New Method for Invertebrate Nervous Histology

General experience has shown that the use of nervous histological techniques requires the application of different modalities in vertebrate and invertebrate material.

MAILLET's[1,2] osmium-zinciodide technique gave the most interesting results and revealed the presence of amyelinated fibres in vertebrates. JABONERO and co-authors[3–7], STACH[8] and GOTHE[9] have wholly succeeded in their respective publications.

The stain appears by unmasked lipids associated with proteins, and MAILLET[10] proved the necessary presence of iodide. According to CRUZ[11], osmical reduction also exists after mediator syntheses has been blocked. In spite of recent works, such as the histochemical interpretation made by STOCKINGER and GRAF[12], KOLB, PISCHINGER and STOCKINGER[13] and WIENKER[14] in ultramicroscopical studies, it would seem that the stain is not greatly specific. But by light microscopy, the particular morphology of the nervous cells is easily distinguished from the other tissues. This method therefore was tried on invertebrates including Mollusca where it proved fully efficient.

Labial palps of *Anodonta cygnea* L. were fixed at a temperature varying between 15 and 17 °C for periods lasting from 15–24 h. After washing and paraffin wax embedding, the sections revealed nervous elements standing out in black on yellowish ground.

The palps are innervated by the tentacular nerve. This nerve has its origin in the anterior-ventral angle of each cerebral ganglion and behind the cerebro-pedal commis-

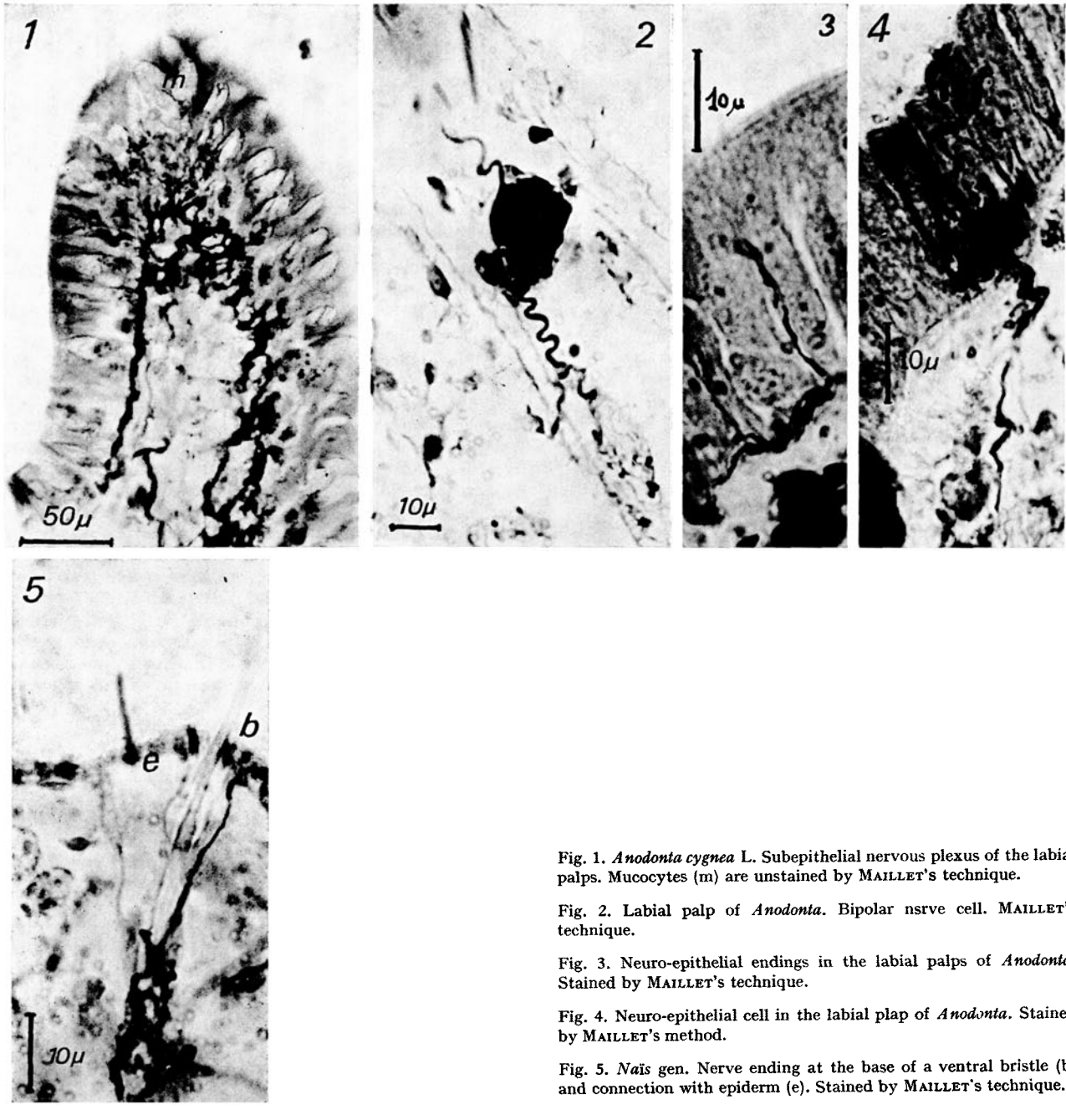

Fig. 1. *Anodonta cygnea* L. Subepithelial nervous plexus of the labial palps. Mucocytes (m) are unstained by MAILLET's technique.

Fig. 2. Labial palp of *Anodonta*. Bipolar nsrve cell. MAILLET's technique.

Fig. 3. Neuro-epithelial endings in the labial palps of *Anodonta*. Stained by MAILLET's technique.

Fig. 4. Neuro-epithelial cell in the labial plap of *Anodonta*. Stained by MAILLET's method.

Fig. 5. *Naïs* gen. Nerve ending at the base of a ventral bristle (b) and connection with epiderm (e). Stained by MAILLET's technique.

sure. Posteriorly, entering in the tentacles, the nervous fibres form subepithelial plexuses (Figure 1) interconnected by bipolar cells (Figure 2). The plexuses give rise to several neuro-epithelial endings (Figure 3) and cells (Figure 4). This technique was also used with much success on annelids (Figure 5).

It would seem, then, that MAILLET's technique could have some use in histological works about peripheral nervous structures on invertebrates. We hope soon to obtain other results concerning this hypothesis[15].

Résumé. La technique de fixation-coloration de MAILLET avait fourni des résultats intéressants pour la mise en évidence des fibres nerveuses amyéliniques chez les Vertébrés. Nous avons appliqué cette technique à des Invertébrés, notamment les Mollusques; elle s'est révélée efficace.

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The Cloacal Gland Complex of the Japanese Quail

In the sexually active male Japanese quail (*Coturnix coturnix japonica*) the outer dorsal portion of the cloaca is greatly swollen and red in color, and when the area is pinched a white foamy mass is extruded[1]. The foam is evacuated during copulation and defecation. This foam may be associated with the mechanics of fertilization[1,2] but there is no direct evidence. Only a slight amount of foam can be expressed from the cloaca of laying quail. When adult males are removed from a photoperiod stimulatory for sexual activity and subjected to a non-stimulatory lighting regimen, the amount of cloacal foam produced and the histological complexity of the gland are depressed[3,4]. Furthermore, no cloacal gland activity was observed when the average weight of the testes was less than 0.75 g[4].

The cloacal gland complex consists of a series of tubular glands located in the dorsal lip of the cloaca, and empty into the proctodeum[2,5]. They begin as solid epithelial buds in the dorsal proctodeum between the eleventh and twelfth day of incubation and establish a lumen by the fifteenth day[5].

Whether these glands are truly cloacal derivative, or members of the anal gland series is questioned[6].

The indications that these glands are sex-dependent[5] stimulated a series of experiments. Sexually mature males exposed at least 8 weeks to a photoperiod of 16 h light to 8 h dark (16L:8D), which is stimulatory for both testicular function and cloacal gland activity[4], were implanted s.c. with a 3 mg pellet of diethylstilbesterol and then killed 10 days post-implantation. Actively laying females on 16L:8D were implanted with 5 mg of crystalline testosterone and killed 10 days later. In another experiment, groups of immature males and females were reared to 4 weeks of age under the limited light condition 8L:16D[4]. Half of each sex were implanted with 5 mg of crystalline testosterone and all were killed 10 days later.

The dorsal lip of the cloaca was removed and processed for histological examination.

Observations and discussion. The cloaca of the Japanese quail differed from that described for the chicken[7]. Columnar epithelium lined only the coprodeum and urodeum. The proctodeum was lined entirely by stratified squamous epithelium, including the entrance into the bursa of Fabricius. The glandular complex was located in the dorsal portion of the proctodeum between the bursa of Fabricius and the anal aperture or vent (Figure 1), and are not the anal glands reported in other birds[6].

Macroscopically, the active cloacal gland complex measured approximately $10 \times 12 \times 2$ mm. Microscopically, the complex comprised a series of branched tubular glands lined by a simple columnar epithelium. Each had a main central lumen that communicated with the proctodeum through a small pore (Figure 2) and gave rise to a series of tubules or sacs. The complex was divided into lobular units by connective tissue septae rich in elastic fibers.

A small, unreported, ventral glandular complex (Figure 3) was also found.

The active cloacal gland (Figure 4) had tall columnar epithelium with basal nuclei and abundant cytoplasm with variable sized vacuoles. The apices of many cells had numerous microprojections, some being pinched off to enter the lumen (Figure 5). Secretory material was abundant, and both cytoplasm and secretory material

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