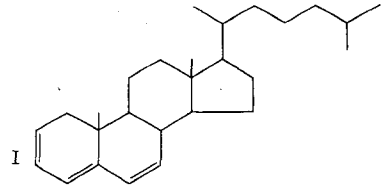
New Evidence on the Function of the Porose Areas of Ixodid Ticks

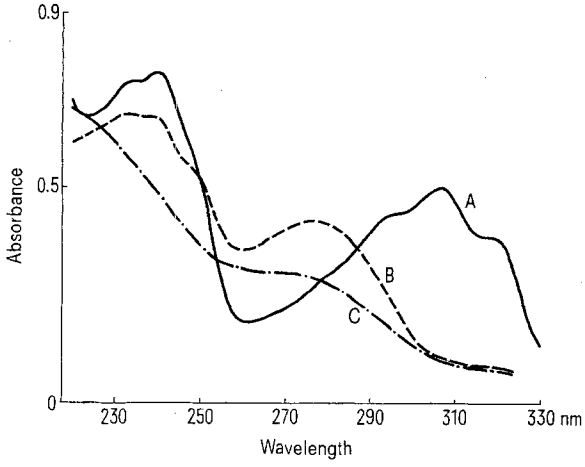
FELDMAN-MUHSAM[1,2] showed that the porose areas of ixodid ticks were connected via fine ducts to secretory glands and suggested that the secretion acts as a lubricant and is necessary for the normal functioning of Gené's organ. Previously the ducts were thought to be nerves[3,4] and the resulting assumption that the function of the porose area is sensory has persisted[5]. Dissection and histology of engorged female ticks from a number of ixodid species confirms that each area consists of cuticular ducts arising from simple acinous glands, the number of glands approximating the number of cuticular ducts. In the engorged female of the cattle tick, *Boophilus microplus*, the secretion arising from these ducts is only visible 1–2 days preceding the eversion of Gené's organ. On eversion, Gené's organ, which waxes the eggs[6], completely covers the porose areas, thereby incorporating the secretion into the egg wax. The secretion is predominantly hexane-insoluble and its incorporation into the wax is readily demonstrated by its appearance as a fine yellow precipitate in hexane washings of normal eggs.

To test the theory that the secretion acts as a lubricant the porose areas of freshly dropped engorged female *B. microplus* were selectively destroyed. The most effective method was electrocautery using a Birtcher hyfrecator. The treated ticks were then incubated at 27 °C and 85% relative humidity and examined periodically for signs of the secretion. Of 60 treated ticks, 2 continued to secrete and were discarded. In the remaining ticks Gené's organ functioned normally and no significant differences in the size or hatchability of egg batches from treated and untreated ticks were observed.

Evidence now suggests an alternative function for the porose area secretion. Surface lipids from eggs of *B. microplus* contain mainly hydrocarbons and cholesteryl esters[7]. Compounds showing UV-absorptions identical to those produced by $\Delta^{2,4,6}$-triene steroids[8] are prominent. One such compound, the major hydrocarbon, has now been identified[7] as $\Delta^{2,4,6}$-cholestatriene (I)[9]. Hexane washings of eggs from *Ixodes holocyclus*, *Amblyomma triguttatum triguttatum*, *Rhipicephalus sanguineus*, *Haemaphysalis longicornis* and *H. bremneri* (but not *Ornithodoros gurneyi* or *Argas persicus*) also produce UV-absorptions characteristic of $\Delta^{2,4,6}$-triene steroids. This suggests that steroids of this type are present in egg-shell lipids of Ixodidae, but absent in Argasidae.



Similar quantities of the $\Delta^{2,4,6}$-triene chromophore, as determined by UV-spectrophotometry, are found in hexane washings of freshly oviposited eggs from both cauterised and untreated *B. microplus*. When eggs from untreated *B. microplus* are incubated and hatched at 27 °C and 85% RH this same quantity of triene is again obtained from the hatched egg-shells. However, when eggs from cauterised *B. microplus* are incubated, the



Observed changes with time, at 64 °C, in the UV-spectrum of hexane-extracted wax from eggs of *B. microplus*. For this experiment the porose areas of the engorged females were selectively destroyed by electrocautery before oviposition. Traces A, B and C represent spectra recorded after 0, 5 and 72 h respectively.

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Induction periods for the autoxidation of triene steroids in *B. microplus* egg waxes, with and without naturally incorporated porose area secretion

Wax sample	Conditions	Induction period (days)	
		With secretion	Without secretion
Intact	a) Incubator (27 °C and 85% RH)	>28	6–8
	b) Laboratory bench (25–26 °C and 40–60% RH)	–	2–3
	c) Laboratory bench (25–26 °C and 100% RH)	12–14	3–4
Hexane-extracted	a) Incubator (27 °C and 85% RH)	>35	0.5–1
	b) Laboratory bench (25–26 °C and 40–60% RH)	3–4	0.5–1
	c) Oven (64 °C)	1–2	0–0.1

Both isolated and intact wax samples were used and induction periods were determined from spectrophotometric measurements at 306 nm.

hatched egg-shells yield little or no triene. This suggested that triene compounds were autoxidised during incubation and that the reaction was inhibited in the presence of porose area secretion. This was confirmed by comparing the stability of triene in the wax, with and without porose area secretion, under various other conditions (Table); the reaction induction periods[10] were significantly longer in those with secretion. The shorter induction periods found under laboratory bench conditions probably result from the influence of light as an initiator of autoxidation[11]. Typical spectra for the autoxidation are in the Figure; absorptions assigned to triene (294, 306, 320 nm) are initially eliminated followed by those in the 220–250 nm region. The removal of absorptions at 220–250 nm reveals that other compounds are also autoxidised, although not as readily as those containing the triene chromophore.

ATKINSON et al.[12,13] have recently shown that *cis, cis*-6, 9-heptacosadiene, the major hydrocarbon of the cuticular wax of the cockroach, *Periplaneta americana*, in contrast to its apparent stability when undisturbed on the cuticle, undergoes extreme autoxidative changes when isolated from the cuticle. On the cuticle the *o*-dihydric phenols involved in sclerotization effectively block such autoxidation. A study of the egg waxes of ixodid ticks has now yielded significant amounts of conjugated triene steroids also susceptible to autoxidation. We have shown that the cattle tick, *B. microplus*, inhibits autoxidation of these steroids and other compounds in its egg waxes by the incorporation of a natural antioxidant during deposition of the wax layer by Gené's organ. The secretion from the porose area is the source of that antioxidant.

Zusammenfassung. Es wird die sekretorische Natur der Area porosa in Ixodesarten bestätigt, jedoch nicht die Annahme, dass das Sekret als Schmiermittel für die normale Funktion des Genéschen Organes notwendig ist. Das Sekret wird offenbar in den wachsartigen Überzug des Eies aufgenommen und verhindert dort die Autoxydation der unbeständigen $\Delta^{2,4,6}$-trien-Steroide.

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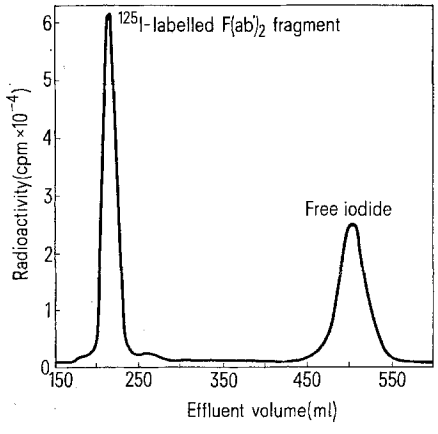
Insolubilized Lactoperoxidase for the [^{125}I]-Labelling of Proteins

The use of lactoperoxidase as a catalyst for iodination by hydrogen peroxide and carrier-free [^{125}I] iodide provides a mild system for labelling proteins to high specific activity with minimum denaturation[1]. Separation of soluble lactoperoxidase (mol. wt. 84,000) from some labelled protein of comparable molecular weight may become a problem, but is easily accomplished with an insolubilized enzyme. Here we present details for the preparation of such an enzyme derivative and describe as an example the labelling of antibody fragments of the type F(ab')$_2$ (mol.wt. 106,000).

Methods. Enzacryl AA[2] (50 mg) suspended in 2 *N* HCl (5 ml) was diazotized by the dropwise addition of 4% (w/v) $NaNO_2$ (2 ml) followed by further stirring for 15 min and centrifuging (all operations for enzyme coupling were carried out at 0°C). The pellet was washed with 4 portions (8 ml) of 0.05 *M* sodium phosphate, pH 7.4 and was resuspended in the same buffer (1 ml) containing lactoperoxidase[3] (1 mg). Coupling was allowed to proceed with stirring for 60 h. After centrifuging, residual diazonium groups were reacted with 0.01% (w/v) phenol in 10% (w/v) sodium acetate (8 ml) for 15 min. Unbound enzyme was removed by 6 washings (20 min each with vigorous stirring) with 0.5 *M* NaCl in 0.05 *M* sodium phosphate, pH 7.4 (6 ml) followed by 3 brief washings with 0.05 *M* sodium phosphate, pH 7.4. Finally, the orange-coloured derivative was suspended in the same buffer (1 ml).

The enzyme suspension (0.4 ml) was mixed with a preparation of F(ab')$_2$ fragments[4] (10 mg) in the same buffer (1 ml). The mixture was transferred to a disposable tube containing carrier-free Na [^{125}I] (2 mCi in 20 µl of water; concentration of iodide, 0.05–0.1 m*M*)[5]. To this was added 0.88 m*M* H_2O_2 (0.2 ml), and the suspension was stirred for 15 min at 22°C. The reaction mixture was filtered through cottonwool by aspiration with a Pasteur



Separation of the reaction products on Sephadex G-100 following [^{125}I]-labelling of F(ab')$_2$ antibody fragments using an insolubilized preparation of lactoperoxidase. For experimental details, see text.

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