A second active phenol was eluted from the column with 50 per cent aqueous alcohol; but the quantity was too small for detailed investigation.

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Epicuticle of Blow-fly Larvæ

IT is now clear that in many insects the epicuticle is the waterproofing layer of the cuticle. Although difficult to define morphologically[1], it often carries a wax layer, removable by abrasion or adsorption. *Rhodnius*[2] and *Tenebrio*[3] undergo desiccation after removal of the wax layer by inert inorganic dusts; but blow-fly larvæ are unaffected[4], suggesting a different constitution of the epicuticle. Indeed, the habits of blow-fly larvæ in liquefying the food in which they burrow suggests that the prime function of the epicuticle is other than the prevention of evaporation from the surface of the cuticle. Since the food is liquefied by the secretion on it of the digestive enzymes, it is desirable to discover whether or not a function of the epicuticle is to protect the underlying protein-containing endocuticle against digestion. The great chemical stability of the outer layer of the epicuticle is in agreement with such a view, for it is not dissolved by saturated potassium hydroxide solution heated to 160° C., or by cold concentrated hydrochloric or nitric acids. On boiling with concentrated nitric acid to which potassium chlorate has been added, however, it disrupts into oily droplets which stain with Sudan Black *B*. It therefore appears to be composed of the material termed cuticulin by Wigglesworth[1,5].

Portions of the cuticle removed from larvæ of *Calliphora erythrocephala* have been treated with the fluid obtained from the semi-liquid food by diluting and filtering. After 24 hr. at 25° C., the cuticle became obviously more delicate and transparent, and failed to give readily the ninhydrin reaction shown by the fresh cuticle. After 48 hr. only a delicate membrane, similar to that remaining after treatment with acid, was left. This membrane is the outer epicuticle. No changes occur if the food extract is previously boiled, and it is clear that all the cuticle except the outer epicuticle is susceptible to digestion.

A further experiment consisted of cutting off the anterior and posterior ends of larvæ and removing the body contents from the tubes of cuticle so left. These tubular cuticles were then slipped over glass rods of suitable diameter, some with the epicuticle outwards and some turned inside out to expose the endocuticle. The ends of the cuticle were bound tightly with thread to prevent ingress of fluid between the rod and cuticle, and cuticles arranged in these ways were exposed as before for 48 hr. to the liquid food extract. The result was unequivocal. In those preparations in which the endocuticle had been protected by the overlying epicuticle, histological examination showed the cuticle to be intact, whereas when the endocuticle had been exposed nothing remained but the outer epicuticle.

This protective function of the epicuticle in blow-fly larvæ is to be regarded as an extension into the post-ecdysial period of the part played at every moult in insects generally, when the epicuticle protects the new integument against dissolution by the moulting fluid.

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Quinone Tanning in the Animal Kingdom

PROF. R. DENNELL suggests in his letter in *Nature* of August 27 that quinone tanning of protein structures may be of wide occurrence in the invertebrates. During investigations of structural proteins in the vertebrates and invertebrates, I have found evidence of quinone-tanned proteins in the central capsule membrane of *Thalassicola*, the external cortical layer of the cuticle in *Ascaris lumbricoides*, the chætæ of *Aphrodite*, the byssus[1] and periostracum of *Mytilus edule*, the byssus of *Dreissensia polymorpha* and in the egg cases of various selachian fishes. There are numerous other structures, such as the winter egg cases of planarians and the egg cases of various molluscs and earthworms which suggest, by their colour, the presence of aromatic-tanned protein. Ellerby[2] has evidence for quinone tanning in the cyst wall of eelworms.

Undoubtedly, therefore, quinone-tanned proteins are of widespread occurrence. While these various structures agree in showing very great chemical stability, there is evidence that the sequence of events leading to the tanning differs in different cases. In the tanning of the cockroach oothecæ, the precursor of the quinone is an alcohol-soluble phenol, protocatechnic acid[3,4]. In *Mytilus*, the precursor is not alcohol-soluble, and its properties suggest that it may be an aromatic amino-acid or protein, and there is evidence of a similar precursor in the hardening of the egg cases in *Fasciola hepatica*. If, in these cases, the side-chain is split off before oxidation to a quinone occurs, then the final method of tanning is not significantly different from that in insects. But if oxidation occurs without the removal of the side-chain, then it suggests a way in which quinone tanning may have evolved. Tyrosine is present in nearly all proteins and probably forms salt links through its phenolic hydroxyl group; and it is a comparatively small step to improve on this mechanism by oxidizing the hydroxyl to a quinone, which can form covalent bonds.

It is hoped to publish elsewhere a full report of this work, which was carried out at the Zoological Laboratory, Cambridge, the Marine Biological Station, Plymouth, and Stazione Zoologica, Naples.

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