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ULTRASTRUCTURE OF THE "*CANNABIS SATIVA*" GLANDS

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Abstract

Some observations have been made on the submicroscopic structure of the perigonial bract glandular hairs of the Cannabis sativa *L. female flowers which are rich in resin. The morphological characteristic shown by the scanning electron microscope have been described.*

The observations under the electron microscope have made evident that the resin-producing cellular elements are to be found almost exclusively in the head of the hairs and in the apex part of the stalk. The secretion makes its exit through the existing pores in the cuticle layer of the gland head's outer cells.

Introduction

The importance which the glandular hairs of the *Cannabis sativa* perigonial bract have upon the production of resin, rich in the active constituents – cannabinoids –, and having the characteristic biological action, is well known.

The study of such elements can consequently arouse noteable interest, not only from the poisonous-legal point of view, considering the great use which is made today of the drugs which are derived from this plant and which, under different names, are used for narcotic purposes all over the world (De Pasquale, 1969), but also after an exact taxonomic classification considering it's noteable polymorphism.

The *Cannabis sativa* L. (Linneo, 1797), according to Engler's classification (1964), belongs to the *Moraceae* family or the subfamily of *Cannaboideae,* whilst in the Flora Europaea (1964) the *Cannabinaceae* form a family of it's own.

Lamarck in 1789 observed that some samples of hemp plants from India, rich in resin, showed different characteristics to the fibre-producing hemp. Therefore, assuming that this was a different species, he gave it the name *indica* after its place of origin.

The nomenclature adopted by Lamarck was followed up by numerous authors and Longo (1936) even attempted to ascertain the differential morpholo-

gical characteristics between the two species: *Cannabis sativa* L. and *Cannabis indica* LAMARCK. Today only one species is generally recognised, the *Cannabis sativa* L. (Flora Europaea, 1964; OMS, 1971), to which consequently the *Cannabis indica* would correspond, and after all according to some authors would be a variety (TSCHIRCH, 1912; AUSTER and SCHÄFER, 1955; TONZIG, 1956; HOPPE, 1958; BENIGNI et al., 1962; MÜLLER and RINTELEN, 1962; COICIU and RACZ, 1962; TREASE and EVANS, 1966; COSTA, 1967; FISCHER, 1968) or a cultural form (BEILLE, 1935; CASAMADA, 1957) of the *Cannabis sativa.*

Present research concern the structure of the stalked glandular hairs of the perigonial bracts of female flowers of the resin producing hemp plants of tried biological activity. Certain observations on the matter had already been a subject of discussion (DE PASQUALE, 1970a) at the XV Italian Pharmacological Society Congress (Milan 2–3 October 1969).

Experimental

Research has been made on fresh material, taken from plants grown from seeds originating from Amindeon in Greece, and cultivated by us in the garden of this Institute of Pharmacognosy. Observations were made on the:

I – Light microscope – The material was treated with the technique of temporary slides. The glandular hair sections were performed with an ultramicrotome on the treated material in the same manner as for the transmission electron microscope. A Photomicroscope II Zeiss was used.

II – Scanning electron microscope (S. E. M.) – The perigonial bract was freeze-dried and then gold vapour-deposited on it's surface with JEE-4B Vacuum Evaporator Jeol. A Scanning Electron Microscope Jeol mod. JSM-S1 was used.

III – Transmission electron microscope (T. E. M.) – Seeing that as far as we know, no research has been carried out on the electron microscope with regards to hemp, and considering the particular characteristics of the resin which this plant secretes, many difficulties have had to be overcome in the specimen preparation and in the carrying out of sections with the ultramicrotome.

Two technique were used: 1) Fixation with osmium, dehydration in an ethanol series of rising graduation, and embedding in Araldite-Epon or methacrylate; 2) Dehydration under hight vacuum in the presence of phosphoric anhydride, embedding of the pieces in methacrylate, sectioning, and coating with evaporated osmium tetroxide.

It was not necessary to contrast the sections as they appeared sufficiently shadowed. The ultra-thin sections were obtained with an ultramicrotome Leitz and the observations were made with an Siemens microscope Elmiscop II. The observations were carried out at the Electron Microscope Centre at this University.

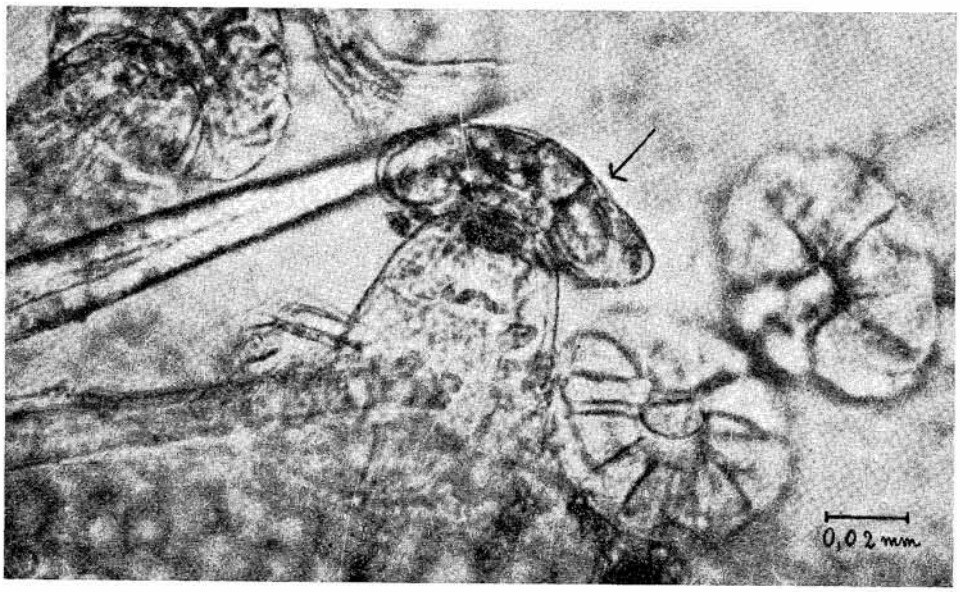

Fig. 1. Perigonial bract: glandular stalked hairs, Light microscope

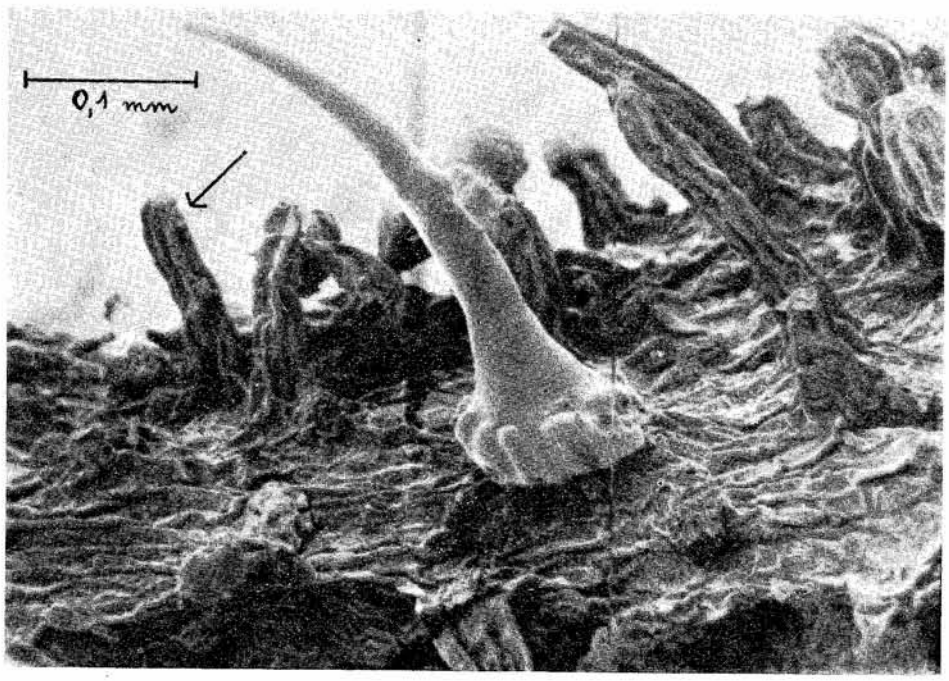

Fig. 2. Glandular stalked hairs without head – S. E. M.

Results and Discussion

The perigonial bract of *Cannabis sativa* female flowers appears course and viscose to the touch and is covered with protective and glandular hairs (De Pasquale, 1970b).

The capitate glandular hairs (Fig. 1), very often without a head (Fig. 2), consist of long stalk of variable dimensions (130–300 μm), according to the stage of development of the hair itself, it is multicellular and multiseriate and is made up, apart from the epidermis layer, by one or more layers of palisade-like tissue, and which terminates in a dome-shaped apex on which the gland head lies. The gland head is rather swollen (Fig. 3 and 4), it has a diameter of 30–90 μm and is formed by 4–16 cells which are covered by a thin cuticle, and are set out in a

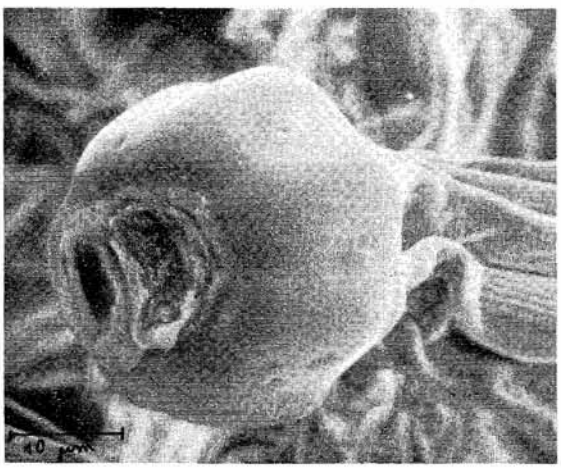

Fig. 3.
Head of the glandular stalked hairs – S. E. M.

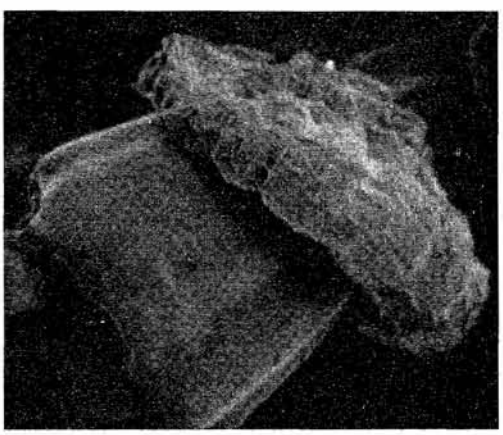

Fig. 4.
Head of the glandular stalked hairs – S. E. M.

rosette-like on two concentric layers, the inner one formed by 6 cells and the outer by 10 larger cells (Fig. 5). The surface of the head can show signs of scars through which the cell content eneiges (Fig. 3).

In addition, other glandular hairs (Fig. 6) were noted, which, unlike the former, were sessile or showed a short stalk originating from only one epidermis. The head is formed by number of cells varying from 1 to 8. Glandular hairs of this type are also seen on the under-epidermis of the leaves, on the outer epidermis of the tepals of male flowers, and on the anthers (DE PASQUALE, 1970b).

From observation under the transmission electron microscope, the cells of the outer layer of the *head* of the long stalked glandular hairs we notice that the

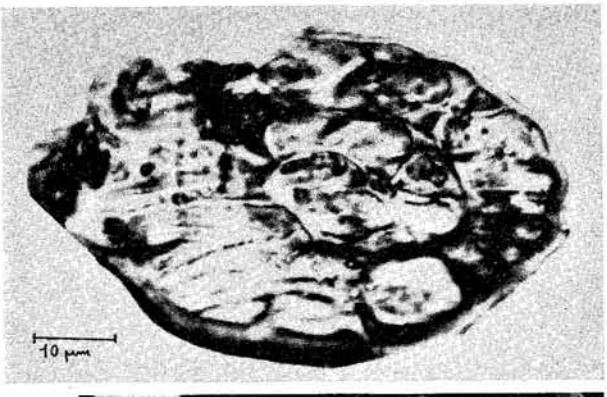

Fig. 5
Section of a glandular stalked hair head – Light microscope

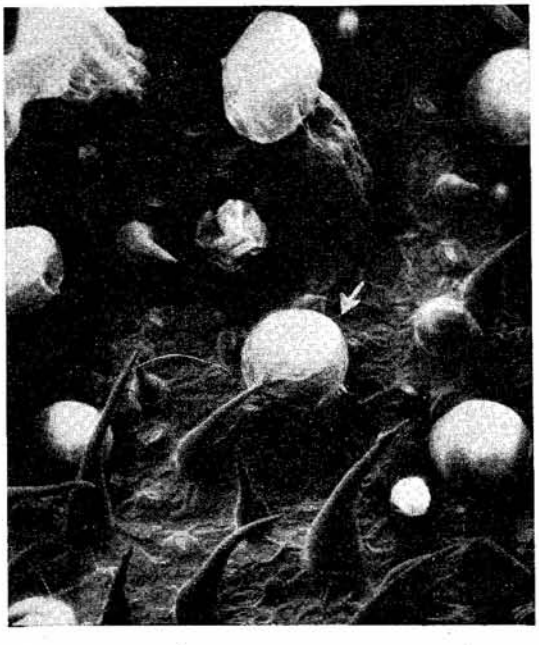

Fig. 6.
Sessile glandular hairs of the perigonial bract – S. E. M.

0,1 mm

outside walls (Fig. 7) are wavy and made up of a cuticle layer which is heavily osmiophilic. This is followed, towards the inside, by a less contrasted pectic layer and then a lighter cellulose layer. Between the cuticle and pectic layer there are very small spaces and the cellular wall is radially crossed by pores, blocked by pectine plugs, through which the resin probably makes its exit. In the older glandular cells on the outer layer (Fig. 8) an irregular separation of the citoplasm from the cell wall ist observed. Therefore a large "extraplasmatic space" is formed, which is present, however, only towards the apex pole and not towards the inner pole of the cells, and it is also lacking completely in the cells of the inner layer of the rosette. In the younger cells there are numerous vacuoles containing

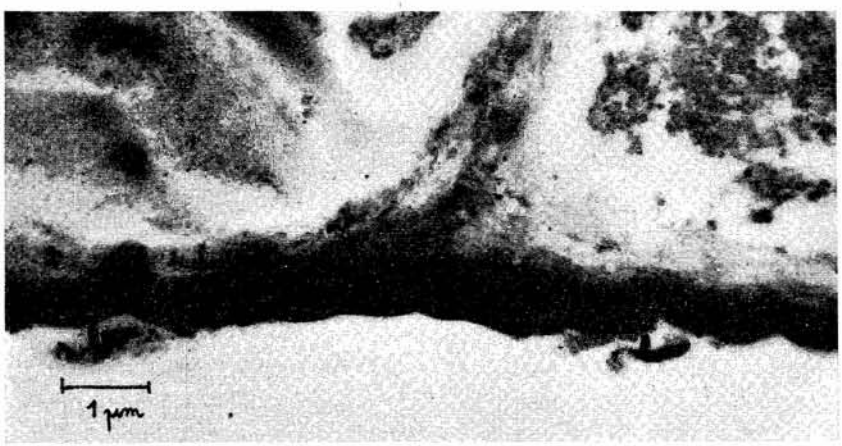

Fig. 7. Glandular hair's head: outer layer cells – T. E. M.

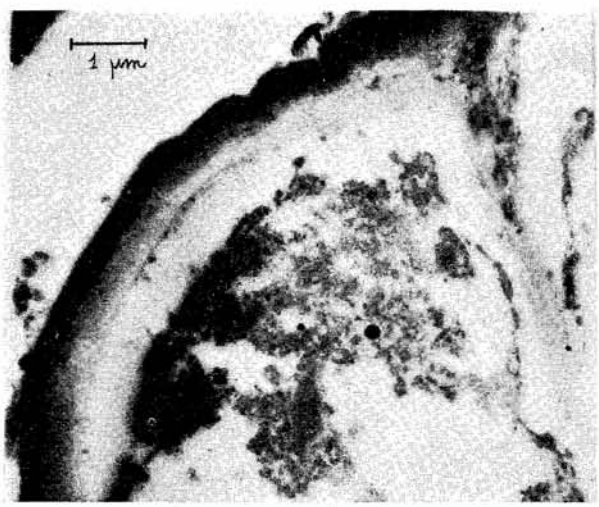

Fig. 8. Glandular hair's head: outer layer cells – T. E. M.

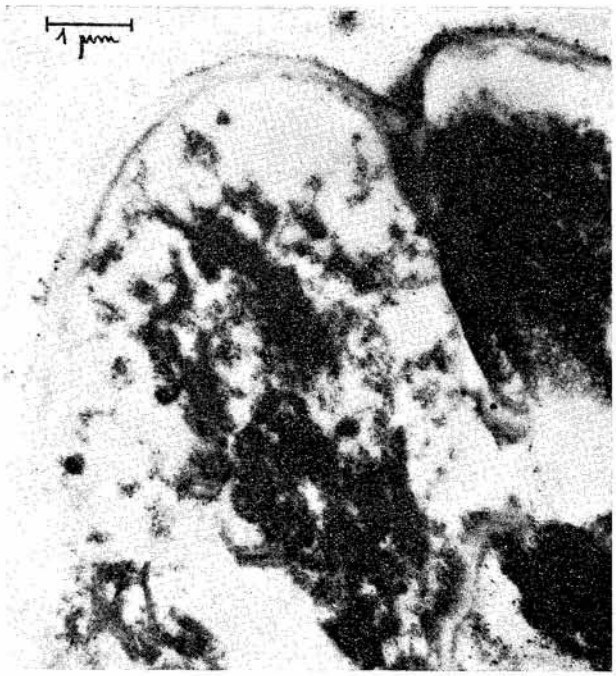

Fig. 9. Glandular hair's head: outer layer cells – T. E. M.

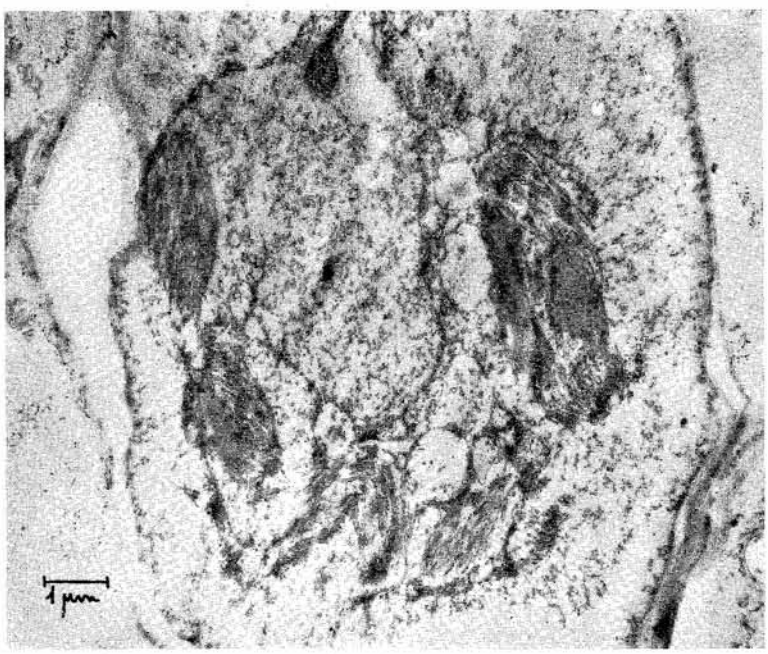

Fig. 10. Glandular hair's stalk – T. E. M.

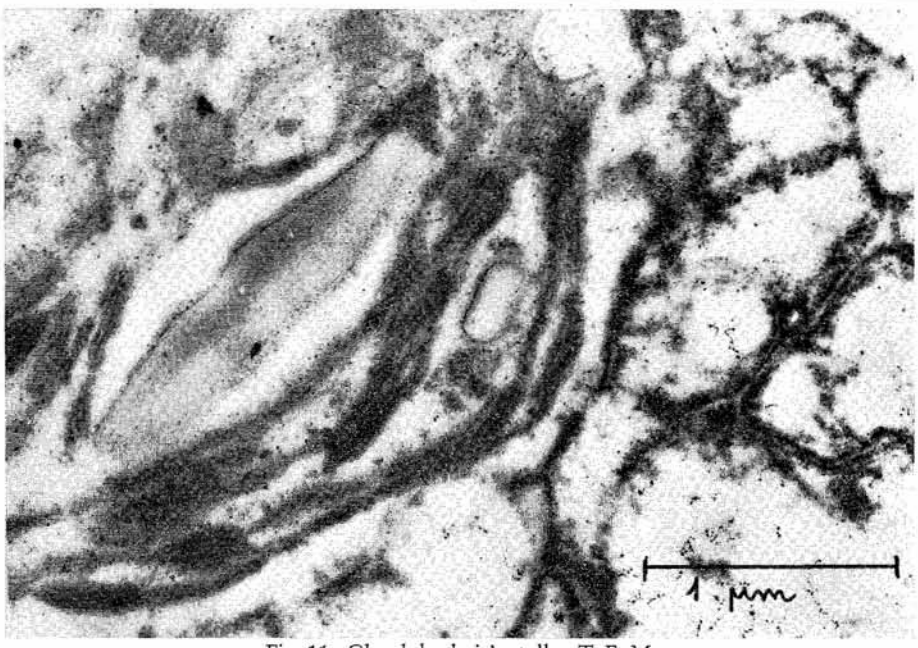

Fig. 11. Glandular hair's stalk – T. E. M.

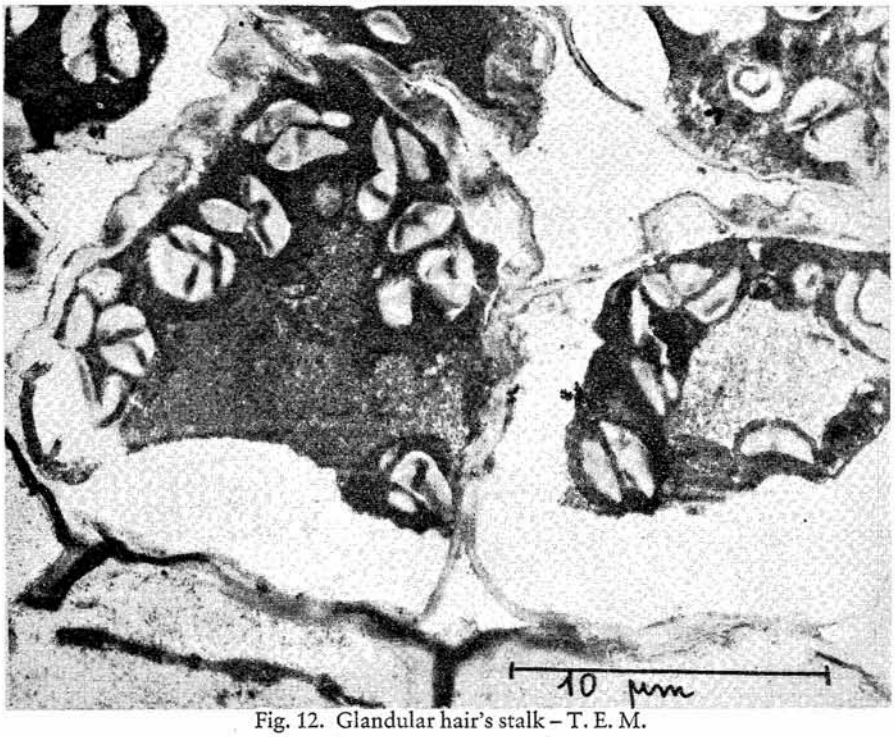

Fig. 12. Glandular hair's stalk – T. E. M.

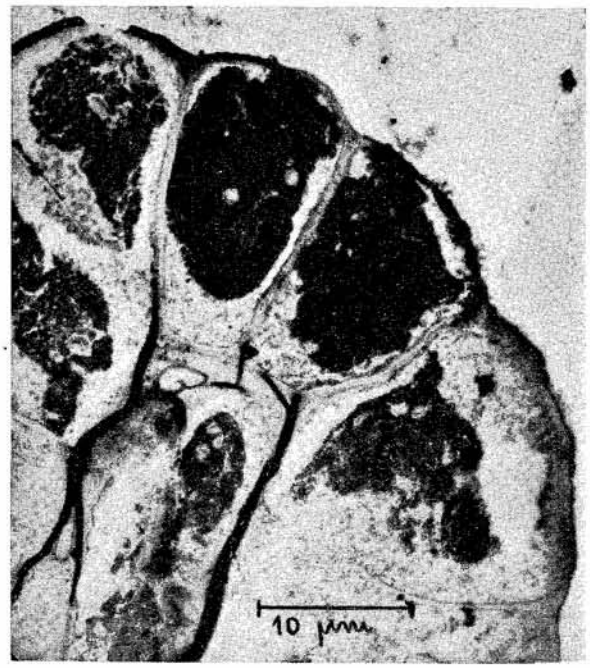

Fig. 13. Apex of glandular hair's stalk – T. E. M.

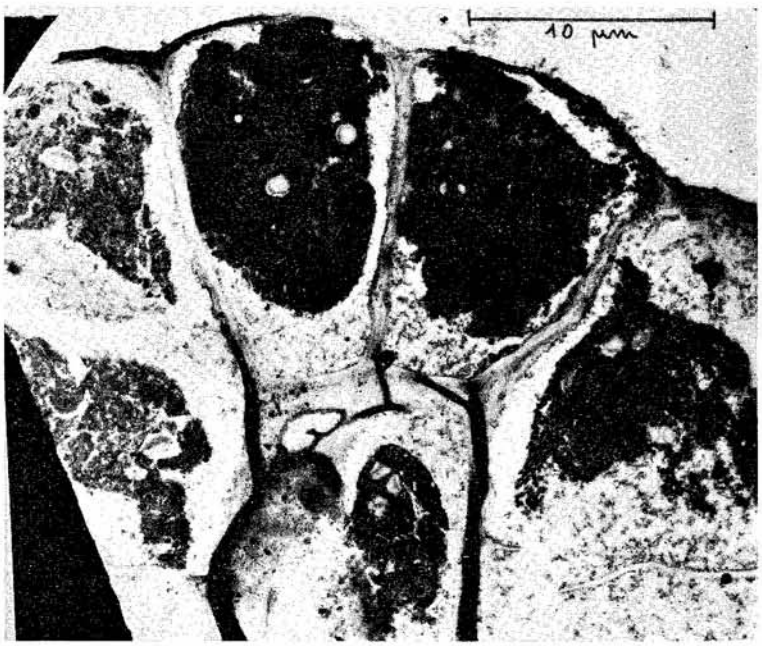

Fig. 14. Apex of glandular hair's stalk – T. E. M.

an osmiophilic substance and many Golgi apparatus, but both of these are of a considerably smaller number in the older cells. The inner cells (Fig. 9) have a cell wall without a cuticle layer and very rich in a highly osmiophilic substance.

In the *stalk* of the hair the series of inner cells have starch-containing chloroplast (Fig. 10 and 11), less in number, however, than the cells in the palisade layer (Fig. 12), from which they originate. In the cells of the outer layer there appear to the empty vacuoles, whilst the apex cells (Fig. 13), upon which the gland head lies, are almost full of highly osmiophilic contents, some areas being more shadowed than others. Also the cells adjacent to the apex (Fig. 14) shown the same contents, notably reduced, and always localised towards the upper pole. The outside walls of apex cells appear to have cuticle layer but only in the surrounding areas, whilst in the central area, where the head is inserted, there are only the cellulose and pectic layers (Fig. 15).

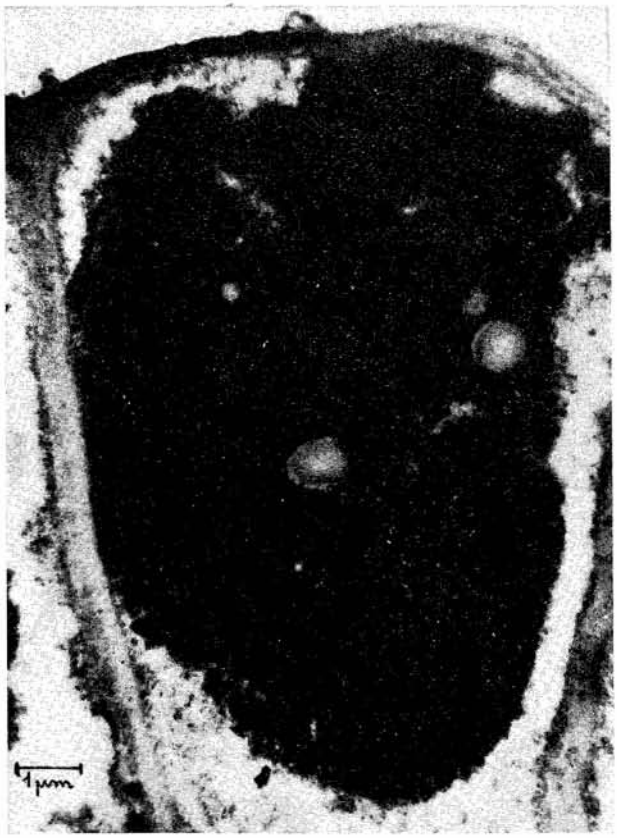

Fig. 15. Glandular hair's stalk: apex cell – T. E. M.

These observations have emphasized that the secretory part of the perigonial bract glandular hairs of the Indian hemp female flowers could be formed almost exclusively by the head. Most probably, however, the cells situated in the apex of the gland stalk and having direct contact with the cells in the head, also take some part in the resin formation process.

The presence of empty vacuoles in the older cells and in those at the base of the stalk, furthest from the gland head, could indicate that there has been an emptying of the vacuole contents in the formation of the secretion. This, then, would make its exit through the pores of the cuticle layer of the outer cells of the head, or through any eventual injuries which may have been caused to the cells themselves.

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