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Cholesterol in the Cuticular Wax of *Boophilus microplus*

TRACES of substances giving colour reactions typical of sterols have been found in the cuticle and cuticular wax of several arthropods¹⁻⁶, but they have not been reported from ticks^{7,8}. I have now shown that both free and esterified cholesterol occur in the wax of *Boophilus microplus*, and I have measured the quantity of each present.

Wax was obtained by washing recently engorged adult female ticks with hexane in standard conditions⁹. Thin-layer chromatography was carried out in the solvent system benzene : ethyl acetate 5 : 1; the dried plates were sprayed with 50 per cent sulphuric acid and heated at 110° C. Magenta pink colour developed in two spots the *R_F* values of which corresponded to those of free and esterified cholesterol, and elution of corresponding areas of unsprayed plates yielded material with the infrared spectra of these substances. Free and total cholesterol were quantitatively determined colorimetrically^{10,11}.

In five samples of wax, free cholesterol lay between 0.8 and 0.9 µg per tick, and total cholesterol between 3.2 and 3.8 µg. Thus approximately 2.6 µg was present in the esterified form. Cholesterol itself accounts for approximately 17 per cent of the total weight because the cuticle wax of the newly engorged female weighs 20 µg (ref. 9). The identity of the fatty acids involved in the ester linkage is not known, but if Gilby's⁸ estimate of an average chain length of C₃₀ is taken for calculation of the weight of cholesterol ester present, free and esterified cholesterol together make up 32 per cent of the wax.

This high proportion was unexpected, because although it has been suggested that sterols may be common constituents of cuticle wax¹², they have not been proposed as major constituents. Cuticle wax provides the main barrier to the passage of water across the tick cuticle⁹, but whether sterols are among the components necessary for the maintenance of the water proofing structure is not known; sterols in this quantity are certainly not involved

in other arthropods. It has been suggested that in *B. microplus* cuticle wax may act as a vehicle for the removal of lipid-soluble material derived from the food⁹. Cholesterol was therefore determined in wax from females weighing about 15 mg, still feeding on the host but before final engorgement, and from females at the peak of egg laying, several days after dropping from the host. Total cholesterol values were 3.5 and 3.0 µg per tick respectively; thus neither engorgement nor the rapid digestion of food which accompanies egg production had increased the quantity already present.

Clearly the origin of the cholesterol and the timing of its secretion must be investigated before any attempt can be made to account for its presence. No information on the ability of acarines to synthesize sterols appears to be available. It is accepted, however, that insects, and probably crustaceans¹³, are unable to do so. If it is assumed that extensive synthesis by *B. microplus* is unlikely, the cholesterol in the wax must have been derived from the food. *B. microplus* passes through larval, nymphal and adult stages on the same host, the female adult increasing in weight from 2 mg to 15 mg over a period of 5 to 6 days after attachment, and then to 150–200 mg during a final period of about 24 h rapid engorgement¹⁴. From estimates of the quantity of erythrocytes and plasma obtained by the various stages¹⁵, it can be calculated that about 1 µg cholesterol is ingested during larval and nymphal feeding, and about 10 µg during adult growth to 15 mg. Possible losses during larval and nymphal stages are unknown. These estimates of intake are probably too low, because the diet also includes components of the epidermis and upper dermal tissue and large numbers of leucocytes¹⁶, all of which have a considerable cholesterol content^{17,18}. It does seem, however, that the cholesterol secreted into cuticle wax may represent a significant proportion of that ingested during early adult growth, the proportion being higher the earlier the secretion takes place.

Arthropods using mammalian blood and skin tissues as food inevitably obtain quantities of cholesterol much greater than those regarded as essential in the diet¹². The uses to which they put this excess, and the routes by which they dispose of it, have not been investigated to any extent. It would be interesting to know whether significant secretion into cuticle wax occurs in other blood-sucking species and, if so, whether this ability can be further correlated with habits of feeding, excretion or egg-laying.

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