THE PRODUCTION OF CUTICLE WAX BY ENGORGED FEMALES OF THE CATTLE TICK, *BOOPHILUS MICROPLUS* (CANESTRINI)

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INTRODUCTION

The layer of wax secreted on to the surface of the cuticle by arthropods plays an important part in protecting these animals against physico-chemical hazards of their environment, and so contributes significantly to the difficulty and expense of controlling economically undesirable species. In spite of this importance, however, very little is known about the biosynthesis of cuticle wax or the quantitative aspects of its production.

Discussions of possible mechanisms for the transport of wax to the cuticular surface have described its synthesis and deposition as a continuous process (Locke, 1965) or as one completed early in development (Lees, 1947). In insects with an unusually well-developed ability to form wax, such as the white wax scale and the honey-bee, a progressive increase in the amount secreted is obvious. Insects (Wigglesworth, 1945) and ticks (Lees, 1947) have been shown to repair damage to abraded cuticle by the deposition of new wax, and to form, under some conditions, a layer thicker than the original. Extra wax may be secreted on to the insect cuticle during moulting (Wigglesworth, 1945). In general, however, it seems to be accepted that the wax layer, once formed, is kept fairly constant in thickness, at least during adult life, and is replenished only as circumstances require.

In the course of an investigation into the origin of the components of the cuticular wax of the cattle tick *Boophilus microplus*, the amount of wax produced by the engorged female was measured at intervals of time between dropping from the host and death at the completion of egg-laying. In an attempt to relate wax secretion to general physiological activity, the weight lost by the tick during egg-laying was measured at the same intervals. This report presents the results of these measurements.

MATERIALS AND METHODS

Ticks were obtained from cultures maintained on stalled cattle at Yeerongpilly. All ticks used were of the same strain and resulted from a single infestation. Engorged females were collected daily at 9.00 a.m. from the floor of the stalls. Since a peak in the numbers of fully engorged females dropping occurs early each morning (Hitch-
coccit, 1955) the majority of those collected would have fallen within a few hours of the
collection time, and daily collections should have been adequately comparable. An
arbitrary zero time of 5.00 a.m. was chosen on which to base measurements of time
after dropping.

The ticks were washed in tap water at 30°C, dried on absorbent paper, counted
into cardboard boxes in lots of fifty and weighed. Ticks of comparable size were
selected and the average weight per tick for each box was between 190 and 220 mg.
The boxes were placed in an incubator at 30°C and high humidity. At 11.00 a.m.
on the day of collection and at 24 hr. intervals thereafter, five to ten boxes were with-
drawn, the ticks were brushed free of any eggs and examined. Dead or abnormal ticks
were discarded. Those remaining were counted, weighed, killed with ammonia vapour,
placed in a filter funnel plugged with glasswool and washed with three volumes of 50 ml.
of redistilled hexane at 40°C. The collected washings were poured back through the
funnel as a fourth wash, then evaporated to dryness on a water bath under a stream of
nitrogen. The residue was extracted with hexane, the extract was evaporated to dry-
ness under nitrogen and the residue from this evaporation was weighed.

This series of washings appeared to remove all hexane-soluble material from the
surface of the cuticle, since further washing gave no weighable residue. A non-
quantitative test for the efficiency of removal was performed by placing washed ticks
and killed unwashed ticks in a desiccator over solid calcium chloride or in a beaker of
water. Washed ticks became dehydrated rapidly in the desiccator, and swelled visibly
in water, while unwashed ticks remained practically unaffected.

Hexane appeared to extract some non-lipid material from the cuticle, since re-
extraction of the residue from the first evaporation left a film of material insoluble in
hexane and only slightly soluble in warm chloroform. The amount present appeared
to increase with the age of the tick, and perhaps reflects changes in the cuticle with
age. The nature of the residue was not investigated further.

Ticks were killed before being washed since contact with solvents caused live ticks
to exude droplets of watery material through the cuticle. This did not occur with dead
ticks.

RESULTS

The results of the weighing of cuticle wax are shown in Fig. 1. The rate of loss of
body weight is shown as a histogram on the same time-scale.

It can be seen that the amount of wax per tick increased rapidly during the first
2 days after dropping, doubling in weight between 6 and 54 hr. Over the next 24 hr.
the increase was slight, but from 78 hr. there was a further increase, to three times the
original weight by 150 hr. The weight then remained virtually constant until the end of
the laying period and the death of the tick. The graph thus described has the shape of
two sigmoid curves, indicating that two maxima occurred in the rate of secretion of
wax during the period of measurement.

The figures obtained for weight of wax are lower than those of Gilby (1957), who
found amounts of 47 and 54 µg. per tick for engorged adult females of B. microplus.
The length of time between dropping and extraction was not specified by Gilby but
the ticks used had not begun to lay, and were comparable to those between 6 and 54 hr.
in the present work (W. J. Roulston, personal communication). The higher figure
Production of cuticle wax by Boophilus microplus may be the result of the use of chloroform for extraction, since in the present work this solvent was found to extract more non-lipid material than did hexane. Chloroform extracts were also jelly-like in physical state, as described by Gilby, whereas hexane extracts on evaporation gave typical waxy solids.

The yellow grease described by Lees (1947) as an exudate from the dermal glands of engorged ixodid ticks was not seen. It is described as insoluble in cold chloroform and would therefore be unlikely to be removed by hexane.

Under the conditions of incubation used, laying commenced approximately 54 hr. after dropping from the host and was virtually completed by the ninth day. The rate of loss of body weight of the tick rose sharply with the commencement of laying, reached a maximum on the fourth day and then declined. This agrees with Hitchcock's (1955) figures for the laying pattern of B. microplus under conditions of constant temperature. Since no external excretion has been recorded as taking place during this period of the tick's life-history, the loss of weight measured represents the weight of eggs laid plus losses of carbon dioxide and water.

The most rapid increase in weight of cuticle wax therefore took place in the period immediately before laying began. The slackening of this increase between 54 and 78 hr. coincided with the first production of large numbers of eggs, and the slower increase between 78 and 150 hr. with the later, lower rate of egg production.
The physiological function of the cuticular wax layer of arthropods is generally accepted to be that of waterproofing the animal in such a way that water is neither lost freely from the interior nor allowed to penetrate freely from the exterior. The actual chemical mechanism involved in maintaining this barrier is not known with certainty for any species, but workers in this field (Beament, 1961; Gilby & Cox, 1963; Locke, 1965) suggest that molecular architecture within the wax layer is responsible rather than simply the quantity of wax present. It therefore seems permissible to speculate that the progressive increase in quantity of cuticle wax in *B. microplus* may serve purposes other than that of waterproofing. Three possibilities suggest themselves when the circumstances of the animal's life-history are considered:

(i) Since the likelihood of abrasion of the cuticle increases and the loss of water as a result of such abrasion becomes a more serious matter when the tick drops from the host to the ground, and since the body wall becomes increasingly fragile as laying progresses, the accumulation of a thicker layer of wax may be a protective device.

(ii) A general stimulus to wax secretion may be linked with the process of egg production. The eggs of most ticks, including those of *B. microplus*, are given their final waterproofing after they are laid, by the application of a waxy secretion produced by Géné’s organ, a glandular structure which is “a proliferation of the general epidermis which is folded inwards from the cuticle” (Lees & Beament, 1948). The level of secretory activity of this gland may be shared by the whole epidermal layer.

(iii) Since no external excretion takes place during this period of the tick’s life history, the main end-product of protein metabolism, guanine, being stored in the Malpighian tubules and rectal sac (Hughes, 1954), non-utilizable lipid-soluble material arising from digestion may be eliminated by solution in and continuous transport to the exterior with cuticle wax.

The deposition of new wax in response to damage by abrasion has been shown to occur in ticks (Lees, 1947), but the regularity of the increase in *B. microplus* and the fact that the wax layer reaches its maximum weight after the peak of egg-laying has been passed suggest that repair and protection are not major functions. In practice, also, the cuticle is far more easily ruptured towards the end of egg laying than at the time of dropping, in spite of the extra weight of wax.

Very little is known about the secretory activity of Géné’s organ. At the commencement of laying, large numbers of eggs are produced in a short time, and laying then continues at a lower rate for some time. When the material required for waterproofing is secreted, and at what rate, is not known. Whether the control of secretory activity and its co-ordination with laying is hormonal or nervous is also not known, and at present it is not possible to say whether general epidermal secretory activity is likely to be under the same control.

The possibility that secretion along with cuticle wax may be a route for the removal of minor metabolites would receive support if analysis of the wax showed these to be present. Gilby (1957), using the techniques of mono-molecular surface films and infra-red spectroscopy, concluded that the wax of *B. microplus* consisted mainly of esters of saturated long-chain acids and alcohol, but did not identify minor components. The pathways of digestion and utilization of lipids by the tick have not been investi-
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gated, but presumably the triglycerides, free fatty acids and phospholipids of cattle blood are metabolized. The fate of such lipid-soluble molecules as carotenoids, steroids and hydrocarbons is not known. The work of Kitaoka & Yajima (1958) and Kitaoka (1961) on the respiratory rate, rate of digestion, and development of the female genital system in B. microplus and B. caudatus shows that metabolic activity is at a maximum in the period between dropping and the onset of laying. A detailed study of changes in the composition of cuticle wax, particularly of minor components, in relation to the rate of its secretion and to metabolic and reproductive activity is therefore being undertaken.

Whatever the physiological function of the increased secretion of wax may be, its production forms an integral part of the metabolic pattern of the animal. Resources of food and energy must be allocated to its formation, and its constituents must be the end products of definite, although at present unknown, biochemical pathways. It seems likely therefore that both the function of the wax and the control of its production may be more complex than has hitherto been suggested.

SUMMARY

1. Weight of cuticle wax produced by the engorged female cattle tick, Boophilus microplus (Canestrini), and loss of body weight during egg production, have been measured at intervals between dropping from the host and completion of egg laying.
2. Wax increased in weight from 20 to 63 \( \mu g \) per tick between 6 and 222 hr. after dropping. This increase took place in two stages, with the steepest increase between 6 and 54 hr. and a smaller increase between 78 and 150 hr.
3. Body weight fell by 71% between 6 and 222 hr. The rate of loss was highest during early egg production, reaching its peak on the fourth day after dropping.
4. The most rapid increase in weight of cuticle wax took place before the onset of oviposition. During peak egg production the increase was slight. During the decline of egg production cuticle wax increased slowly in weight.
5. The possible physiological function of the increased wax secretion, and its relation to general metabolic activity, are discussed.

REFERENCES
