and that the agent in question is a wax. Now, although it is known that the integuments of many insects (Wigglesworth, 1945; Beament, 1945) and ticks (Lees, 1947) owe their waterproof properties to a thin superficial layer of wax which is secreted through the pore canals, the mode of transport of the wax through the cuticle remains obscure. The occurrence of a waterproofing organ, from which small quantities of material can be isolated, therefore provides a very favourable opportunity for examining the wax precursors. At the same time, however, a more detailed investigation of the structure of Géné's organ has been needed in order to determine the precise site of secretion of the waterproofing agent.

Other questions have also been raised. The unusual nature of the waterproofing process called for a more extended examination of the properties of the wax in relation to those of the substrate, namely, the egg-shell. And this in turn has led us to follow the development of the egg-membranes and to examine some of their chemical and other properties.
Most of our observations have been made on two species of ticks, *Ixodes ricinus* L. and *Ornithodoros moubata* Murray, which were selected as representative of the Ixodidae and Argasidae respectively. All ticks lay their eggs in large clusters. An engorged female *Ixodes* deposits a single cluster of some 2,000 eggs before she is spent. *O. moubata* lays, between successive blood meals, as many as 6 egg batches, each containing about 100 eggs. The small egg of *Ixodes* is elliptical in shape, the much larger egg of *Ornithodoros* ovoid or sub-spherical.

**Oviposition Movements**

Oviposition has been observed most closely in *I. ricinus*. Engorged female ticks were laid, ventral side uppermost, on the floor of a moist chamber and were secured in this position by means of plasticine bands; they were then left in darkness under a binocular microscope until egg-laying began. Mechanical disturbance or the access of light causes the suspension of oviposition, but usually a few eggs are laid in the light before the rhythm is interrupted. The sequence of events is illustrated in Text-fig. 1.

In the normal position of rest between the delivery of successive eggs, Géné's organ is not visible, the capitulum is directed anteriorly, and the vagina is slit-like (Text-fig. 1A). The delivery of an egg is heralded by the downward movement of the capitulum, the tip of which describes a vertical arc and finally comes to lie closely applied to the ventral body-wall just in front of the vagina (Text-fig. 1B). Géné's organ is then everted from between the dorsal posterior borders of the capitulum and the anterior margin of the scutum. Owing to the movement of the capitulum it protrudes ventrally and comes to lie over the hypostome and palps. The organ itself, when fully extended, is seen to consist of a balloon-like base which is produced on each side into two short horns which contain a translucent fluid (Text-fig. 1C). The whole organ is invested with an exceedingly delicate glistening cuticle, capable of a high degree of folding.

When oviposition is about to take place the hypostome is tucked firmly against the ventral body-wall, the palps are splayed out, and Géné's organ is inflated to its maximal extent. At the same time the inner lining of the vagina begins to unroll like the finger of a glove (Text-fig. 1D). The prolapsed vagina or 'ovipositor' forms a tube which, when fully extended, nearly touches Géné's organ and is a most effective instrument for delivering the egg. After depositing the egg between the horns, the 'ovipositor' is quickly retracted. Géné's organ with the egg attached is then partially deflated and inflated several times and the palps are often worked sideways in an active manner. Finally, the organ is retracted completely within the body and the egg is left adhering to the dorsal surface of the hypostome (Text-fig. 1E, F). The cycle is completed when the hypostome swings into the dorsal position again, carrying the egg with it (Text-fig. 1G). Because of these events the eggs tend to accumulate on the dorsum of the tick and not on the ventral surface, and as
the egg-laying female rarely moves after the onset of oviposition the eggs are deposited in a dense cluster.

The rate of egg production under favourable conditions is not easy to determine, as after any disturbance laying is not resumed for some hours. One female tick laid as many as 246 eggs during 48 hours, that is, at the rate of 1 egg every 12 minutes. According to Wheler (1906), however, the sheep tick may sometimes lay eggs at the rate of 1 every 3 minutes.
The movement of the palps and of Géné’s organ itself may sometimes have the effect of rotating the egg and so promoting increased contact with the shell. However, careful observation has convinced us that as a general rule only a small area of the shell—certainly less than half the total surface—establishes contact with Géné’s organ. At this time the egg usually lies between the horns which may embrace it as the organ is retracted. Nevertheless contact with the horns is not essential, for eggs which chance to be deposited on the dorsal aspect of the organ, where the horns cannot reach them, undergo normal development subsequently. Once oviposition is under way the cuticle of Géné’s organ soon acquires a greasy glistening appearance and the film of grease gradually extends to the legs and the cuticle near the vagina. However, spreading of the grease to the egg-shell cannot be observed under the microscope.

The oviposition movements in *Ornithodoros moubata* have not been followed in detail as this tick is even more sensitive to disturbance, but the appearance of females which have been interrupted at different stages of egg-laying suggests that the process is the same in all essentials. Co-ordinated movements involving the eversion of Géné’s organ, the prolapse of the vaginal lining, and the rotation of the hypostome probably take place as in *Ixodes*. Since the hypostome is shorter, however, and the eggs less sticky, the latter are not carried dorsalwards but instead accumulate in a heap beneath the anterior region.

**Visual Demonstration of the Secretion of Wax by Géné’s Organ**

The role of Géné’s organ in secreting a wax is most simply demonstrated in *O. moubata*. In this species the organ can be caused to evert if the body of the tick is compressed between two microscope slides so that only the proboscis and camerostome project freely (Pl. I, fig. 1). After the slides have been clipped together with Cornets forceps the organ can be held everted for further detailed examination. A full description will be deferred until a later section.

In the normal egg-laying female the surface of Géné’s organ is always devoid of large accumulations of wax. If, however, engorged ticks are kept for 3 or 4 weeks at 15° C.—a temperature just too low for oviposition—large amorphous deposits of wax appear on the surface of the cuticle, usually around the base of the horns. These can easily be collected on a needle after evertting the organ by pressure. Wax accretions on three organs are shown in Text-fig. 2; not all individuals secrete wax in such quantity.

That Géné’s organ in *Ixodes* is also concerned in secreting a wax can be shown by evertting the organ and touching it against a clean glass slide. The demonstration is less striking than in *Ornithodorus*, however, for in this species the wax fails to accumulate in any quantity owing to its greater mobility (see p. 302).
The Fate of Eggs Laid out of Contact with Géné's Organ

The function of Géné's organ in waterproofing the eggs has been confirmed by occluding the opening through which the organ is everted and following the subsequent fate of the eggs when exposed to different humidities. In Ornithodorus, eversion was prevented by covering the camerostomal fold and proboscis with cellulose paint. This somewhat delays the onset of oviposition but afterwards egg-laying continues at the normal rate. The procedure adopted was to allow the same ticks, isolated singly in specimen tubes, to lay successive small batches of eggs with the opening free, then occluded, then finally free again. The laying ticks were exposed to a humidity of 70 per cent. R.H. in an incubator at 25 °C. where the eggs remained undisturbed for 14 days. At this temperature all normal eggs hatch within 12 days.

Some of the results obtained are given in Table 1. As a general rule (e.g. ticks, nos. 1–3) very nearly all the eggs laid with Géné's organ free hatch normally, while those laid without the intervention of the organ are all completely shrivelled and hard. Sometimes, however, a tick which at first lays normal eggs continues to lay shrivelling eggs after Géné's organ has been freed (e.g. no. 4). This may be ascribed to the disturbing influence of covering and uncovering the camerostome which probably upsets the synchronous eversion of the organ. The eggs failing to touch Géné's organ will therefore remain unwaterproofed. A few shrivelling eggs are sometimes laid among or preceding an otherwise normal batch (e.g. no. 1). No doubt this is also caused by an occasional faulty eversion of Géné's organ. The same phenomenon is seen when egg-laying ticks are kept in crowded cultures where mutual disturbance is often sufficient to lead to the production of large numbers of unwaterproofed eggs.

Unless contact with Géné's organ influences the viability of the egg in other ways, a proportion of the eggs deprived of contact would be expected to hatch if the atmosphere were kept sufficiently moist. A further group of egg-laying females with Géné's organ obstructed was therefore set aside in saturated air and was examined only when hatching should have been complete. Six females laid a total of 386 eggs of which 23 hatched, the remainder, even in
the damp atmosphere, gradually collapsing and darkening. One hundred and eighty-four eggs, which were laid by 3 ticks, yielded no nymphs. One female, on the other hand, laid 28 eggs of which as many as 14 hatched. Normal egg-masses kept in a damp atmosphere remain free from moulds almost indefinitely. It was noteworthy that eggs laid without the intervention of Géné's organ rapidly acquired a thick felt of hyphae growing on the surface of the egg-shell. This is a further result of the absence of wax from the shell.

Parallel observations have been made on *Ixodes ricinus*, with generally similar results (Table 1). Egg-laying females were secured on their backs with the hypostome firmly embedded in plasticine. As this eliminates the hypostomal movements, as well as preventing the eversion of Géné's organ, the eggs collect in a pile round the opening of the vagina. Normal egg batches of this species are far more susceptible to desiccation than are those of *Ornithodorus* (p. 301) and for complete hatching must be exposed continuously to saturated air. The results of observations on the hatching of small egg-clusters, laid consecutively, with or without the aid of Géné's organ, are summarized in Table 1. Again, as the first column shows, there is always a small number of shrivelling eggs in every normal egg-cluster, probably resulting from the failure of the organ to evert properly. With the organ blocked the great majority of the eggs, even in the saturated atmosphere, gradually collapse and darken and very few hatch. After removal of the block greater numbers of eggs hatch, although in many individuals an abnormally high proportion of the eggs prove to be non-viable. As in *Ornithodorus*, therefore, the interference with Géné's organ appears to hinder its proper functioning, even when the obstruction has been completely removed.

**DEMONSTRATION OF THE WATERPROOFING LAYER ON THE EGG**

If, as has been suggested, a waxy covering is applied to the outside of the egg by Géné's organ, wax solvents and detergents should be capable of

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**Table 1. The hatching of successive egg-batches laid with or without the intervention of Géné's organ**

<table>
<thead>
<tr>
<th>Species</th>
<th>Géné's organ uncovered</th>
<th>Géné's organ blocked</th>
<th>Géné's organ again uncovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of eggs</td>
<td>No. of eggs</td>
<td>No. of eggs</td>
</tr>
<tr>
<td></td>
<td>Laid</td>
<td>Hatched</td>
<td>Shrivelled</td>
</tr>
<tr>
<td><em>Ornithodorus moubata</em></td>
<td>1</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td><em>Ixodes ricinus</em></td>
<td>5</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>859</td>
<td>822</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>218</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>171</td>
<td>151</td>
</tr>
</tbody>
</table>
attacking this superficial layer and therefore of exerting a pronounced effect on transpiration through the shell. As Table 2 shows, this expectation is fulfilled.

**TABLE 2.** The effect of chloroform and Co9993 on the water loss from small egg-batches (average weight 26 mg.) laid during the previous 24 hours

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Per cent. loss of weight during 1 hr. in dry air at 25° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ornithodorus moubata</em></td>
<td>None</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Extracted in chloroform at 15° C. for 1 minute</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>Smear with Co9993</td>
<td>24.0</td>
</tr>
<tr>
<td><em>Ixodes ricinus</em></td>
<td>None</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Extracted in chloroform at 15° C. for 1 minute</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>Smear with Co9993</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Water loss from newly laid egg-masses is greatly increased if they are extracted with cold chloroform or smeared with the wax emulsifier Co9993 (see Wigglesworth, 1945). The effect of chloroform is particularly dramatic on the relatively small eggs of *Ixodes*. Of the normal waterproofed eggs about 50 per cent. shrivel during 24 hours in dry air at 25° C. After washing in chloroform for 30 seconds all the eggs shrivel and dry out completely within 5 minutes. As a result of the removal of the wax, the eggs no longer stick together and tend to roll about freely.

That Géné’s organ is concerned with the deposition of the wax layer can be shown by extracting, with chloroform, egg batches which have been laid with or without the intervention of the organ; the relative effect of this treatment on water loss can then be compared. Results with *Ornithodorus moubata* and *Ixodes ricinus* are set out in Table 3. The number of eggs that are shrivelled after a given interval of time can be used as a convenient index of water loss. As it has been found that the permeability of *Ornithodorus* eggs, when devoid of the wax layer, may differ conspicuously from one batch to another, comparisons have always been made using eggs deposited by the same tick.

Normal eggs of *Ornithodorus* never show the slightest shrivelling after 24 hours in dry air at 25° C., while those washed with chloroform are all shrivelled, and some are dry, after only 30 minutes (Table 3). Eggs laid in moist air without the intervention of Géné’s organ also shrivel rapidly in dry air; and the rate of shrivelling is not increased after chloroform treatment. Extraction with chloroform also makes little difference to the rate of water loss from eggs dissected from the uterus. Thus it is clear that in this species, the waterproofing material on the outside of the egg is derived solely from Géné’s organ.

This is not the case with *Ixodes*, however. Egg-masses deposited with
Géné's organ covered are certainly more permeable than those laid with the assistance of the organ. Thus after 4 hours in dry air at 25° C. none of the eggs in a normal batch of 50 were shrivelled and only a few had begun to dimple, whereas of the eggs laid by the same tick without the organ about half were completely shrivelled (Table 3). Nevertheless, the permeability of the eggs laid without Géné's organ is greatly increased by washing in chloroform; shrivelling is then as rapid as it is after extraction of normal eggs. These results indicate that the egg, before establishing contact with Géné's organ, must already have acquired a covering of some chloroform-soluble material capable of reducing the permeability of the shell. Eggs dissected from the oviduct, on the other hand, are highly permeable; and the permeability is not noticeably increased by chloroform (Table 3).

**Table 3. The effect of extracting eggs from various sources with chloroform at 15° C. for 1 minute**

The rate of shrivelling after treatment was observed in dry air at 25° C.
All laid eggs were from batches less than 24 hours old

<table>
<thead>
<tr>
<th>Species</th>
<th>How laid</th>
<th>Treatment</th>
<th>No. of eggs</th>
<th>No. of eggs shrivelled after</th>
<th>5 min.</th>
<th>30 min.</th>
<th>4 hrs.</th>
<th>24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ornithodorus mouhata</em></td>
<td>With Géné's organ</td>
<td>None</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>20</td>
<td>14</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Without Géné's organ</td>
<td>None</td>
<td>20</td>
<td>8</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>20</td>
<td>9</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Eggs from uterus</td>
<td>None</td>
<td>20</td>
<td>16</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>20</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><em>Ixodes ricinus</em></td>
<td>With Géné's organ</td>
<td>None</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Without Géné's organ</td>
<td>None</td>
<td>50</td>
<td>0</td>
<td>5</td>
<td>24</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Eggs from oviduct</td>
<td>None</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

It follows that the egg of *Ixodes* is waterproofed in two stages: the egg receives first an external covering of waterproofing material during its passage down the common oviduct and vagina, and then acquires an additional coating from Géné's organ. Further evidence, which is presented below, suggests that both waterproofing agents are waxes with very similar, if not identical, properties. This two-stage application of lipoid can easily be detected if egg-masses laid by the same tick, with and without the intervention of Géné's organ, are compared under the binocular. It is then obvious that although both are visibly greasy, the former are provided with more liberal quantities of wax. The eggs from the oviduct have, in contrast, a smooth, highly polished appearance with no trace of grease.

The greater permeability of eggs that are prevented from touching Géné's organ is due principally to the fact that the first wax layer is always incomplete.
Since the egg-shell contains reducing substances (p. 323), this can be demonstrated by immersing the eggs in 5 per cent. ammoniacal silver nitrate. The shell and yolk of normal eggs always remain completely unstained by this treatment, whereas eggs that have failed to touch the organ invariably show some staining of the shell but little blackening of the yolk. After washing in chloroform there is widespread staining of the shell and very rapid blackening of the yolk as the stain penetrates into the interior of the egg. Whether the thickness of the wax layer also influences the permeability has not been determined.

The eggs laid by different females with Géné's organ out of action vary greatly in permeability. The following is an example. The eggs deposited by 2 ticks on 3 consecutive days were collected and exposed separately to dry air at 25°C for 4 hours. The egg batches of the first yielded 10, 5, and 2 per cent. of shrivelling eggs, those of the second 35, 44, and 49 per cent. respectively. Individual differences in the coverage of the eggs with wax are probably the cause of this variability.

The presence of the first coating of wax, conferring on the egg a certain degree of impermeability, may account for statements in the literature to the effect that Géné's organ is unnecessary for normal oviposition. Smith (1945) records an example of a specimen of *I. dentatus* which came away from the host with so large a piece of skin still attached to the hypostome that the eversion of Géné's organ was prevented. Yet the eggs deposited by this female appeared normal. With *I. ricinus*, however, we have shown that the first wax covering is insufficient, even in damp air, to prevent the collapse of most of the eggs over the long period required for normal development.

**The Effect of Temperature on Water Loss from the Egg**

Previous work with insects (Ramsay, 1935; Wigglesworth, 1945; Beament, 1945), with ticks (Lees, 1947), and with an insect egg (Beament, 1946b) has shown that the waxes which are responsible for the impermeability of the cuticle or egg-shell undergo transitional changes at a certain 'critical temperature' and permit water to pass more readily. An evaporation curve exhibiting a pronounced break at a certain temperature can therefore provide confirmatory evidence that a wax layer is present. But in addition it has been found that the critical temperature bears a close relation to the other physical properties of the waxes, which in fact show great variability in different species. Among ticks, species from dry environments, such as the Argasidae, have low rates of transpiration and high critical temperatures; whereas the reverse is true of species from damper environments, like the majority of the Ixodidae.

The effect of temperature on water loss has now been investigated in a representative series of tick eggs. Owing to the small size of the egg it is impracticable to estimate water loss in relation to surface area; for the present purpose of determining the approximate critical temperature it has sufficed to plot loss of weight against temperature. The usual experimental procedure adopted was to expose small clusters of eggs taken from a single egg-mass (weighing about 20 mg. and containing up to 200 eggs) to dry air at different
temperatures for periods of 30 minutes. The eggs were contained in a small gauze basket which was suspended over phosphorus pentoxide in a conical flask immersed up to the neck in a water bath. Owing to the rapid desiccation above the critical temperature, fresh lots of eggs from the main egg-cluster were often used to obtain separate points on the curve.

TEXT-FIG. 3. The effect of temperature on the evaporation of water from the eggs of different species of ticks.

The evaporation curves for the eggs of five species are shown in Text-fig. 3. The rate of water loss from egg-masses of comparable weight after exposure to dry air at 25° C. for 24 hours and the approximate critical temperature of the eggs are recorded in Table 4. We also include for comparison the critical temperatures previously obtained for the cuticle of the parent species (Lees, 1947). It should be borne in mind, however, that the values for the cuticle were read off from curves relating transpiration and surface area.

The results bring out several points. First, the different species can be arranged in a graded series according to the resistance of the eggs to desiccation at 25° C. When this is done, the sequence corresponds closely with a similar series based on the order of resistance of the adult tick itself (Lees, 1947). In other words, susceptible species, such as *Ixodes ricinus*, also lay susceptible eggs; more resistant species, like *Ornithodorus*, lay comparatively resistant eggs, and so on. Secondly, the critical temperature is related to the rate of water loss below the critical temperature (e.g. at 25° C.). Thirdly, the
critical temperature of the egg in Ixodidae is nearly identical with that of the cuticle (the difference of 3° C. in the case of *I. ricinus* is of doubtful significance); whereas the critical temperature of the egg in Argasidae is much lower than that of the cuticle. In *O. moubata*, for example, we obtained values of 45° C. and 62° C. for the egg and cuticle respectively. The egg critical temperature is only 1° C. above that of the most resistant Ixodid, *Hyalomma savignyi*.

**Table 4. Water loss from small batches of eggs (weighing about 20 mg.) and their approximate critical temperatures**

The critical temperature of the female ticks are included for comparison.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Per cent. loss of weight in dry air during 24 hrs. at 25° C.</th>
<th>Critical temperature, °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg</td>
<td>Cuticle</td>
</tr>
<tr>
<td>Ixodidae</td>
<td><em>Ixodes ricinus</em></td>
<td>27.2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td><em>I. canisuga</em></td>
<td>15.6</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td><em>Dermacentor andersoni</em></td>
<td>9.0</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td><em>Hyalomma savignyi</em></td>
<td>8.0</td>
<td>44</td>
</tr>
<tr>
<td>Argasidae</td>
<td><em>Ornithodorus moubata</em></td>
<td>7.9</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td><em>Ornithodorus delanoei</em></td>
<td>2.5</td>
<td>46</td>
</tr>
</tbody>
</table>

Besides differing in their transitional temperatures, the egg waxes in this range of species probably exhibit parallel differences in their other physical properties. We have already recorded that in *I. ricinus* the lipoid secreted by Géné’s organ is a labile grease, whereas in *O. moubata* it is a viscous wax. Species with intermediate critical temperatures no doubt possess egg waxes with intermediate physical properties. A difference in ‘stickiness’ can indeed be easily appreciated merely by handling the egg-masses, for the eggs adhere to one another solely by virtue of their waxy covering. The egg-masses of *I. ricinus*, for example, stick together most tenaciously; those of *H. savignyi* appear much less greasy and fall apart quite readily; while the eggs of *O. moubata* and *O. delanoei* show only the slightest mutual coherence.

We have already pointed out that in the *Ornithodorus* egg the entire waterproof covering is supplied by Géné’s organ, whereas the *Ixodes* egg is partly waterproofed before leaving the vagina. Confirmation is provided by the effect of temperature on the water loss from egg-masses of the two species laid with and without the intervention of Géné’s organ. Eggs of *Ornithodorus* laid in a damp atmosphere with the organ covered lose water rapidly at all temperatures when exposed to dry air (Text-fig. 4). *Ixodes* egg-masses, laid without Géné’s organ, although distinctly more permeable than eggs touched by the organ, still show a definite break in the evaporation curve at about 31° C. (This type of curve may be compared with those obtained by Wigglesworth (1945) for soil-inhabiting larvae of *Tipula* and *Hepialus*. Although the cuticular wax layer of these insects is more or less severely scratched by soil particles, a distinct break in the evaporation curves may still be detected.)
Normal eggs laid by the same ticks had a critical temperature of about 34° C. (Text-fig. 4). This is additional evidence that the partial waterproofing effect is also due to the presence of a wax layer. Probably the wax is similar in nature to that secreted by Géné’s organ, although smaller in quantity.

Text-fig. 4 also shows the evaporation curves of *Ornithodorus* eggs 0–1 days after laying and after incubation for 6 days at 25° C. Eggs of both ages have a critical temperature of about 44° C. But the older eggs lose water at a lower rate, particularly at temperatures above 44° C. Some possible explanations of this effect are discussed later (p. 324).

**The Morphology of Géné’s Organ in *Ornithodorus moubata***

The external appearance of Géné’s organ in *Ixodes* has already been described briefly. Because of the greater ease of evertting the organ in
304 Lees and Beament—An Egg-waxing Organ in Ticks

Ornithodorus, we have chosen this species for more detailed study. Much of the internal structure is visible through the transparent cuticle.

The everted organ consists of a broadly sessile stalk surmounted by two large crescentic horns (not four as in Ixodes). When it is fully inflated the stalk is completely occupied by the white tissues of the gland (Text-fig. 5 A). The horns are particularly conspicuous as they are usually filled with a clear refractile liquid—evidently the secretion of the gland—which extends also round the margin of the organ at the base of the horns and occupies the space between the gland and cuticle. The amount of secretion in the horns varies considerably: sometimes there is only a little at the tips and the gland then extends well into the base of each horn.

On retraction the entire organ is pulled through the narrow slit in the camerostome and turned inside out. The gland then comes to lie in a series of pleats inside the body-cavity, while the cuticle of the stalk and horns is contained in a highly folded state within the gland itself (Text-fig. 5B).

Géné’s organ has a powerful system of retractor muscles which penetrate between the cells of the gland and run back through the stalk to insertions in the dorsal body-wall. Most of the distal attachments of these muscles are found on the cuticle between the horns, but each horn is provided also with a single retractor attached near the tip (Text-fig. 5B). These observations resolve the difficulty experienced by Robinson and Davidson (1914) in accounting for retraction. These authors, who studied only the invaginated organ in Argas
persicus, believed that the retractor muscles were attached only to the epidermis or gland (‘hypodermal sac’) and not to the cuticle (‘chitinous sac’).

The mechanics of the process of eversion have not been made out with certainty. Géné’s organ in Ornithodorus is devoid of any intrinsic musculature capable of causing eversion. There is, therefore, a certain resemblance with the blow-fly ptilinum which also lacks intrinsic protractors (Laing, 1935). On the other hand, there are no externally visible signs such as pulsations of the cuticle which would suggest that Géné’s organ, like the ptilinum, is inflated by locally increased blood-pressure. Robinson and Davidson held that eversion is brought about by the rapid secretion of fluid between the ‘hypodermal’ and ‘chitinous’ sacs. But this again is unlikely as the amount of secretion in the horns during normal oviposition appears to be no greater at the moment of eversion than at any other time between the successive delivery of eggs when the organ is retracted. It is more probable that a number of indirect muscles are concerned: the most important is certainly the depressor of the hypostome which is also inserted on the dorsal body-wall just posterior to the point of insertion of the gland retractor. This muscle appears to form a partial septum which, as it shortens and depresses the hypostome, impinges against the invaginated gland. It is noticeable that the organ can be everted with a much smaller application of pressure if the hypostome is held simultaneously in the depressed position.

Nuttall (1908) mentions that the inflated vesicle of Géné’s organ in Haemophysalis is covered with minute punctuations which he took to be pits from which the secretion of the organ escaped. In Ornithodorus papilla-like processes are sometimes present near the base of the horns. Examination of serial sections showed, however, that these corresponded merely to the points of the muscle insertions. There is no specialized channel by which the secretion can escape through the cuticle of the organ. We consider this question in greater detail below.

**The Site of Wax Secretion on Géné’s Organ**

Although, under certain conditions, large external deposits of wax may be formed (p. 295), the points of secretion cannot easily be determined by inspection owing to the spreading propensities of the wax. The internal structure has, therefore, been examined in greater detail with the object of determining the site of transfer through the cuticle.

Sagittal and horizontal sections of whole organs fixed in the everted state show clearly that the gland is actually a specialized region of the epidermis which is continuous with the epidermis underlying the general body cuticle (Pl. I, fig. 2). There is no differentiation into ‘hypodermal sac’ and ‘gland’ such as Robinson and Davidson (1914) describe. At the base of the stalk the epidermal cells are relatively attenuated but at a point just proximal to the base of the horns the epidermis becomes greatly thickened and is folded inwards, so forming the pleats and convolutions of the gland.
The relations of the gland and epidermis to the cuticle are as follows. Over the basal region of the stalk the epidermis is closely applied to the cuticle, but near the point where the unspecialized epidermis passes into the thickened glandular tissue it becomes detached from the cuticle, thereby leaving a conspicuous lumen wherein the secretion of the gland accumulates (Text-fig. 5A; Pl. I, fig. 2). We have already stated that neither gland nor epidermis as a rule extends far into the horns. The walls of the horns were examined carefully in sections stained heavily with iron haematoxylin, but no trace of an inner cytoplasmic lining could be detected. There seems no doubt, therefore, that the secretion of the gland must be regarded as extracellular, accumulating between the cell wall and a part of the cuticle which is non-living.

The histology of the gland epidermis is shown in Pl. I, figs. 3 and 4. The component cells are columnar or wedge-shaped with well-defined cell boundaries. The nuclei lie near the margin of the cell abutting the haemocoele. In egg-laying females the gland cytoplasm is often distended with droplets, some of which appear to be on the point of discharge into the lumen (Pl. I, fig. 3).

The cuticle investing Géné’s organ includes layers of smooth epicuticle and of endocuticle which pass without interruption into the cuticular layers of the general body-wall. The endocuticle is about 20 μ in thickness over the stalk region, but at those points where the secretion is stored it becomes very attenuated, only attaining a thickness of about 5 μ in the horns. Sections, cut at 6 μ and mounted in tap-water, were examined with an oil immersion objective for the presence of pore canals. Their approximate distribution is shown in Text-fig. 6B. Although there are numerous pore canals traversing the endocuticle of the stalk, they are more sparsely distributed near the point at the base of the horns where the epidermis becomes detached from the cuticle and pore canals are entirely absent from the endocuticle of the horns (Pl. I, fig. 7). In such regions, which are devoid of pore canals, it seems unlikely that the gland cells have any cytoplasmic connexions with the cuticle.

Previous work (Lees, 1947) has shown that in Ornithodorus the epicuticle of the general body cuticle is itself made up of successive thin layers of cuticulin, polyphenols, wax, and cement. Whether the pore canals penetrate the cuticulin layer, as in Rhodnius (Wigglesworth, 1947), is not known. If this is the case, their free ends are presumably covered over by the wax layer. The thin cement layer in turn forms an external protective covering over the wax. Now, once the cement has been laid down, no further deposits of wax can be secreted by the pore canals unless both cement and wax are removed (by abrasion, for example). Yet, as we have seen, the production of wax over Géné’s organ takes place freely at intervals throughout the life of the egg-laying female. It is, therefore, important to know whether the cement layer extends on to the organ.

The presence of cement on the general body cuticle can be demonstrated by extracting the whole tick with cold chloroform for 30 minutes and staining
Lees and Beament—An Egg-waxing Organ in Ticks

in 5 per cent. ammoniacal silver nitrate. The cement is insoluble in cold chloroform and protects the underlying wax from solution. Therefore the polyphenols beneath the wax layer cannot reduce the silver reagent where cement is present and these areas remain unstained. Since the epicuticle of Géné’s organ also contains a polyphenol layer (covered in this case by the wax secreted by the organ itself) this method can also be used here for mapping the distribution of cement.

If a tick, with Géné’s organ everted, is immersed in ammoniacal silver without previous extraction, there is no staining of the cuticle, showing that the wax layer is complete (Pl. I, fig. 5). On the other hand, if the tick is previously extracted for 30 minutes in cold chloroform, the cuticle round the base of the horns, and the horns themselves, stain intensely (Pl. I, fig. 6); nevertheless, like the general body cuticle, most of the stalk still fails to stain. This shows that the cement layer extends on to Géné’s organ as far only as the base of the horns (Text-fig. 6A). The line of demarcation between the staining and non-staining areas is very sharp and corresponds with a line drawn round the circumference of the stalk which is well to the proximal side of a similar line marking the point of detachment of the epidermis from the cuticle. This result, which is of particular significance, was checked on sections of organs previously stained with silver (Pl. I, figs. 8, 9). There is a zone of varying width running round the circumference of the stalk between the bases of the horns where pore canals are present and cement absent (Text-fig. 6C). In view of the marked chemical dissimilarity of the precursor from the horns and the wax from the surface of the cuticle (see p. 313), there seems no doubt that the former is first subjected to transformations by the living cells. If such is the case the wax must be secreted through those areas of the cuticle provided with pore canals (which contain, presumably, cytoplasmic processes extending from the living cells) and not through the non-living cuticle of the horns which is devoid of pore canals. According to this

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TEXT-Fig. 6. Géné’s organ in Ornithodoros moubata showing the area covered by the cement layer (A), the distribution of the pore canals (B), and the probable site of wax secretion (C).
view, the area shown diagrammatically in Text-fig. 6c also represents the site of the secretion of wax.

**Properties of the Ornithodorus Waxes**

The waterproofing waxes examined by us have been derived from the following sources. The egg wax was obtained in its natural state from deposits occurring on the outside of Géné’s organ; and small quantities of this wax were also extracted from vacated egg-shells. In addition, we have made some comparisons of the properties of the egg and cuticular waxes. The latter may be obtained in natural form from living ticks which have regenerated large quantities of this material after their cuticles have been thoroughly abraded with alumina dust (Lees, 1947), and the cuticular wax may also be extracted from cast nymphal skins.

**Properties of the Natural Waxes**

The colourless transparent wax removed from Géné’s organ has a somewhat syrupy consistency at 25° C. Small filaments of the wax, when warmed in a melting-point capillary, undergo rather indefinite optical changes between 36° and 46° C. and melt at 50-54° C. (Table 5). In view of the fact that the critical temperature of the egg is about 45° C. (p. 302), it is interesting to recall that Beament (1945) has found that increased evaporation from the cuticle of insects is associated more closely with changes in the optical properties of the wax, which occur below the melting-point, than with the melting-point itself.

The egg wax appears brilliantly luminous when viewed in polarized light and, as with other polycrystalline lipoids, exhibits no position of extinction between crossed nicols. It dissolves instantly in cold or hot chloroform or xylene but is insoluble in alcohol. Lumps of wax stain deeply with sudan black B but not with sudan III. The protein colour tests (xanthoproteic, Sakaguchi, ninhydrin, and Millon reactions) are all negative. The significance of this latter observation is discussed on p. 329.
The wax recovered from the surface of the cuticle after abrasion has entirely different physical properties. At 25°C, it is a hard white crystalline solid which shows no tendency to 'creep' along surfaces. As judged by the optical properties, transitional changes occur at 38° and 53° C. and the wax melts at 65°C. The critical temperature of the cuticle, as we have previously noted, is approximately 62°C. (Table 4).

Properties of the Extracted Lipoids

The waterproofing substances from the cuticle were obtained by extracting the exuvia with chloroform. The cast skins were washed several times in cold water and dried in a desiccator. After repeated extraction in boiling chloroform under a reflux condenser, the solution was filtered through a hot-jacket funnel, evaporated to dryness at room temperature, and the total residue weighed.

The extracted material is not homogeneous, for two lipoids—a white waxy solid and a soft yellow grease—separate partially in the evaporating dish. Complete separation can be effected by treating the residue with acetone, in which only the grease is soluble. The white wax and the yellow grease are present in the total chloroform extract in the ratio of about 6:1 by weight.

The white crystalline wax has the following properties. Although the purified substance only melts to a clear liquid at 107°C, it undergoes optical changes, such as clearing, darkening, and loss of crystalline form, at lower temperatures (Table 5). These changes may be associated with crystalline transformations. The wax is soluble in boiling chloroform or benzene, and also in hot pyridine, but is sparingly soluble in the cold. It is insoluble in water, hot or cold alcohol, and hot or cold acetone. There are some indications that the material is capable of forming an oriented layer. Thus, if a crystal of the wax is dropped on to the surface of boiling water it forms a fine film which may be obtained by dipping a glass-slide through the surface; and this film has similar properties to the crystalline material save that it is now wetted by 50 per cent. alcohol. The white wax is not stained by either of the sudan stains, or by Ciba's B.Z.L., nor is it darkened by osmium tetroxide.

The second component, the yellow grease, is readily soluble in cold lipophilic liquids, including acetone and absolute alcohol; it stains with sudan III and with sudan black B and darkens with osmium tetroxide. The optical changes with temperature are indefinite.

Hot chloroform extracts of egg-shells (separated first from the larval exuvia) also yielded two fractions comprising white wax and yellow grease in approximately the same proportions as in the cuticle extracts. Their properties also appear to be very similar (for temperature changes see Table 5).

It would appear that the white wax may be a mixture of very long chain paraffins, acids and esters, while the grease may be composed of much shorter molecules, with unsaturated bonds along the chain.1 In the natural waxes—the regenerated material from the cuticle or the wax from Gené's organ—the

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1 Private communication from Prof. A. C. Chibnall, F.R.S.
two lipoids seem to be present in some stable form of association, although this may not necessarily be of a strong chemical nature. This state does not appear to be automatically reconstituted when the two substances have been extracted with lipoid solvents. Nevertheless it is noteworthy that when the two components derived from the cuticular extracts are intimately mixed, the resulting melting-point of the mixture is considerably lower than that of the purified white wax and approaches the melting-point of the natural regenerated cuticular wax (Table 5).

**The Spreading Properties of the Egg Wax**

*Spreading on the Egg.* Since a comparatively small part of the egg-shell appears to come in contact with Géné's organ during the normal oviposition movements (see p. 295), the newly secreted wax must be capable of spreading over the surface of the shell. The spreading properties of the wax were therefore tested in *O. moubata*. Unwaterproofed eggs were obtained as usual by allowing ticks with Géné's organ covered to deposit egg-clusters in damp air; and the wax was obtained from ticks kept continuously at 15°C, organs bearing massive deposits of wax being selected for use. Each egg was removed individually from the egg-mass with forceps, brought into contact with the wax on the everted organ, and manipulated so that the wax was thoroughly spread over the required surface. Sometimes the eggs of a batch were completely smeared with wax; sometimes, in order to test the spreading powers, approximately half the surface was smeared. After treatment the eggs were placed on damp filter-paper for 2 hours and were then exposed in a watch-glass to dry air at the same temperature. The control eggs, which remained unwaxed, were always drawn from the same egg-mass.

**Table 6. The effect of smearing unwaterproofed eggs of O. moubata with wax taken from Géné's organ of the same species**

The rate of shrivelling was observed in dry air

<table>
<thead>
<tr>
<th>Female no.</th>
<th>Treatment</th>
<th>Temperature, °C.</th>
<th>No. of eggs</th>
<th>No. of eggs shrivelled after 30 min.</th>
<th>4 hrs.</th>
<th>24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>Wholly smeared</td>
<td>20</td>
<td>20</td>
<td>6</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>25</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>Half-smeared</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>30</td>
<td>20</td>
<td>13</td>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>Half-smeared</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>19</td>
<td>20</td>
<td>2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Half-smeared</td>
<td>20</td>
<td>20</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

The results, which are given in Table 6, show that the rate of shrivelling is considerably reduced when the eggs are completely smeared with wax. Nevertheless, in spite of the fact that the amount of wax transferred to each
egg is probably greatly in excess of the normal quantity, and that a greater surface area of the shell is brought into contact with the wax than during the natural manipulation by Géné's organ, the final degree of impermeability is always inferior to that of the egg when waterproofed naturally.

Results with half-smeared eggs proved to be very variable. In some egg batches (e.g. females 2 and 3) the permeability was greatly reduced, indicating that considerable spreading of the wax had taken place. In others (e.g. female 4) the eggs shrivelled as rapidly as the controls, thus providing no evidence of effective spread. No certain correlation between temperature and the rate or efficiency of waterproofing could be established. But the waterproofing effect is usually more pronounced if eggs are selected whose shells, even before the application of wax, are relatively impermeable (e.g. female 5, Table 6).

No evidence of spread was obtained with smaller areas of contact. A number of eggs, each with a large lump of wax adhering at one point, shrivelled as rapidly as the controls at an incubation temperature of 30° C. The limited spreading powers of the wax are also shown by its inability to spread effectively from egg to egg. A number of unwaterproofed eggs were, for example, placed in contact with normal waterproofed eggs or were thrust into the centre of a normal egg-mass. Yet these always shrivelled on subsequent exposure to dry air. If a female tick is interrupted in the process of oviposition, Géné's organ never bears any large wax deposits although there is always a film of wax bounding the epicuticle (as can be shown by the failure of the epicuticle to stain with silver from the outside). This film of wax is also incapable of limiting evaporation from unwaterproofed eggs if these are brought into intimate contact with the organ. It would appear, therefore, that the production of wax by Géné's organ may, to some extent, be co-ordinated with the delivery of the egg by the vaginal 'ovipositor'.

An additional factor influencing the spread of wax is probably the smoothness of the substratum. It was noted that waterproofing was invariably poor if any fungal hyphae were growing on the surface of the shell.

It is possible also to reduce the permeability of *Ixodes* eggs by applying the egg wax of *Ornithodoros*. Eggs laid without Géné's organ (and therefore already partially covered with *Ixodes* wax) were completely smeared with wax on an everted organ of *Ornithodoros*. Water loss was then found to be slightly less than from normal *Ixodes* eggs. The following are examples of the results obtained with batches of 20 eggs laid by the same tick. In a normal batch, laid with Géné's organ, 4 eggs had shrivelled after 4 hours, and 20 after 24 hours, in dry air at 30° C. In a second batch, laid with Géné's organ covered, every egg was completely dried out within 4 hours. In a third, laid without Géné's organ, but wholly smeared with *Ornithodoros* wax, no eggs had shrivelled after 4 hours and only 15 after 24 hours. The effectiveness of the waterproofing is, however, hardly comparable with that exhibited by the normal *Ornithodoros* egg.

*Spreading on Membranes.* Beament (1945) has shown that extracted insect cuticular waxes may be deposited on prepared membranes from chloroform.
solutions. Provided that the surface film is continuous the membrane then reproduces many of the physical properties (e.g. critical temperatures) of the normal insect cuticle from which the wax was obtained. Experiments were carried out to determine whether natural or extracted waxes of *Ornithodorus* could also be used in a similar manner. The membranes consisted of lipoid-free butterfly wings, chloroform-extracted wings of *Rhodnius prolixus*, and large wax-free nymphal skins of *O. moubata*. These were mounted in holders of the type described by Beament (loc. cit.); the surface area exposed was adjusted so as to range from 3 to 50 mm.². The sources of the waxes and the methods of application were as follows: (i) Natural wax from Géné’s organ was smeared in the centre of the membrane. (ii) Wax from Géné’s organ was deposited on the membrane from cold chloroform. (iii) The total chloroform extract from egg-shells, the acetone-soluble fraction (yellow grease), and the chloroform-soluble fraction (white wax) were deposited separately from cold and hot chloroform. (iv) The same procedures were also carried out with regenerated cuticular wax and with the cuticle extract.

Because of several initial failures, each waxed membrane was subsequently heated in an oven at different constant temperatures which varied from 30° to 70° C. It was hoped that heat would assist spreading in an uneven deposit of wax and promote an alinement of the wax molecules more consistent with impermeability. The steady water loss from each membrane was recorded at room temperature before and after heat treatment.

In none of the membranes could any reduction in permeability be detected, with the exception of the following. A lipoid-free *Ornithodorus* nymphal skin, 3 mm. in diameter (and therefore about three times the surface area of an *Ornithodorus* egg) had an initial permeability at 20° C. of 20 mg./sq.cm./hr. After smearing it with the natural wax from Géné’s organ and heating to 50° C. the rate of transpiration at 20° C. fell to 5 mg./sq.cm./hr. Nevertheless, this membrane was still too permeable for testing the critical temperature. It seems clear from these results that the natural egg wax must spread relatively slowly, even at high temperatures. And chloroform appears to have the effect of separating the wax system into two components neither of which can be redeposited on a membrane in the form of a waterproofing layer.

**Spreading on Water.** The spreading pressures of substances on water may be compared in the apparatus described by Adam (1945). Water is introduced into a large funnel immersed in a water bath and is flushed through until the surface is clean. This is then covered with a fine film of lycopodium powder which will indicate changes in the surface when any foreign material is added.

If Géné’s organ is everted and applied to the centre of the prepared water surface, the powder is displaced slowly and a film of wax spreads from the organ over a small area. We have noted that the wax is not very mobile at room temperatures; the secretion spreads more rapidly if the temperature of the water surface is raised, but it appears to cover only the same small area.

If the eggs of *O. moubata* are dropped on to the prepared water surface, slow spreading over a limited area takes place at all temperatures between
This phenomenon was obtained with newly laid eggs and with those about to hatch. It is obvious that the waterproofing lipoid is not completely bound or otherwise immobilized on the shell surface.

Material spreads much more rapidly from the surface of eggs of *I. ricinus* at room temperature and extremely rapidly at higher temperatures, but the waxy film again fails to cover a very large area. By comparison with the spreading powers of the waterproofing grease from the cockroach cuticle (Beament, 1945), the surface activity of the tick waxes is slight. Such spreading powers as the egg waxes possess seem to be associated with the presence of the acetone-soluble material (the yellow grease) which spreads actively. The white wax is devoid of spreading properties.

**The Amount of Wax present on the Egg**

The average weight of waterproofing material on the egg can be derived from a knowledge of the weight of extracted material and the total number of egg-shells extracted. Assuming the relative density to be 0.96 g./c.c. (Lewkowitsch and Warburton, 1921), the average volume of wax covering these eggs can be found. The approximate surface area of the egg may be arrived at from camera lucida drawings and geometric considerations. The thickness of the extracted materials, if spread evenly over the surface of the egg, can then be calculated.

The thickness of the wax layer in the *Ornithodorus* egg was found to be approximately 0.47 μ and in the *Ixodes* egg 1.76 μ. On the other hand, the thickness of the cuticular wax layer in the nymph of *Ornithodorus* is no more than 0.29 μ.

It will be noted that in both species of ticks the waterproof covering of the egg is considerably thicker than the wax layer encountered on the inside of the *Rhodnius* egg-shell (Beament, 1946); and it is also of greater thickness than the waterproofing layers on the cuticles of several insects (Beament, 1945), and of the nymphal tick itself. The calculation made above assumes an even thickness of wax on the egg. In fact, visual inspection shows that this is by no means always true, for eggs may often be seen in the egg-masses of *Ornithodorus* which have definite mounds of wax adhering at one or more points. Nevertheless, the figures may be taken to indicate that each egg receives a slight excess of wax.

**The Nature of the Contents of Géné’s Organ**

The nature of the glandular secretion of Géné’s organ is of considerable interest as it is evident that the secretion is associated in some way with the production of wax on the outside of the organ and may in fact be the wax precursor.

The contents of the horns, a clear refractile liquid, can be examined *in situ* with the 1/12th oil immersion objective. Apart from pieces of tissue (derived from fragmentation of the gland), which are abundant in some organs but completely absent in others, no particles or droplets are visible within the limits of resolution.
It is possible to collect small quantities of the liquid by applying a fine
ligature of silk thread at the base of each horn and another stouter ligature
round the base of Géné’s organ, then cutting it free and pricking each horn
separately on to a glass slide. The method is illustrated in Text-fig. 7.

By careful selection of the ligatured organs it is possible to obtain the
secretion virtually free of organic debris from the gland.

The liquid from the horns can be shown to contain water by pricking the
ligatured horn on to a small crystal of anhydrous cobalt chloride in a dry
atmosphere. The crystal instantly turns red. If the water is allowed to
evaporate after pricking on to a glass slide, a speck of solid material is left.

This is perfectly transparent and is sometimes slightly brittle, but has a waxy
feel when scratched with a needle. On adding droplets of warm or cold water
the material redissolves instantly. Xylene and chloroform, which are excellent
solvents for the wax deposits on the outer surface of the horns (p. 308), fail
even to wet it.

Protein or protein derivatives are present in the contents of the horns. The
following tests, which are summarized in Table 7, were performed on specks

of the material redissolved in distilled water. The ninhydrin reaction is
positive indicating the presence of amino groups. The Sakaguchi reaction
for arginine, which was performed by adding successive droplets of 5 per cent.

---

**Table 7. Some properties of the material isolated from the gland of Géné’s organ in O. moubata**

<table>
<thead>
<tr>
<th>Test</th>
<th>Contents</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Solid</td>
<td>Dissolves instantly</td>
</tr>
<tr>
<td>Arginine reaction</td>
<td>Aqueous solution</td>
<td>Strongly positive</td>
</tr>
<tr>
<td>Ninhydrin</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Xanthoproteic reaction</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Aldehyde reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millon’s reaction</td>
<td></td>
<td>White flocculant precipitate</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphotungstic acid</td>
<td></td>
<td>Yellow precipitate</td>
</tr>
<tr>
<td>Phosphomolybdc acid</td>
<td></td>
<td>Solution remains clear</td>
</tr>
<tr>
<td>Picric acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjust to pH 8 or pH 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan III and sudan black B</td>
<td>Solid</td>
<td>No staining</td>
</tr>
<tr>
<td>Heat</td>
<td></td>
<td>Does not melt at 100°C</td>
</tr>
<tr>
<td>Crossed nicols</td>
<td></td>
<td>Faintly luminous</td>
</tr>
</tbody>
</table>
NaOH, 1 per cent. alcoholic α-naphthol, and 10 per cent. sodium hypochlorite, is invariably strongly positive. Other colour reactions, including the sulphur reaction for cystine, the aldehyde reaction for tryptophane, the Millon and xanthoproteic reactions, are negative. The material must, therefore, be poor in tyrosine and tryptophane.

The protein in the horn contents is rapidly coagulated by the addition of ethyl alcohol forming a flocculent white precipitate. It is not precipitated by concentrated nitric acid in which the solid dissolves to yield a clear solution; neither is it readily heat-coagulable. If the solid is warmed on a slide at 100° C. for 1 hour, it immediately redissolves on the addition of water and this also occurs after warming in the presence of a droplet of 1 per cent. acetic acid. With stronger heating over a flame the solid material is gradually coagulated and fails to redissolve completely in water. It does not melt.

The protein is readily precipitated in aqueous solution by alkaloidal reagents such as picric, phosphotungstic, phosphomolybdic, and trichloracetic acids and fails to redissolve in water.

We seem to be dealing here with a protein rich in di-amino-acids. The high arginine content, solubility in water, and the resistance to coagulation by heat suggest some affinities with the protamines or histones. The reactions detailed above could not all be ascribed to the presence of free amino-acids, peptones, or proteoses (which might conceivably be present as emulsifying agents).

With the minute quantities of material available for tests, it has not been possible to isolate from the contents of the horn any material whose identity with the wax could be proved beyond doubt. Two observations do, nevertheless, lend some support to the view that the horns contain the wax precursor. First, a speck of the solid material treated with 5 per cent. trichloracetic acid becomes difficult to wet with water, the surface developing at the same time a glistening waxy appearance. However, although the coagulum is now readily wetted by chloroform little appreciable solution takes place. Secondly, the appearance in polarized light also affords some slight evidence of the presence of long chain lipoids. A small patch of material from the horns is isotropic when viewed normal to the surface but particles scraped up on a needle are decidedly birefringent in all quadrants. The material is therefore polycrystalline, as the wax itself is (p. 308).

Certain facts suggest that the wax is not present in the horns as a simple emulsion. For example, the horn contents are not readily precipitated by changes in the reaction of the medium. If the solution is adjusted to pH 8 or pH 3 by adding, respectively, droplets of borate or phthalate buffer, no trace of milkiness develops. Further, the solution remains perfectly translucent in the presence of excess polyvalent ions (e.g. calcium, aluminium, citrate).

The horn contents have no powers of emulsifying waxes in bulk. The absence of any effective agent can be shown by pricking a ligatured horn on to a small lump of wax removed from the outer surface of Géné’s organ. No visible emulsification takes place and the watery contents of the horn fail even to wet and mix with the wax.
THE MORPHOLOGY OF THE FEMALE GENITAL SYSTEM IN TICKS

Several detailed accounts of the morphology and histology of the genital system are available. Among Argasidae we have descriptions for *Ornithodoros savignyi* (Christophers, 1906) and *Argas persicus* (Robinson and Davidson, 1914); and among Ixodidae for *Ixodes ricinus* (Samson, 1909) and *Dermacentor andersoni* (Douglas, 1943). Some of the salient features will be recapitulated below. Our inquiry has, however, been directed principally towards two specific questions. In the first place, a rather more detailed treatment of the development of the ova in *O. moubata* has been a necessary preliminary for an extended examination of the structure and properties of the egg-shell. And secondly, the genital tract in *I. ricinus* has been re-examined in an attempt to discover the site of application of wax to the egg, for, as we have shown, a waxy covering is already present on the egg before it is received by Géne’s organ.

**The Genital Tract and Ovary in O. moubata**

The genital system of an egg-laying female is shown in Text-fig. 8. There is, as in all ticks, a single garland-like ovary which passes at each end into the...
long coiled oviducts. The posterior wall of the ovary is studded with developing ova of all sizes, but the anterior wall is sterile and consists of undifferentiated cells only. This type of tubular ovary with a parietal germarium occurs throughout the Arachnida and may represent an archaic feature of their organization. The tick ovary, indeed, closely resembles that of the Onycophora (Manton, 1938).

The oviducts pass into the large sac-like uterus from which arises the short vagina leading to the genital aperture. Robinson and Davidson (1914) distinguish two sections in the vagina, a posterior or cervical region, provided with a thick investment of muscles, and a short vestibular region without circular muscles. Two small tubular accessory glands enter the vagina near their junction.

The general course of development of the eggs appears to be as follows. The ova arise from small undifferentiated cells lying in the germinal ridge. This part of the ovary is itself invested externally by a fine hyaline non-chitinous membrane, the tunica propria. As the egg grows in size it begins to stretch this membrane and projects outwards from the surface of the ovary (Text-fig. 9A). By the time a diameter of 100 μ has been attained the egg has acquired a stalk or funicle whose walls are made up of a single layer of undifferentiated epithelial cells (Text-fig. 9B). These cells stain blue in Mallory, whereas the cytoplasm of the immature egg acquires a strong affinity for acid fuchsin. Having attained a diameter of about 500 μ the egg is finally ovulated into the lumen of the ovary. This process appears never to have been observed, but there is little doubt that, as Christophers (1906) implies, the continuous growth of the egg must impose an increasing strain on the elastic tunica propria so that eventually pressure forces apart the cells of the funicle and the egg passes through.

The appearance of the shell can first be detected when the egg is 25 μ or less in diameter. According to Nordenskiöld (1909) the shell in I. ricinus is first laid down in the form of adjacent granules which coalesce to form a continuous membrane, the last region of fusion being in the region of the funicle. This author held therefore that the funicle played some part in secreting the shell; and also that nutritive material for the developing egg is drawn from the lumen of the ovary via the funicle, as well as from the haemolymph through the tunica propria. In our sections of the ovary in Ornithodorus the shell first appears as an exceedingly tenuous pellicle which is continuous over the funicular region, as elsewhere. It is to be doubted whether the funicle plays any part in secreting the shell for the two are not firmly attached to one another. This can be shown if the ovary is dissected in saline and the tunica propria is slightly torn with a needle. Developing ova of all sizes then roll out freely into the dissecting-dish. Yet the shell clearly increases both in surface area and in thickness as the ovum grows in size within the tunica propria. It is apparent from this that the shell must be secreted by the oocyte itself. Among the insects the term ‘chorion’ is best reserved for those parts of the shell secreted by the follicle cells (Beament, 1946a). We shall not therefore apply this term to the tick egg-shell.
Lees and Beament—An Egg-waxing Organ in Ticks

As the egg passes down the ovary into the oviduct it absorbs water, finally reaching a diameter of about 800 μ. The eggs are stored for a short while in the uterus before being laid.

The outer surface of the tick egg is completely smooth and featureless; there is no micropile. Robinson and Davidson (1914), finding sperms only as

![Text-fig. 9. The structure of the ovary in Ornithodoros moubata: A, transverse section through a mature ovary; B, section through an immature egg developing in the germinal ridge.](image)

high as the upper oviduct in *A. persicus*, suggested incorrectly that the shell was laid down by the oviduct shortly after fertilization. But we have seen that in *O. moubata* the shell rudiment is acquired very early in development. How then is the egg fertilized?

In this species, as in other Argasidae, the uterus also serves as a receptaculum seminis, often containing as many as a dozen spermatophores. As these are ruptured, the sperms, which are relatively gigantic objects some 400 μ in length, make their way up the oviducts, presumably by means of the slow gliding movements of which they are capable. The genital tracts of fertilized ticks, which were dissected in Ringer and examined with a 1/6th objective, often contained isolated sperms in the lumen of the ovary as well as large clusters of sperms in the upper oviducts. There would appear to be no
escape from the conclusion that the oocyte is fertilized in situ within the
germinal ridge before the rudiments of the shell are laid down at the beginning
of the growth phase. \(^1\) It is of considerable interest in this connexion that
Christophers (1906) has figured a developing egg in *O. savignyi* only about
one-quarter grown and yet containing the clearly recognizable remains of a
spermatozoon. In spite of careful search, however, we have found no similar
traces within the eggs of *O. moubata* at any developmental stage. Neverthe-
less, in view of the resistant nature of the egg-shell (p. 322), it is highly
improbable that sperms could enter the egg after the deposition of the shell
has begun. Uterine eggs are usually surrounded by spermatozoa but they
never penetrate the shell.

The Genital Tract in *Ixodes ricinus*

The ovary in this Ixodid is much longer than in *O. moubata* and contains
greater numbers of developing eggs. On the other hand, the general course
of ovulation and of egg development is probably very similar and need not be
described here.

There are, however, important morphological differences in the distal
regions of the genital tract. There is no uterus in *I. ricinus* and the two
oviducts join to form a short common oviduct leading directly to the vagina
(Text-fig. 10). Further, there are two types of accessory glands: short, club-
shaped or tubular glands, probably homologous with the tubular glands in
*Ornithodorus*, and a larger gland whose numerous irregular lobes are disposed
on the dorsal and lateral sides of the vestibular portion of the vagina. The
latter is without a homologue in *Ornithodorus*. Douglas (1943) refers to this
lobed accessory gland in *Dermacentor andersoni* as a receptaculum seminis and
regards it as comparable with the uterus in Argasidae—a term which, in his
opinion, is a misnomer. In *Ixodes*, however, the lobed accessory glands never
contain spermatophores; on the contrary, these seem to be passed directly
into the common oviduct.

The general arrangement of these organs is shown in the sagittal section
(Text-fig. 11). The cervical and vestibular regions of the vagina, with the
tubular accessory glands opening near their point of junction, can again be
distinguished. The entire vagina is lined by thin cuticle which is continuous
with the general body integument. Over the vestibular region, epicuticle and
a thin layer of endocuticle is present; it is highly folded and in places bears
minute recurved spines. In the cervix the intima is reduced to a tenuous layer
of epicuticle which closely follows the contours of the vaginal cells. The latter
are closely invested with a deep layer of circular muscle-fibres which, by their
contraction, are probably responsible for the prolapse of the vagina during
oviposition (Pl. I, fig. 12).

We have shown that the eggs dissected from the oviducts are completely
unwaterproofed but that they acquire an incomplete layer of wax on the

\(^1\) The precocious uptake of sperms by the young oocyte is described in *Peripatopsis* by Manton
(1938), but here the function is stated to be that of providing nutriment for the growth of the ovum.
surface of the shell during their passage down the vagina. The source of this wax may therefore be (i) the cells lining the cervical region of the vagina, (ii) the tubular accessory glands, or (iii) the lobed accessory glands. Their histology affords convincing evidence of the close affinity of one of the organs—the lobed accessory glands—with Géné’s organ.

A section through the wall of one lobe of Géné’s organ is shown in Pl. I, fig. 10. The histological appearance is completely different from that of the corresponding organ in *O. moubata*. The gland-cells are very large by comparison, are often widely spaced from each other, and are irregular or polygonal in shape. The cytoplasm is dense and deeply staining, the nuclei are large, and there is a well-developed ‘honeycomb’ border. Not infrequently an irregular meshwork of fibres extends from the cell margin into the gland lumen.

The tightly packed columnar epithelial cells lining the cervix have poorly defined cell boundaries and small nuclei disposed at different levels in the cell (Pl. I, fig. 12). As already mentioned, the cuticular intima maintains close contact with the free borders of the cells.
The tubular accessory glands are built up of wedge-shaped cells bordering a narrow central lumen (Pl. I, fig. 13). Their cytoplasm usually contains large droplets of colloid which stain a pale grey with iron haematoxylin. The lumen appears to open freely into the genital tract.

On the other hand, the histological appearance of the lobed accessory gland (Pl. I, fig. 11) and the gland of Géné’s organ is so similar that the two cannot easily be distinguished unless the arrangement of the lobes is followed carefully in serial sections. Probably, therefore, the former is responsible for secreting the wax. It may be noted that this gland bears the same relation to the vaginal cuticle as does the gland of Géné’s organ to the cuticle of the horns, for the lumen of the accessory gland is partitioned off from the genital tract by the cuticular lining of the vestibule, which is in contact with the cells only near the margins of the gland. The accessory gland—also like Géné’s organ—represents an intucked region of the epidermis which has become specialized for a particular glandular function.

An additional point of interest is the absence of a homologous organ in *O. moubata*. In this species, as we have seen, there is no evidence that waxes are secreted on to the egg during its passage down the vagina.
Three distinct layers can be distinguished in the shell of the newly laid egg: (i) an inner 'shell layer'; (ii) an incomplete layer of granules with reducing properties; and (iii) the outer covering of wax which is applied by Géné's organ (Text-fig. 12A). A fourth layer, the 'inner membrane' (iv), is secreted after the egg has been incubated for 2–3 days (Text-fig. 12A, B). The properties of these layers may now be outlined; those of the wax have already been described.

**The Shell Layer.** One-day-old eggs were punctured, freed from yolk in distilled water, dried, and extracted in boiling chloroform to remove soluble lipoids. The observations on this material were repeated on shells of eggs dissected from the ovaries at various stages of growth and on eggs which had been laid without establishing contact with Géné's organ. The latter are, of course, free from wax and need not be extracted first in chloroform.

The lipoid-free shell layer is a uniform membrane with striking elastic properties, smooth on its outer and inner surfaces and 3–4 μ in thickness. It is colourless, the tint of the egg depending entirely on the pigment in the yolk. There are no pore canals.

The shell layer contains no chitin but it gives a strong xanthoproteic reaction and turns pink when heated with Millon's reagent. The ninhydrin reaction is negative and it fails to stain with cold and hot p-benzoquinone. The shell material stains deeply and rapidly with basic and acid fuchsin, borax carmine, haematoxylin, and picric acid. It fails to take up colour from alcoholic solutions of sudan III or sudan black B, nor does it darken with osmium tetroxide.

The shell membrane is insoluble in cold concentrated nitric acid, but dissolves on heating, leaving a granular residue; no oil is released. It is partially soluble in cold concentrated nitric acid saturated with potassium chlorate and on warming dissolves completely with the evolution of gas. The shell material dissolves readily in hot potash and is also rapidly broken down by 14 per cent. sodium hypochlorite.
This evidence shows that the shell layer is composed of a protein which, unlike the ‘cuticulin’ of *Rhodnius* epicuticle (Wigglesworth, 1947), does not incorporate a lipoid.

*The Granular Layer.* If whole eggs, laid without Géné’s organ, are immersed for 15 minutes in 5 per cent. ammoniacal silver nitrate, the outer surface of the shell layer, after mounting, is seen to be covered with scattered granules and granule aggregates which have stained a deep reddish-brown (Pl. I, figs. 14, 15). The nature of these granules is uncertain for they are also stained by 1 per cent. silver nitrate (but not by silver nitrate in the presence of nitric acid). They appear on the shell layer shortly before the ovulation of the egg. Two further points are worthy of note. First, the shell layer has the appearance of a material which is partially tanned. Thus the shells of eggs newly ovulated into the lumen of the ovary are decidedly flabby and elastic, but they lose much of this elasticity and become much more rigid as they pass down the genital track into the uterus. Secondly, the reducing granules of the tick egg, although not identical with the polyphenol granules which constitute the substrate for the waterproofing layer in the *Rhodnius* egg-shell (Beament, 1946a), occupy the same functional position with respect to the wax layer.

*The Inner Membrane.* After incubation for 2–3 days at 25° C. a further layer, also secreted by the embryo, is added to the inner surface of the shell. It is easily demonstrated if empty egg-shells are treated with sodium hypochlorite solution: this dissolves the shell layer, leaving a tenuous transparent membrane which retains the same outline. The inner membrane is absent in the one-day egg.

The chemical properties of the inner membrane are mainly negative. It gives no definite colour with Millon’s reagent and the xanthoproteic and ninhydrin reactions are negative. It does not contain chitin. The material is insoluble in cold and hot nitric acid but eventually soluble in hot nitric acid saturated with potassium chlorate. No oil is liberated when solution takes place. It is insoluble also in sodium hypochlorite and aqueous potash, but dissolves in fused potash. Water-soluble dyes such as borax carmine, basic or acid fuchsin fail to stain the membrane and it remains uncoloured after treatment with *p*-benzoquinone and ammoniacal silver nitrate.

The resistance of this material to attack by chemical agents therefore recalls the properties of the fertilization membrane of the *Rhodnius* egg (Beament, 1948a).

**Permeability of the Egg-shell**

Eggs which have received no wax from Géné’s organ are highly permeable to water and shrink rapidly in dry air (p. 296). As might be expected, un-waterproofed eggs of *O. moubata* also show notable swelling properties when immersed in distilled water. Developing ova taken from the germinal ridge of the ovary swell to nearly three times their normal diameter in 5 minutes before bursting (Text-fig. 13D, E); those from the uterus, because of their more rigid shells, swell more slowly, a hyaline zone appearing between
the yolk and the shell (Text-fig. 13A, c). Swelling may finally cease altogether.

We have already noted that water loss from the one-day egg, waterproofed in the normal manner by Géné's organ, is enormously hastened by extraction in cold chloroform. This statement, however, does not necessarily hold good for eggs in a later stage of incubation. It has been found indeed that the effect on transpiration gradually diminishes as the period of incubation lengthens and that the eggs also develop increasing resistance to treatment with abrasive dusts. Some of the results obtained with eggs of known age are set out in Table 8.

After incubation for 3 or 4 days at 25° C., short extractions (1 min.) in cold chloroform, which undoubtedly dissolve all the wax from the surface of the shell, leave the egg almost as resistant to desiccation as previously. Longer periods of extraction have a noticeable effect on older eggs, while the longest extraction (4 hrs.) completely destroys the waterproof properties, even in those eggs which are on the point of hatching. Penetration is particularly noticeable after 4 hours for chloroform gradually displaces the water in the egg and, without any accompanying change in the egg volume, forms a refractile zone round the shrivelled yolk (Text-fig. 13B). In a comparable manner, the one-day egg is highly sensitive to abrasion and shrivels immediately in dry air after it is rubbed with alumina dust or even if it is merely sprinkled with dust: whereas the older eggs again develop considerable resistance to this treatment, sometimes being seemingly unaffected by the most thorough abrasion (Table 8).

Two explanations of this phenomenon seem possible. On the one hand, the mobile wax applied by Géné's organ to the outside of the shell may gradually sink in and impregnate the shell layer; it would then still be liable to extraction by prolonged treatments with chloroform but would be in-accessible to the influence of abrasive dusts. Or, alternatively, one could postulate the deposition of a secondary waterproofing layer by the embryo during the course of incubation—an event which occurs during the develop-

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**TEXT-FIG. 13 A-E. Eggs of Ornithodoros moubata after various treatments:**

- **A**, normal egg;
- **B**, waterproofed egg extracted for 4 hrs. in cold chloroform;
- **C**, unwaterproofed egg from uterus after 30 min. in distilled water;
- **D**, egg dissected from germinal ridge;
- **E**, a similar egg after 5 min. in distilled water;
- **F**, egg of Ixodes ricinus in outline.
ment of the Rhodnius egg (Beament, 1948b). Indeed the inner membrane, which is secreted after 2–3 days of incubation, might possess the necessary waterproof properties. In order to resolve this question further evidence was sought regarding the permeability of the layers of the egg-shell both to water and to other substances.

Table 8. The number of eggs, in batches of 10, that shrivelled in dry air at 25°C after the following treatments: a, b, c, extraction in cold chloroform for 1 minute, 30 minutes, and 4 hours, respectively; d, dipping in alumina dust; e, rubbing with dust

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Treatment</th>
<th>No. of eggs shrivelled after 30 min.</th>
<th>No. of eggs shrivelled after 4 hrs.</th>
<th>No. of eggs shrivelled after 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>a</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>10</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>10</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>10</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>1–2</td>
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<td>3</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>7</td>
<td>10</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>10</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>8</td>
<td>10</td>
<td>..</td>
</tr>
<tr>
<td>3–4</td>
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Permeability of the Shell Layer. Unwaterproofed eggs laid during the previous 24 hours were exposed to solutions of many different dyes, solvents, oils, and salts, but it was soon apparent that particles of a very wide variety of molecular sizes, and having either hydrophilic or lipophilic affinities, were capable of passing rapidly into the yolk. Stains, such as basic fuchsin, penetrate the shell layer and stain it intensely; and it is also stained if the dye is injected into the egg. Large protein molecules will also penetrate the shell. If, for example, the egg is immersed in laked blood for 24 hours the colour of the contents deepens as oxyhaemoglobin enters the yolk. Now it is known that the normal brownish tint of the Ornithodorus egg is due to the presence of haemoglobin pigments (Wigglesworth, 1943). As the shells of ovarian eggs at the stage of yolk deposition are no less permeable than one-day eggs, there is clearly no obstacle to the absorption of these pigments through the shell
layer from the haemolymph. The large pore size might also facilitate the penetration of the relatively small wax molecules into the lipophilic shell layer.

The waterproofed egg in the first day of incubation is, by contrast, entirely impermeable to all staining solutions unless they are incorporated with vigorous wax solvents. After treating the egg with the wax emulsifier CO9993 or cold chloroform for increasing periods, the permeability increases progressively and the staining properties of the shell layer are restored. At first the eggs swell osmotically in distilled water but fail to shrink in saturated sodium chloride, indicating that the shell is now permeable to small ions as well as to water. Extraction for 30 minutes in cold chloroform renders the shell permeable to sucrose but it still stains only in small and isolated patches. However, after extraction in boiling chloroform for 5 minutes the original permeability of the shell is completely restored and it then stains deeply and uniformly. These observations must imply that there is considerable affinity between the wax and the shell material.

Permeability of the Inner Membrane. Normal eggs incubated for 3 days at 25°C (and therefore possessing a completed inner membrane) were extracted for 5 minutes in boiling chloroform. After this treatment the egg-shell as a whole was found to be permeable both to water and to monovalent ions, but, although the shell layer stained deeply with aqueous basic fuchsin, the dye no longer entered the yolk. This suggested that the inner membrane was permeable to water and salts but not to larger molecules.

The properties of the inner membrane may be tested more directly by carefully puncturing and stripping off the shell layer from the three-day waterproofed egg. The inner membrane, which is left entire as a delicate pellicle surrounding the yolk, is then found to be very permeable to water, as is shown by its swelling properties. Its outer surface, which is matt and seemingly homogeneous, is fairly readily wetted by water. On the other hand, if the shell layer is removed from an egg in the eighth day of incubation, the inner membrane is distinctly greasy and hydrofuge; and the egg swells much more slowly in distilled water.

Although this evidence does not entirely exclude the possibility that the inner membrane is impregnated from within by lipoid secreted by the embryo, it seems much more probable that the wax deposited on the outside of the egg gradually migrates through the shell layer as far as the inner membrane. Since the inner membrane is at first freely permeable to water, the diminishing influence of chloroform and abrasive dust on transpiration cannot be ascribed to the deposition of this layer. It is also noteworthy in this connexion that the effect of these agents gradually declines throughout the period of incubation—a result which might be expected from the slow infiltration of wax into the shell layer—whereas the inner membrane is laid down at a particular stage of incubation. These changes in the egg-shell during the incubation period are indicated diagrammatically in Text-fig. 12A, B.

If no secondary waterproofing layer is secreted after the onset of incubation, there should be no marked change in the critical temperature of the egg. The
evaporation curves of batches of eggs incubated for 6 days at 25° C. is shown in Text-fig. 4. The critical temperature is approximately 44° C. and compares with a value of 43° C. for the one-day eggs. Nevertheless, the lower rate of water loss above the critical temperature, which is brought out clearly in this figure, is a very constant feature of the older eggs. It is possible that we are observing here a further effect of the impregnation of the shell layer with wax (see p. 330).

**DISCUSSION**

Our observations have shown that the tick egg is waterproofed by an external covering of wax which is secreted by the female tick after the shell layers are complete. This is the reverse of the condition found in *Rhodnius* and other insect eggs where the impermeable wax layers are laid down by the oocyte or embryo after the formation of the chorion and therefore lie inside it (Beament, 1946b, 1948b). From the superficial position of the wax layer it follows that tick eggs are very readily attacked by wax solvents, detergents, or abrasive dusts; whereas these agents often exert a remarkably small effect on the transpiration from many insect eggs.

An additional property of the waxy covering is to cause the eggs to adhere in a cluster—a function often assumed by the cement-secreting glands in insects. Among the Argasidae the wax layer responsible for the impermeability of the general integument is protected by an external covering of cement (Lees, 1947). Nevertheless, the manner in which the egg is waterproofed is obviously incompatible with the acquisition of a further protective layer. And our results show clearly that the waxes are in fact freely exposed on the surface of the egg.

In *I. ricinus* wax is applied to the outside of the egg in two stages. An incomplete layer, secreted probably by the lobed accessory glands, is first smeared over the egg during its passage down the vagina; and a further complete covering of wax is applied when the egg touches Géné’s organ. On the other hand, there is no evidence that the egg of *O. moubata* is even partially waterproofed before it is laid: here Géné’s organ is solely responsible for secreting the waterproofing layer. This difference may possibly be related to the size of the egg. In *Ixodes* (as indeed in all Ixodidae) the egg is relatively small and has an approximate volume and surface area of only 0.045 mm.³ and 0.61 mm.² respectively, as compared with values of 0.33 mm.³ and 2.3 mm.² for the *Ornithodorus* egg (Text-fig. 13a, f). Whereas the rate of water loss from the egg may be expected to vary according to the surface area, the amount of water that can be lost before desiccation becomes critical, and also the rate of shrinkage of the egg, will be determined by the volume. It is indeed an observed fact that unwaterproofed eggs of *Ixodes* (but not those of *Ornithodorus*) shrink so rapidly that there would clearly be some danger of excessive desiccation taking place during the manipulations of the egg by Géné’s organ, were the first incomplete wax layer not already present.

Evidence, both of an observational and experimental nature, shows clearly that the function of Géné’s organ is to transfer a waterproofing wax to the
surface of the egg. The gland is a proliferation of the general epidermis which is folded inwards from the cuticle in this region. This relationship provides a simple explanation of the fact—which at first sight appears as a striking example of parallel physiological adaptation—that the eggs of a given species of tick have the same order of resistance to desiccation as the parent species itself. The production of a waterproofing agent is a general property of the epidermis and Géné's organ can be regarded as a region specialized for waterproofing the egg. Nevertheless, there are important differences in the properties of the egg and cuticular waxes which can, in turn, be related to their specific functions (see below).

A consideration of the nature of the wax precursors raises some interesting questions which are worthy of further study by more exact chemical methods. Although it has proved impossible to isolate the wax itself from this situation, it is highly probable that the wax precursor is present in the watery contents of the horns of Géné's organ. Protein is also present, but the nature of its association with the wax is not clear. Certainly the wax is not dispersed as a coarse oil-in-water emulsion stabilized by protein (in a manner analogous to the dispersion by protein of the poly-isoprene aggregates in rubber latex). Against such an interpretation may be cited the absence of visible droplets in the horn contents, the insensitivity to changes in pH and to the presence of excess polyvalent ions, and the stability to heat. An alternative suggestion that the horns may contain emulsifying agents, other than protein, receives no support from the experimental results, for the secretion from the horns is found to be entirely devoid of emulsifying properties when tested against the wax deposits from the outer surface of Géné's organ. It is more likely that the wax is intimately associated, and probably chemically linked, with protein when it is secreted by the gland. Although the nature of such lipo-protein complexes is but imperfectly understood (Chargaff, 1944), the action of proteins in dispersing water-insoluble lipids, like cholesterol, is well known.

The stability of the wax-protein association is such that it is difficult to believe that the wax could be liberated by any purely physical process, for example, by ultrafiltration through non-living cuticle. And the cuticular wall of the horns is devoid both of pore canals and of any cytoplasmic lining. We have shown, however, that pore canals, penetrating the cuticle, are present around the base of the horns where the gland epidermis becomes applied to the inner surface of the endocuticle; and there is also a zone where the pore canals remain uncovered by the cement which forms an external covering over the basal part of the stalk. If, as appears probable, this is the site of wax secretion on the outer surface of Géné's organ, the horns must serve as reservoirs for containing the relatively large quantities of wax precursor needed for the rapid waterproofing of a batch of eggs. Such a function must also imply that the precursor secreted by the gland is first stored in the horns and is then again taken up by the gland-cells as required and passed through the cuticle by the cells which maintain connexion with pore canals. The form in which the wax is transported through the cuticle is, of course, unknown,
but the highly soluble wax-protein complex would appear to be a material very suitable for transport by the cell. At the cell surface, perhaps at the tip of the pore canals, the complex appears to be broken down and the wax in some way released. The protein moiety must then be retained by the cell, for, as we have seen, the wax deposits on the outside of Géné's organ are protein-free.

Since Géné's organ makes contact with only a small part of the total surface area of the shell during normal oviposition movements, the newly secreted wax must have the ability to spread and complete the waterproof layer over the egg. We have shown that although wax from the deposits on Géné's organ does possess definite powers of spreading on unwaterproofed eggs and, under very favourable circumstances, on prepared membranes, these powers are unexpectedly feeble. By comparison, for example, with the waterproofing grease from the cockroach cuticle (Beament, 1945), spreading of the egg waxes is slow and inefficient. The lipophilic properties of the shell material and the uniformly smooth surface of the shell layer are undoubtedly factors which favour spread. There is no reason to suppose, however, that the extension of the wax film is also assisted by the presence of specific spreading agents on the egg surface. Perhaps the wax undergoes further chemical changes after secretion, resulting in a partial loss of the spreading properties.

The comparison of the critical temperatures of tick cuticles and eggs also suggests points of interest in relation to the spread of waterproofing materials. In two species of Ornithodorus the critical temperatures of the egg, although comparatively high, are about 20° C. lower than are those for the cuticle of the parent species. These differences are correlated with the physical properties, the natural wax from the cuticle being hard and crystalline whereas that from the egg is soft and viscous. It would seem likely that the properties of the egg wax are largely dictated by the need for spreading over the surface of the shell. In general, it has been found that species of insects with high critical temperatures are waterproofed by hard apolar waxes (Wigglesworth, 1945; Beament, 1945). One might suggest from the knowledge at present available that a wax with good spreading powers is unlikely to have a high critical temperature; and that this property will be achieved only at the expense of some increase in permeability. Unlike the egg waxes, the cuticular lipoids have little need for mobility as spreading may be limited to the distance between neighbouring pore canals. Among Ixodidae there are comparatively slight differences only between critical temperatures of cuticle and egg in the same species. Nevertheless, preliminary observations suggest that the waxes concerned differ in their physical properties as widely as in the Argasidae; the egg waxes always appear to be more mobile.

A notable feature of the development of the tick egg is the fact that the shell is secreted not by follicle cells, but by the oocyte itself. The microscopic structure of the shell is comparatively simple. Only three layers are present when the egg is laid, the outer wax layer, a layer of granules with reducing properties, and the shell layer; a fourth layer, the inner membrane, which,
like the shell material, is composed of protein, is added after several days of incubation.

The unwaterproofed shell layer is remarkably permeable both to water and to large molecules with either lipophilic or hydrophilic affinities. Our experimental results point to the conclusion that the mobile wax gradually invades and impregnates the shell layer and in places reaches the inner membrane. The changes in the permeability of the egg around the critical temperature are probably related to these events. It has been suggested that increased transpiration through the insect cuticle at the critical temperature is associated with the destruction by thermal agitation of the parallel alinement of the wax crystallites at the protein-wax interface (Beament, 1945). If the shell layer is pictured as a loose protein meshwork, the wax will at first be entirely superficial and temperature will exert a correspondingly profound effect on transpiration. As the wax infiltrates into the shell layer and not only occupies the protein interfaces throughout the thickness of the layer, but also fills the intermolecular pores, the permeability to water may be expected to fall. This may be the explanation of the lower transpiration rate of 6-day eggs above the critical temperature. There is no evidence that a similar relationship is ever established between the cuticular waxes of ticks and the cuticulin-polyphenol substratum on which they are deposited. Here the waterproofing layer remains entirely superficial and therefore continues to be accessible to such agents as abrasive dusts (Lees, 1947).

Summary

1. During the oviposition of ticks a glandular organ—the organ of Géné—is everted and touches the egg. If it is prevented from everting most of the eggs shrivel rapidly; few hatch even in a humid atmosphere.

2. The waterproofing properties of the normal egg are conferred by a superficial coating of wax, 0.5–2.0 μ in thickness. In Ornithodorus moubata the wax is secreted and applied solely by Géné’s organ. In Ixodes ricinus waterproofing takes place in two stages: an incomplete covering of wax, probably secreted by the lobed accessory glands, is first smeared over the egg during its passage down the vagina; waterproofing is then completed by a further application of wax from Géné’s organ after the egg has been laid. Owing to its superficial position on the egg the wax layer is readily attacked by solvents and emulsifiers.

3. The morphology of Géné’s organ in O. moubata is described. The gland is a proliferation of the epidermis which lies detached from the cuticle. Its secretion, a watery refractile liquid containing the wax precursor, accumulates between the gland and the cuticle in two horn-like extensions. The wax is probably secreted through pore canals distributed over a narrow zone of cuticle below the horns; the cement covering-layer of the epicuticle does not extend to this zone.

4. The transparent, heat-stable material isolated from the horns of Géné’s organ is regarded as the wax precursor. Solubility in water is probably con-
ferred by chemical linkage with protein. The precursor is taken up from the horns, where it is stored, and is presumably broken down within the gland cells. The wax is then secreted through the pore canals while the protein moiety is retained by the cell.

5. The critical temperatures of the eggs of Ixodidae range from 35° C. in *I. ricinus* to 44° C. in *Hyalomma savignyi*; only slightly higher critical temperatures were recorded for Argasidae (45° C. in *O. moubata*). Eggs with lower critical temperatures are more susceptible to desiccation. The susceptibility of the eggs of a given species is of the same order as that of the parent species; but whereas in Ixodidae the critical temperatures of the egg and the cuticle of the female tick are approximately the same, in Argasidae the critical temperatures of the cuticle are much higher (62° C. in *O. moubata*). These differences are related to the physical properties of the waxes. The cuticular wax in *O. moubata* is hard and crystalline (m.p. 65° C.), whereas the egg wax is soft and viscous (m.p. 50–54° C.).

6. The natural wax from Géné’s organ has definite powers of spreading on the surface of the egg and so completing the waterproofing layer.

7. The material extracted with boiling chloroform from egg-shells or from nymphal cuticles separates spontaneously into two fractions, a hard white wax (c. 85 per cent. by weight) and a soft yellow grease (c. 15 per cent.). The properties of these two lipoids differ conspicuously from those of the natural wax. Attempts to deposit the extracted materials on membranes in the form of a waterproofing layer were unsuccessful.

8. Ovulation is described in *O. moubata*. The shell of the tick egg is secreted by the oocyte itself and not by follicle cells. Three layers can be distinguished in the 24-hour egg: (i) an outer wax layer; (ii) an incomplete layer of granules which reduce ammoniacal silver nitrate; (iii) a shell layer. A fourth layer, the inner membrane (iv), is secreted by the oocyte after incubation for 2–3 days.

9. Both the shell layer and the inner membrane are composed of resistant, elastic protein and are devoid of chitin. The shell layer of the unwaterproofed egg is highly permeable to water and to large molecules with either hydrophilic or lipophilic affinities. The inner membrane is at first freely permeable to water and to inorganic ions. During the course of incubation the wax gradually migrates into the shell material and may reach the inner membrane. As this occurs, the effectiveness of abrasive dusts and of chloroform in promoting increased transpiration through the shell is notably reduced.
REFERENCES

— 1948b. Ibid. (in the press).
ROBINSON, L. E., and DAVIDSON, J., 1914. Parasitology, 6, 382.

EXPLANATION OF PLATE I

Fig. 1. O. moubata. Female tick, with Géné’s organ everted, clipped between glass slides. X7.

Fig. 2. O. moubata. Sagittal section through the centre of an everted Géné’s organ to show continuity of epidermis (indicated by arrows) with the gland. X50.

Fig. 3. Géné’s organ in O. moubata. Gland epithelium showing secretory droplet. X210.

Fig. 4. The same, showing relation of gland to the thin cuticle of the horns. X210.

Fig. 5. Whole mount of Géné’s organ in O. moubata. The tick with the organ everted was immersed for 30 minutes in ammoniacal silver nitrate. Note absence of staining. X20.

Fig. 6. A similar organ extracted for 30 minutes in cold chloroform and stained with ammoniacal silver. Note intense staining over the horns where cement is absent. X20.

Fig. 7. O. moubata. Cuticle of the horns heavily stained with silver after chloroform extraction. There are no pore canals in the thin unstained endocuticle. Section photographed in water. X430.

Figs. 8, 9. O. moubata. Sections of the cuticle from the zone between silver-staining and non-silver-staining areas of Géné’s organ. The thickness of the cuticle is indicated by an ink line, pore canals in the endocuticle and silver-staining areas of the epicuticle by arrows. X430.

Fig. 10. I. ricinus. Section through one lobe of the gland of Géné’s organ. Honeycomb border indicated by arrow. Iron haematoxylin. X210.

Fig. 11. I. ricinus. Portion of lobed accessory gland, to show similarity with gland epithelium of Géné’s organ. Iron haematoxylin. X210.

Fig. 12. I. ricinus. Longitudinal section of vaginal cells and circular muscles. Iron haematoxylin. X210.

Fig. 13. Transverse section of tubular accessory gland. Iron haematoxylin. X210.

Figs. 14, 15. O. moubata. Surface view of shell fragments from two unwaterproofed eggs treated with 5 per cent. ammoniacal silver nitrate. X210.