Role of Lipid in the Hardening of the Cuticle in the Silkworm, *Bombyx mori*

Malek[1], and Dennell and Malek[2], have stated that the cuticle of *Periplaneta* becomes impregnated with a sterol and a protein rich in tyrosine, and Dennell[3] has given evidence that, in the development of the puparium of *Calliphora*, destruction of localized areas of the epidermis prevents the passage of sterol to the cuticle and also causes lack of tanning of the cuticle immediately above the operated areas.

Table 1. HARDENING AND PIGMENTATION OF THE PUPAL CUTICLE TREATED WITH SOME ORGANIC SOLVENTS AT 25° C.

Solvent	Pigmentation	Hardening	Remarks
Methanol	(±)*~+	(±)~+	10–20 per cent of treated pupæ survived
Ethanol	(±)~+	(±)~+	,, ,,
Butanol	−~(±)	−	Died in a few hours
Acetone	(±)~+	(±)~+	10–20 per cent of treated pupæ survived
Ether	±~+	−~±	Died in 2–3 days
Chloroform	−~(±)	−	Died in a few hours
Carbon tetrachloride	−~(±)	−	,, ,,
Benzol	−~(±)	−	,, ,,
Xylol	−~(±)	−	,, ,,
Ethyl acetate	−~(±)	−	,, ,,

* Brackets indicate that the effect was not usual. + = pigmentation (or hardening); ± = slight pigmentation (or hardening); − = no pigmentation (or hardening).

The lipid layer of the epicuticle of very young and soft pupæ of the silkworm *Bombyx mori* (race *N*124 × *C*124) has been removed within 30–150 min. of pupation either by immersion in ether for 10–15 sec. or by rubbing with cotton wetted with ether. After these treatments, pigmentation of the treated parts proceeds only slowly or imperfectly, and the cuticle remains unhardened. The pupæ thus treated shrank due to the evaporation of water, but some remained alive for a few days and showed an almost normal amber colour in the whole body or only in the region of the dorsal blood vessel (Fig. 1, *b* and *c*). This did not happen when methyl alcohol, ethyl alcohol, or acetone was substituted for ether. Pupæ treated with chloroform, xylol, benzene, ethyl acetate or carbon tetrachloride died within a few hours (Table 1).

Histochemical observation shows that the cuticle treated with ether is almost deprived of epicuticle lipid (see ref. 4). Replacement of the lipid by the ether extract from the cuticle or by 'Vaseline' prevented the pupæ from shrinking, but did not cause cuticle hardening to proceed.

In agreement with Malek[1], and Jones and Sinclair[5], these results indicate that hardening and pigmentation of the cuticle are independent processes. However, it is not clear whether the lipid is directly involved in the hardening process, or whether it merely plays some part in facilitating the process. Microanalysis by paper chromatography has shown that the ether extracts from cuticles contain no phenolic substances.

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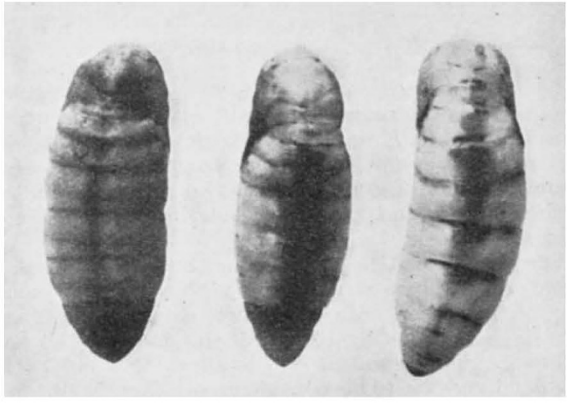
[1] Malek, S. R. A., *Nature*, **170**, 850 (1952). **180**, 237 (1957).
[2] Dennell, R., and Malek, S. R. A., *Proc. Roy. Soc.*, B, **143**, 414 (1955).
[3] Dennell, R., *Ent. exp. et app.*, **1**, 268 (1958).
[4] Takahashi, *Bull. Nagano Seric. Exp. Stat*, **58**, 1 (1959).
[5] Jones, B. M., and Sinclair, W., *Nature*, **181**, 926 (1958).



(*a*) (*b*) (*c*)

Fig. 1. Pupæ 6 hr. after ether treatment. (*a*) Control; (*b*) and (*c*) treated pupæ

Action of Neuraminidase on Haptoglobin

IN the course of work on the chemical structure of haptoglobin (Hp)[1], we have had occasion to investigate the action of neuraminidase on the Hp and on the complex of Hp with hæmoglobin (Hb-Hp). The neuraminidase employed was prepared as described by Mayron and Rafelson[2,3] from influenza virus, Asian strain (Jap. 305), and is free of proteolytic activity and glucosidase activities other than neuraminidase action. The Hp II preparations utilized were prepared as previously described[4], and are considered to approach the stage of theoretical purity. Such preparations contain 5·1 ± 0·1 per cent N-acetylneuraminic acid (NANA)[1]. The liberation of NANA from Hp II and Hb-Hp II was determined by the method of Warren[5] using three-times crystallized NANA as a standard. A typical enzyme incubation at 37° employed 2–5 mgm. of Hp II or Hb-Hp II and 0·1 to 0·3 unit of neuraminidase in 0·1 *M* phosphate buffer, *p*H 6·5, total volume 2–3 ml. One unit of enzyme activity is defined as that amount of enzyme which liberates 1 mgm. of NANA from an excess of neuramin-lactose in 1 hr. at 37°, *p*H 6·5.

Fig. 1 shows the percentage of NANA liberated as a function of time from Hp II and Hb-Hp II by the

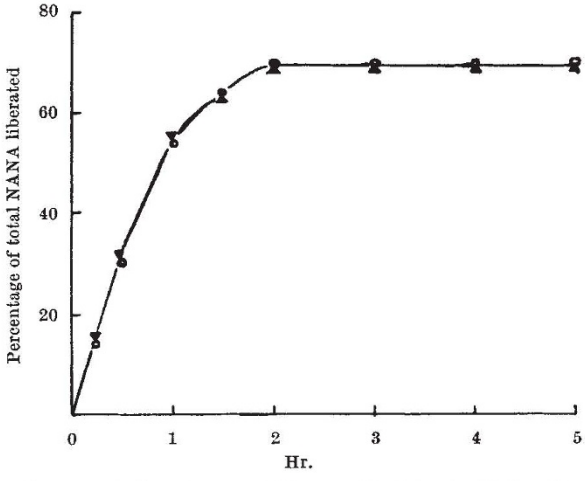


Fig. 1. Action of neuraminidase on Hp II and Hb-Hp. ○, Hp II; ▲, Hb–Hp II