Accessory Glands of Gene's Organ in Ticks

During dissection of gorged adult females of *Hyalomma excavatum*, *Rhipicephalus sanguineus* s.str. and *Boophilus annulatus*, we noted two hitherto undescribed groups of accessory glands, one on each side of Gené's organ.

The glands become evident soon after the onset of feeding and continue to develop during the period of digestion of the blood meal. In *Ripicephalus*, which oviposits 2–3 days after gorging, the development is completed during this interval; in *Hyalomma*, in which oviposition occurs ten days after gorging, the development is correspondingly slow. The glands attain full development by the time oviposition begins. They were not detected in the starved female or the nymph. We did not find these glands in adult *Ornithodorus tholozani*.

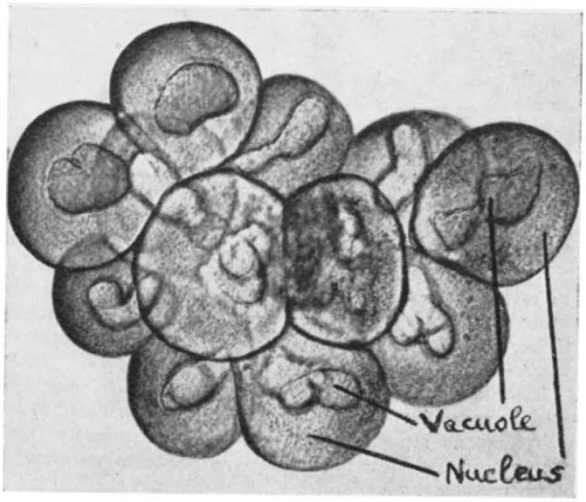

Fig. 1. New accessory gland from a female of *Rhipicephalus sanguineus* s.str., on the third day of oviposition. (× 140)

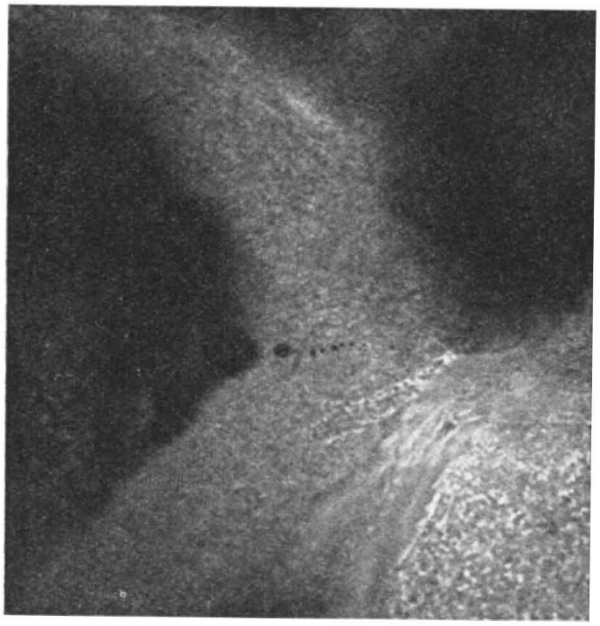

Fig. 2. Droplets of secretion on their way through the intracellular channel from a laying female of *Rhipicephalus sanguineus* s.str. (× 660)

Each group of glands consists of about 40 units in the case of *Hyalomma* and between 20 and 25 in the case of *Rhipicephalus*.

Each gland consists of about 10–13 cells. In general the cells are arranged roughly in a circle (Fig. 1). The large nucleus (up to 85μ) is situated in the lateral part of the cell; it contains several nucleoli. In each cell there is a large vacuole which grows continuously and attains a size of about 100–130μ at its maximum development. Each gland has a duct issuing from its centre; a narrow intra-cellular channel passes from each vacuole to the narrow main duct. On staining with methylene blue, droplets can be traced on their way from the vacuole via the narrow intra-cellular channel into the duct (Fig. 2).

These glands apparently contribute to the secretions of Gené's organ which protect the eggs in their external environment.

This investigation was supported by Research Grant No. 4531 from the National Institutes of Health, U.S. Public Health Service.

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CYTOLOGY

Induced Fluorescence with Acridine Orange, after Osmium Fixation

The differential staining of nucleic acids with fluorochromes[1] has provided a useful tool for the study of virus-infected tissue cells[2]; but its value has been limited by the fact that the common fixatives which permit the use of this method also tend to produce artefacts, such as the gross nuclear vesiculation observed in cells infected with some types of adenovirus[2], but not observed by phase-contrast examination of the living infected cell[3].

This serious limitation could be overcome if the technique could be adapted for use with fixation by osmium tetroxide, which has been shown to preserve faithfully most of the structural features of the living cell[4,5]. Up to the present, however, this has not proved possible, and reports indicate practically no affinity between the diamino-acridines and nucleic acids fixed in osmium tetroxide[3].

Recent work in this laboratory has shown that this lack of affinity may be overcome if staining is carried out with acridine orange in alcoholic solution, in place of the buffered aqueous solution, as usually practised. The technique described is simple and uniformly successful, giving vivid colour differentiation and even staining over the entire preparation. Acridine orange *R* (2,8-*bis*-dimethylamino-5-phenylacridine hydrochloride, Colour Index No. 792) in 1 : 1,000 dilution in industrial methylated spirit ('Methcol') was used, and this was filtered before use. The end result was found to be the same, however, using dilutions of lesser or greater strength, and allowing for an appropriate alteration in the staining time.

Cells of the 'ERK' cell line[6] were grown on glass microscope slides to form a near-confluent monolayer. The medium was washed off with phosphate-buffered saline, *p*H 7·2, and sample slides were then fixed either in the vapour from 1 per cent osmium tetroxide