PRELIMINARY RESULTS OF ANATOMICAL AND HISTOLOGICAL EXAMINATION OF THE *VARROA JACOBSONI* OUD. MITE *)

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The parasite mite *Varroa jacobsoni* Oudemans, 1904, relatively recently introduced into Europe — around 1950, causes the *Varroa* disease in bee brood and adults, with serious consequential effects on the biology of the bee colony.

At present, most authors include this mite into *Dermanyssidae* family, on account on the generally occurring disproportion between shields and polytrichia characteristic of many genera of this family. O. HARAGSIM & SAMSINAK (1972) included this mite into the new subfamily *Varroinae* — of *Dermanyssidae* family, thus providing for the possibility of incorporation into the classification scheme of EVANS & TILLE (1966). In support of their opinion, the authors bring forth morphological characteristics : the form of chelicerae — like a stylet ; the mobile palp with 3 teeth, in the apical end ; lack of the 2nd hair on hypostomum, the serrate polytrichia, a single dorsal scutum (shield) ; the sternal scutum merges into the metasternal scutella ; the genito-ventral scutum is large and covered by serrate hairs ; the other ventral scuta are also large, covering almost completely the posterior ventral zone of the body ; the peritremes are transversal.

We studied a number of anatomo-histological elements, being primarily concerned with the biology of the *Varroa jacobsoni* female.

Reports in the specific literature up to now (GROBOV, 1976, and the book "*Varroasis, A Honey Bee Disease*" released by the APIMONDIA Publishing House, 1977) include a few data about this subject. Preliminary observations were made by A. V. SADOV (1976) — of the anatomy of the digestive and excretory systems, and A. B. LANGE *et al.* (1976) described the traheal system.

Material and methods

The material investigated was from the apiary No. 9 of the Resca beekeeping section (Caracal), sampled on 30.07.1977. The mites were collected from the bodies of worker bees and drones, or from the comb cells containing drone larvae and pupae. Only *Varroa* adult females (186) were found, and one male.

The females which had to be examined histologically and histochemically — photon microscopy, were fixed in Cornoy solution (3 hrs) with direct dehydration in absolute alcohol ; in Bouin solution with dehydration in normal ethyl and butyl alcohol (72 hrs) ; in Bouin-Hollande solution with dehydration in butyl alcohol and formol 10% (3 days).

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For sealing we used Merck paraffin, and paraffin-beeswax 5% mixture.

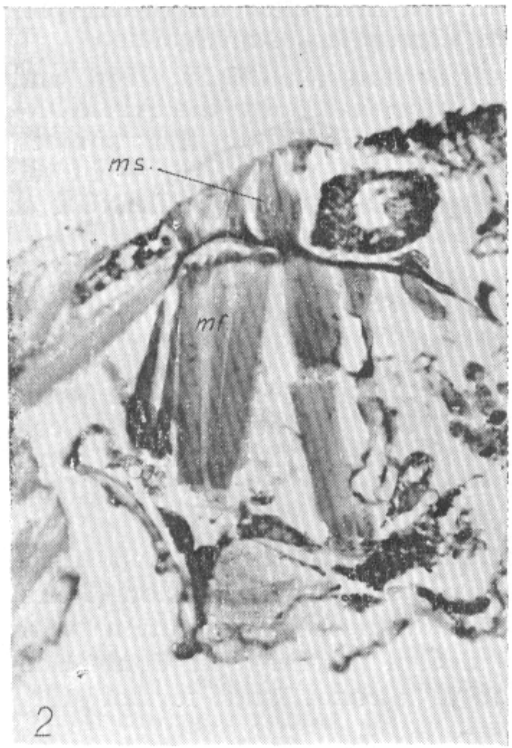
The sections of 3—10μ thickness were sagittal, frontal and transversal. 22 adult females were sectioned.

The following staining methods and of histochemical reactions were used : hemalum, hemalum Mayer + eosin ; Feulgen reaction for nucleic acids (ADN) ; chromic hematoxylin (Bargman) for revealing neurosecretions ; Pas (Per-iodic acid + Schiff) for identifying neutral polysaccharides, and Mowry staining with alcian blue for acid polysaccharides ; the method of alizarin red S for identifying the intracellular calcium.

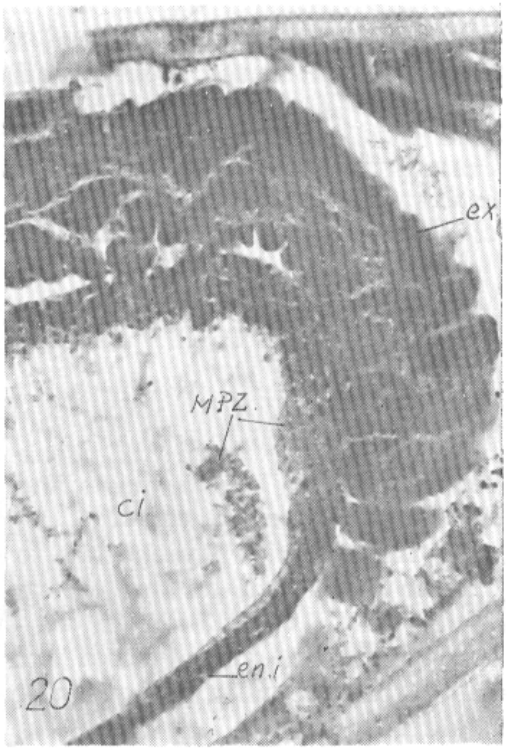
Anatomo-histological structure

The general structure of the *Varroa jacobsoni* adult female is the same as of chelicerae arthropods, and its particularities — as those of *Gamasoidea*. All organs are inside the only cavity of the body (hemocoelome) which extends itself into the apendix.

T e g u m e n t. The body is covered, just as in all *Gamasoidea*, by a 0.4—0.12 μ thick and hard three-layer cuticle (ectostracum, mesostracum, and hypostracum). It is thicker in the dorsal scutum area, and less thick in the area of gnathosoma (0.09μ) and of the ventral scutum (0.17μ). Under the cuticle is the thin hypodermis (endostracum), consist-



Musculature : ms — suspensory muscles of endosternite ; mf — flexor muscles of legs



Digestive apparatus: ci — intestinal caecum; ex — excretory duct; MPZ — muccopolysaccharides ; eni — intestinal endothelium

ing of small, elongated cells whose limits are not always visible, with oval and intensly chromatic nuclei. Cytoplasm extensions from hypodermis cells can be seen sporadically reaching the cuticle — at the basis of hairs.

M u s c u l a t u r e. In the anterior part of the body there is a strong musculature — mainly dorso-ventral muscle bundles.

In many sagittal and cross sections, one can clearly see 4 pairs of long muscles fixed laterally on the underside of episternum (endosternite) — the leg flexors. A bundle of oblique muscles (suspensory muscles of the episternum) are attached on one side to the episternum, and to the cuticle (hipostracum) on the other side.

In the series of sagittal sections one could see a dorso-ventral muscle bundle, laterally, separating the posterior end of the opistosoma, rich in conjuctive tissue and large amibocytes, from the rest of the body cavity.

On a series of cross sections, visible on each side of the body — from podosoma zone to the posterior one, of opitosoma, are dorso-ventral muscle bundles (dorsal adducent muscles), placed obliquely, and in some sections one can see extensions of lateral stomach caecums. On these sides, the body cavity is full of relaxed conjunctive tissue and many amibocytes.

In frontal sections, the strong musculature of appendix (chelicerae, pedipalps, legs) are visible.

N e r v o u s s y s t e m. In *Varroa jacobsoni* the nervous system is highly concentrated ; it consists of a nervous mass or brain which lies in the central and ventral part of prosoma. Nervous trunks are branching out from it.

The brain is of elongated shape, 1.93—2.14μ high and 2.04—2.95μ long — in sagittal sections. It consists of a supraoesophageal nervous mass (brain ganglions), a suboesophageal ventral mass, and a pair of segmental ganglions. The two nervous masses are connected by a perioesophagel ring consisting of nervous fibres, so close to the oesophagus as it appears to go throughout the brain. The suboesophageal mass is better developed than the supraoesophageal one.

The brain is covered by cortex (neurilema), a thick layer with quite well developed conjunctive tissue around it. The limits of the cortex cells are not visible, nuclei are relatively small (0.02—0.03μ) and intensely chromatic. Neurilema extends itself along the nervous trunks.

The medullary part of the brain (neuropil) is a network of thin fibres crossing the brain longitudinally, transversally and dorso-ventrally, and connecting the paired ganglions on the left and right sides of the brain.

In the sagittal and frontal sections of the suboesophageal mass one can clearly see the 4 pairs of pedious nervous centres and the nerves branching out from them.

Also extending from the suboesophageal mass are : a pair of anterior nerves (nerves of palps), and a pair of posterior nerves (splanchnic nerves). In cross and frontal sections, the commissures connecting the pairs of pedious ganglions are visible.

In a series of frontal sections, the nervous centres of gnathosoma (probably of chelicerae) are separated from the supraoesophageal mass to which they are connected by a thin bridge. A nervous trunk branches out on one side of each centre.

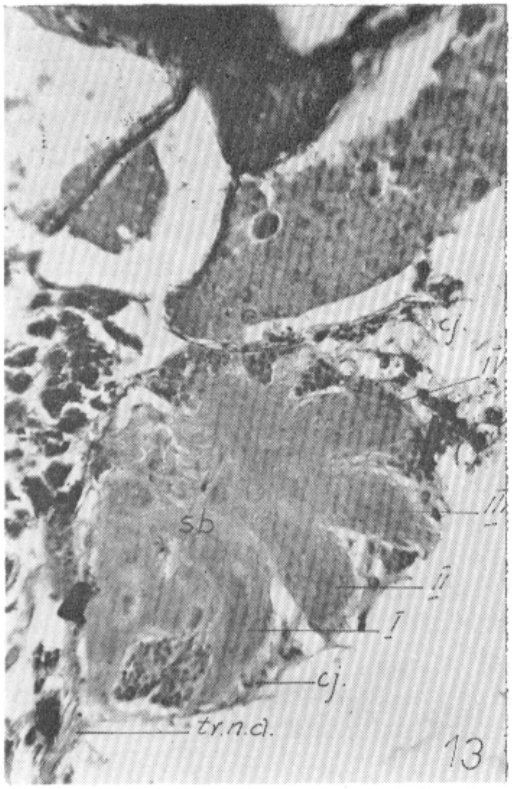
No neurosecretion was identified with hematoxylin staining.

The digestive apparatus has the already known structure (GROBOV, 1976), consisting of pharynx, oesophagus, ventriculus or midgut with 3 pairs of caecums, and the hindgut.

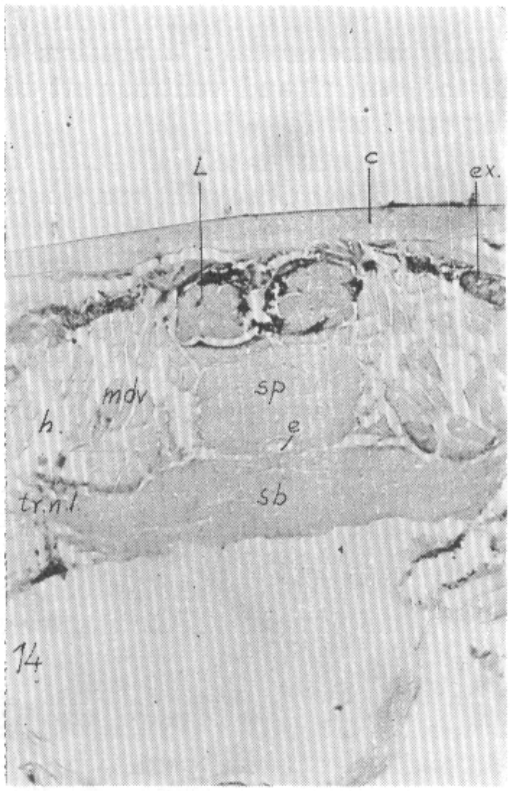
The oesophagus, after passing through the brain, winds in a large curve dorsally, and reaches the ventriculus. Histological examination showed that the wall of oesophagus consists of flat and elongated cells, with small, flat nuclei. No chitin exists in the segment passing through the brain.

Ventriculus. The pair of anterior caecums reach the dorsal part of the brain, in some parts even protruding over its anterior part. The posterior caecums extend to the hind part of the body ; they are longer and larger than the former. Just like the whole ventriculus, the posterior caecums may be compressed because of the development of the female genital organs and especially because of the eggs with embryo.

In histological sections, the wall of the ventriculus and of its caecums have an outside layer consisting of flat cells, whose shape is hardly visible, with rare nuclei, intensely coloured when stained with



Nervous system : cj — conjunctive tissue ; I—IV — nerve centres and pedious nervous trunks ; tr.n.a. — anterior nerve trunck



Nervous system : L — lyriform organ ; c — cuticle; ex — excretory duct; mdv — dorsoventral musculature ; sp — supraoesophageal nerve mass ; h — haemocoel ; e — oesophagus ; tr.n.l. — lateral nerve trunks; sb — suboesophageal nerve mass

hemalum and hematoxylin ; the endothelium consists of several layers of cells of irregular shape, sometimes high, with small oval, chromatic nuclei, with vacuolar cytoplasm. In a number of sections, free cells are seen in the caecum lumen, in which nuclei still exist.

Pas and Mowry staining for muco-polysaccharides have revealed the presence of secretions containing neutral muco-polysaccharides and acid muco-polysaccharides respectively, in the apical end of the high cells, in the free cells, and in the caecum lumen.

The midgut continues with the hindgut (DOUGLAS, 1943 — rectal duct). A rectal valve separates the rectal duct from the rectum. The cells of the rectal duct are large and well defined, and have many oval nuclei. In the anal pouch, guanine granules from the Malpighian tubes and other substances are accumulated.

The excretory organs are two tubes (Malpighian tubes) which pass through the body, on both sides, reaching the ventriculus. In some individuals, their closed end reaches the anterior part of proterosoma. At the posterior part, they reach the ventral zone and parallel the intestine and genital ducts, opening into the rectum.

The cells of the walls of the excretory tubes are large, their nuclei are intensely coloured and with nucleoles. In cross sections, it is seen that at the distal, open end of the tubes the wall consists of several large cells which delimit a large lumen.

Numerous highly refringent guanine granules occur both in cells and lumen all along the excretory ducts.

The reproductive system in the females of various mite species has a complex structure. It consists of an ovary prolonging to the fore part by the lyriform organ ; of an oviduct, vagina, and the vaginal orifice. In addition there are the bell-shaped ducts, the spermatheca, the chamber and the cornua of the pouch which are all connected with the ovary ; the function of all these organs have been a highly disputed problem (JAKEMAN, 1961).

We describe only the organs which were distinctly visible in our sections.

The single ovary lies in the posterior and central or dorsal part of opistosoma. It has the shape of a banana and is a compact mass. Its wall consists of a thin endothelium of flat cells, under which germinal cells in various stages exist. The nuclei of oocytes have a diffuse chromatin lying, in the first stage, in the peripheral zone ; the it begins to concentrate into a higly basophilic pellet ; then it fractures itself into granules which accumulate in the peripheral zone of the nuclei. The secondary oocytes lie in the posterior and dorsal part, while the egg — in the central ventral part.

The lower part of the ovary consists of a mass of ovules whose characteristics are those of a syncitium with many nuclei (feeding stroma).

The lyriform organ consists of two long tubes which, in some individuals, reach the anterior end of the body, sometimes the basis of gnathosoma. The cells of their walls have a polygonal shape, and their nuclei are large and have nucleoles. At the distal end the tubes are very close to one another. The tubes of the lyriform organ are almost parallel

to the excretory ducts, but they cannot be mistaken for the latter because of the differing structure of the cells of their wall, and of the guanine present in the lumen of the latter. One of the speculative assumptions about the function of this organ is that of MICHAEL (1892) according to whom it has a germinative function. In fact two hypotheses are commonly accepted : that of a vitellogenous function and that of a germinative function.

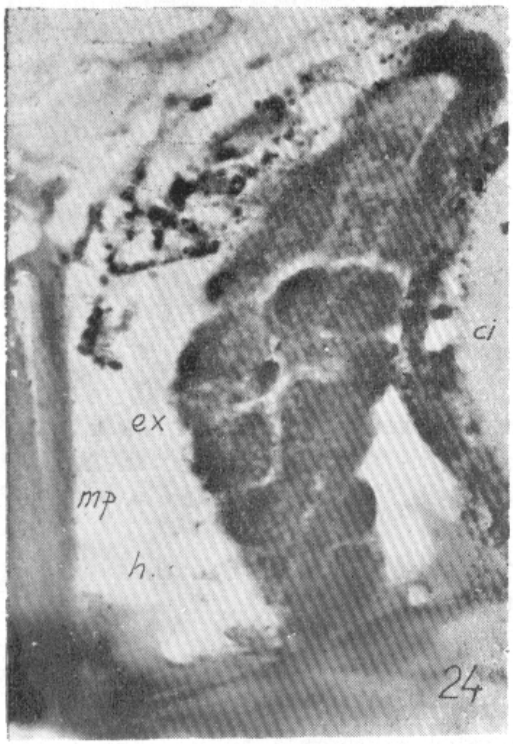
Close to the ovary, dorsally, there is the spermatheca *(sacculus faeminaeus,* MICHAEL). Its wall is thin, with the cells having small nuclei visible only when the spermatheca is full of sperm.

The oviduct (uterus according to some authors) contains one single egg with embryo ; it is large and rich in vittelus. The egg stretches over a great part of the body cavity.

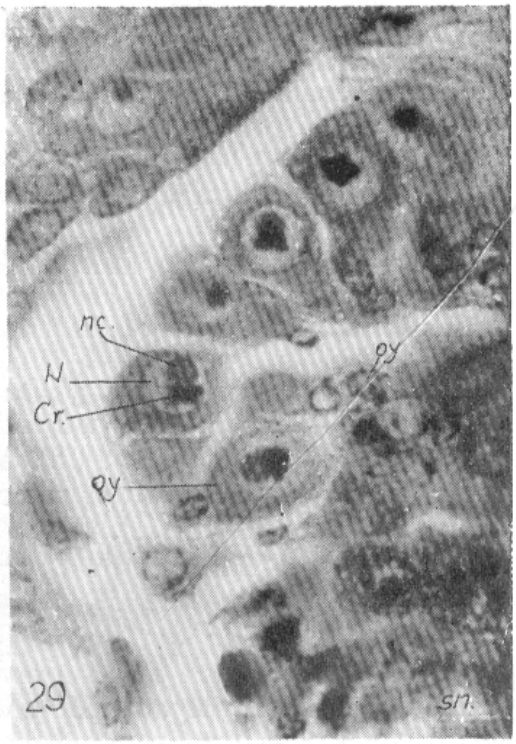
The vagina opens into a vaginal orifice, transversally in the front part ; it is covered by the genital-ventral scutum (shield).

Discussion

In our first study of the anatomo-histological structures and of a number of functions of various types of cells in the *Varroa jacobsoni* Oud. adult female, we have constantly compared our observations and findings with the conclusions reached by other authors for other genera



Excretory organs : ex — excretory duct ; mp — posterior musculature ; h — haemocoel ; ci — intestinal caecum



Genital system : nc — nucleolus; N — oocyte nucleus ; Cr — chromatin ; oy — oocytes ; sn — nutritive stroma

and species (BELOZEROV, 1957 ; JAKEMAN, 1961 ; SADOV, 1976 ; WARREN, 1941 ; YOUNG, 1968, 1970).

In *Varroa jacobsoni* the tegumentum consists, just as in all *Gamasoidea*, of a three-layer cuticle (ectostracum, mesostracum, and hipostracum), which is very thick and strong, and a thin hypodermis — the cytoplasm of a number of its cells reaching as far as the basis of hairs.

The musculature is strong, particularly in the anterior part of the body where the muscles of appendices (chelicerae, pedipalps, and legs) are visible. The well developed episternum, in the anterior-dorsal part, serves for insertion of the leg flexors and of the suspensory muscle of the episternum whose other end is fixed directly into the cuticle. The same manner of insertion of muscles was described to exist in *Haemogamasus ambulans* (YOUNG, 1970).

The nervous system has the characteristics common to all *Gamasoidea* (JAKEMAN, 1961 ; ANDRE, 1968). In *Varroa jacobsoni* it distinguishes itself not only by its high concentration and the large size of the brain, but also by the fact that the supraoesophageal and suboesophageal mass of ganglions is more precisely defined. In the series of sections examined, we located some of the nervous centres in the brain (the pedious centres), and the place of origin of a number of nervous trunks. In addition to the existing specific literature, we identified the presence of a number of anterior nervous centres from which nerves are branching out on both sides, separately from the supraoesophageal mass (structure visible in frontal sections).

Use of BORGMAN method for identifying neurosecretions gave no positive results.

The structure of the digestive system, as described in literature (ANDRÉ, 1968 ; BELOZEROV, 1957 ; JAKEMAN, 1961 ; SADOV, 1976 ; YOUNG, 1968), is similar to that in the mites of *Mesostigmata* suborder. The absence of cuticle in the oesophagus and rectum seems to be a common characteristic in *Mesostigmata* (HUGHES, 1969 ; JAKEMAN, 1961).

In the anterior dorsal zone, close to the anterior intestinal caecum we identified a globulous gland (probably a salivary gland) whose function we could not determine.

The Pas and Mowry staining methods revealed the presence in the cells of the ventriculus, of the caecum and of their lumina, of a quite great amount of neutral and acid muco-polysaccarides respectively. These substances come from the mite's secretion or from their food which certainly is the haemolymph of their hosts.

In the *excretory* ducts, both in their cells and lumen, guanine was identified, which is probably the only substance excreted ; ANDRÉ (1968) reported that uric acid was found only in very rare cases.

The reproductive organs, just like in all *Gamasoidea*, are of a very complex and constantly disputed structure, some of the classical structures and functions described by MICHAEL, 1892 being questioned (JAKEMAN, 1961).

In our sections we have distinctly identified : the ovary, the lyriform organ, the spermatheca, the oviduct, and the vagina. There was

some difficulty in identifying the connections between various organs. Oocytes in various development stages were seen, remarkable by the relatively large amount of compact chromatin in them. The egg is large and lies over a considerable area of the body cavity, therefore compressing the other organs ; it contains a great number of vitellus spherules.

Eggs with embryo were only identified in a small number of females examined.

Final assessment of the structure and function of various component parts of the reproductive system is not yet possible because further observations are necessary of the various development stages — larva, nymph, and adult female, as well as throughout the year.

Varroa jacobsoni has no circulatory system of its own, the circulation being lacunary. In the body cavity and between organs, a rich conjunctive tissue exists, many of its cells being amibocytes.

Our observations are an additional contribution to the anatomical and histological knowledge about this mite and open new possibilities for further investigation.

Also, our description of the anatomical and histological structure is yet another evidence coming in support of the classification of the mite in *Mesostigmata* suborder, *Dermanyssidae* family, — based up to now only on external morphological characteristics, and of the need to include it into another sub-family — *Varroinae* (HARAGSIM, SAMSINAK, 1972).

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