The trichomes and glands of *Cannabis sativa* L.

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The main object of this paper is to describe a new type of sessile gland in cannabis and to report gas chromatographic results on the presence of cannabinoids in the glands, but the opportunity is taken to include descriptions, with scanning electron micrographs, of all the trichomes.

Clothing trichomes

It has long been known that two types occur: (*a*) squat unicellular cystolith trichomes which are abundant on the upper surface of the bracts and leaves (figures 1, 2 and 9); (*b*) elongated unicellular covering trichomes which occur on the lower surface of the bracts, leaves and bracteoles (figures 6, 8 and 9).

Glandular Trichomes

The long recognised stalked glands or glandular trichomes consist of a multicellular stalk surmounted by a hemispherical head (figure 3). This head consists of 8-16 cells which secrete resin " between the cuticle and the central part of the cell " (1). My own observations indicate that these occur mainly on the lower (outer) surface of the bracteole, which encloses the flower, and on the upper surface of the bracts subtending the bracteole. I have also noticed smaller stalked glands with a bicellular head and these are described by Jackson and Snowden (2). However, they may merely represent stages in the development of the normal stalked glands: several such stages can be seen in figures 4 and 5, although the latter may represent a mature stalked gland of a different type than the normal ones.

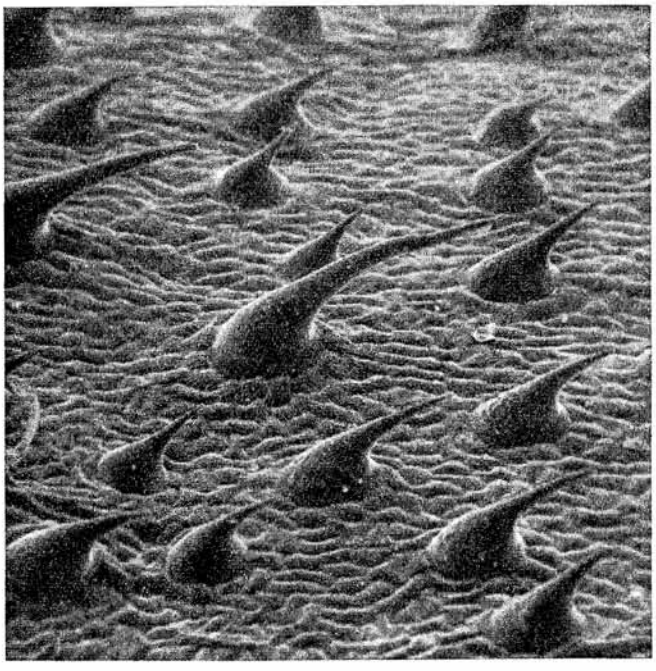

Figure 1

Cystolith trichomes; upper surface of leaf; coated with gold-palladium (× 175)

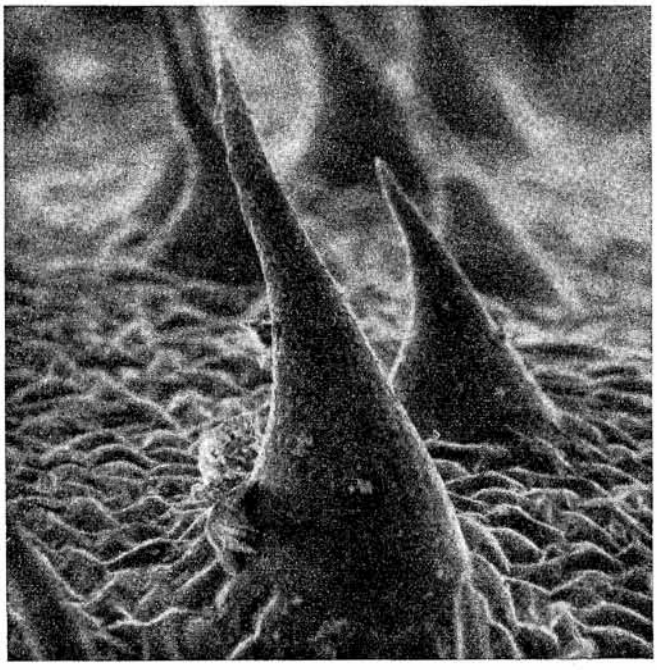

Figure 2

Cystolith trichomes; upper surface of leaf; uncoated (× 450)

A new type of gland

Using a stereoscopic light microscope (X 75) and fresh plant material, I have frequently observed shining spherical glands on the lower surface of bracts and leaves: they are very abundant in some varieties of

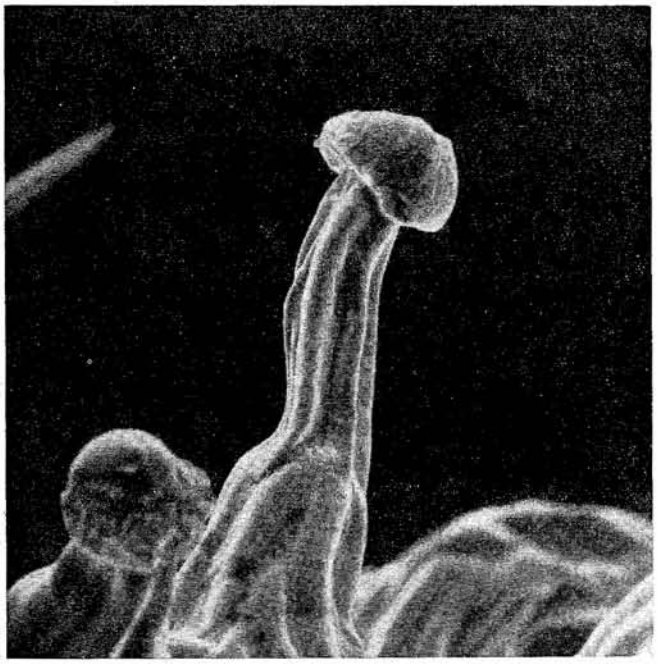

FIGURE 3

Stalked glandular trichome; bracteole lower surface; coated with gold-palladium (× 310)

cannabis. They are about 45-70 µm in diameter, appear to lie on the surface below the covering trichomes and have a very smooth surface, even after coating with gold palladium, as revealed by the scanning electron microscope (figures 6 and 7). Some leaves were treated with glutaraldehyde, ethanol, amyl acetate and liquid carbon dioxide; this would dissolve out most of the resin from the glands. Figure 8 shows that after this treatment there is definite evidence of a membrane surrounding the glands. In one of the varieties examined (SP5) male, female and monoecious plants were available: abundant sessile glands were present on the leaves of all the plants. In addition very large, spherical to irregularly oval sessile glands were found on the anthers.

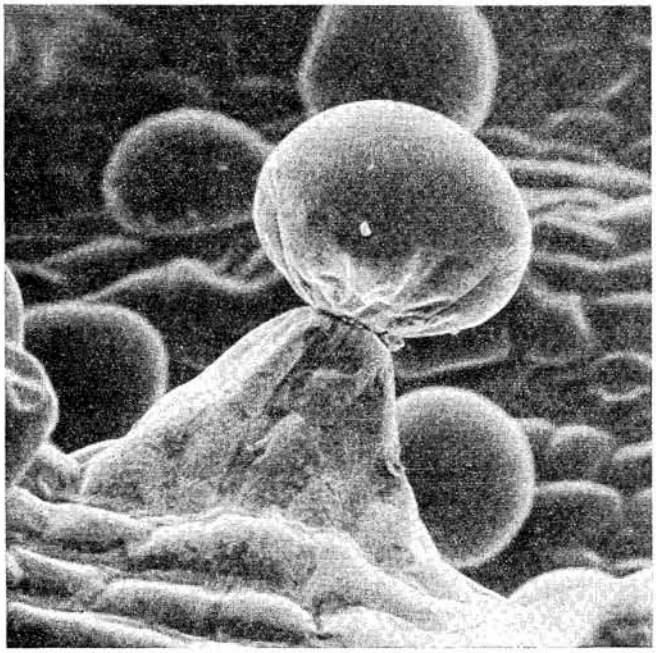

FIGURE 5

Stalked glandular trichome with spherical head; bracteole, lower surface; coated with gold-palladium (× 400)

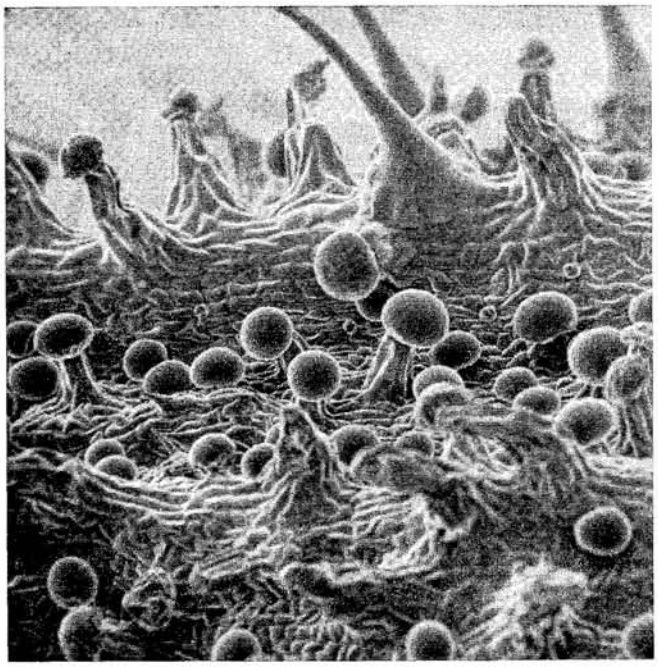

FIGURE 4

Stalked glandular trichomes at various stages of development; also two covering trichomes; very young bracteole, lower surface (× 100)

Bouquet (1) probably refers to similar structures " stuck on the surface of the epidermis ", but states they consist of a flat round head of 8-10 cells and implies they are the normal glandular trichomes whose stalks have not developed. Nordal (3) also describes similar multicellular sessile glands but refers to them as " developing glandular hairs ". The sessile glands which I have observed, however, differ from the normal stalked ones as follows: (*a*) there is no evidence of intracellular structure. Transverse sections cut from fresh material with a freezing microtome only show a sphere filled with globules of resin (figure 9); the globular forms of the resin may be produced by the lactophenol mountant. Surface preparations of the leaf and bracteoles, cleared in lactophenol, also show the globular effect in contrast with the normal stalked glands which exhibit in addition intra-cellular structure. (*b*) No stalked glands were ever found associated with the sessile ones even in quite mature leaves. In contrast, similar structures in very young bracteoles and subtending bracts are associated with stalked glands at various stages of development

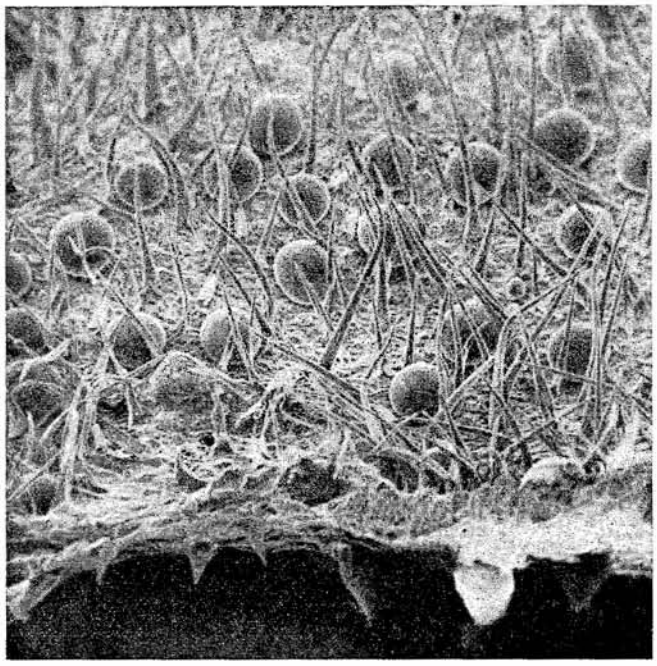

FIGURE 6

End-view of sessile glands and covering trichomes on lower surface of leaf; cystolith trichomes of upper surface visible at bottom of the figure. Coated with gold-palladium (× 115)

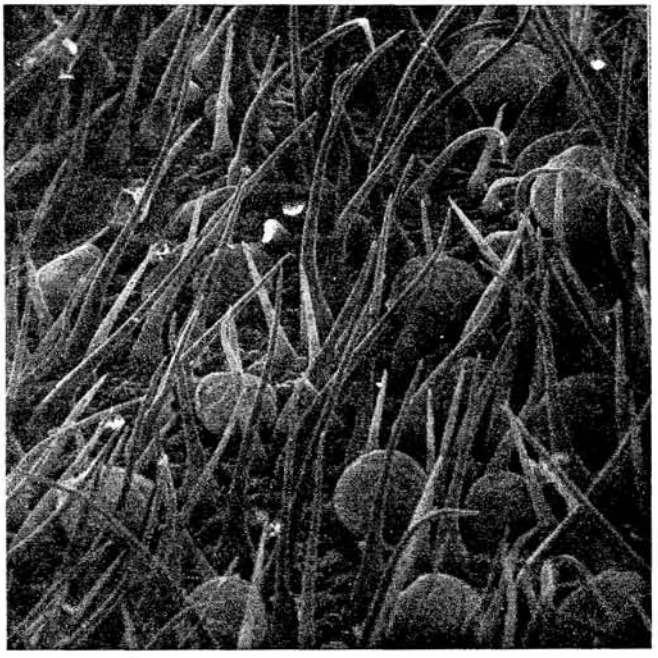

FIGURE 8

Sessile trichomes after removing some of cell contents; note, cracked collapsed membranes. Lower surface of leaf (× 200)

(figure 4) indicating that the apparently sessile glands are incipient stalked glands. (*c*) Transverse sections show no sign of a stalk (figure 9). Unfortunately, if sections less than 60 μm are cut the glands are knocked off, or badly distorted, so it was not possible to see in exact detail how they are attached to the leaf. However, they seem to lie in slight hollows of the leaf surface and are lightly attached as they can readily be removed with a fine needle.

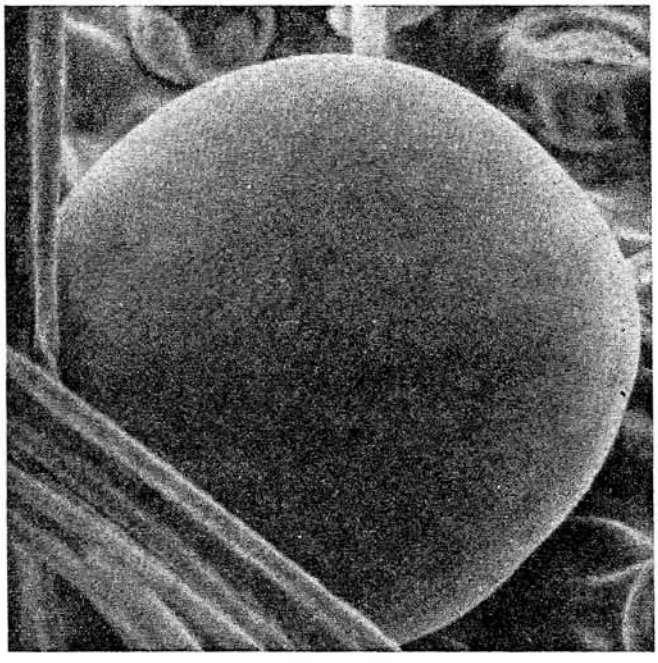

FIGURE 7

Sessile gland from figure 6 enlarged ten-fold (× 1150)

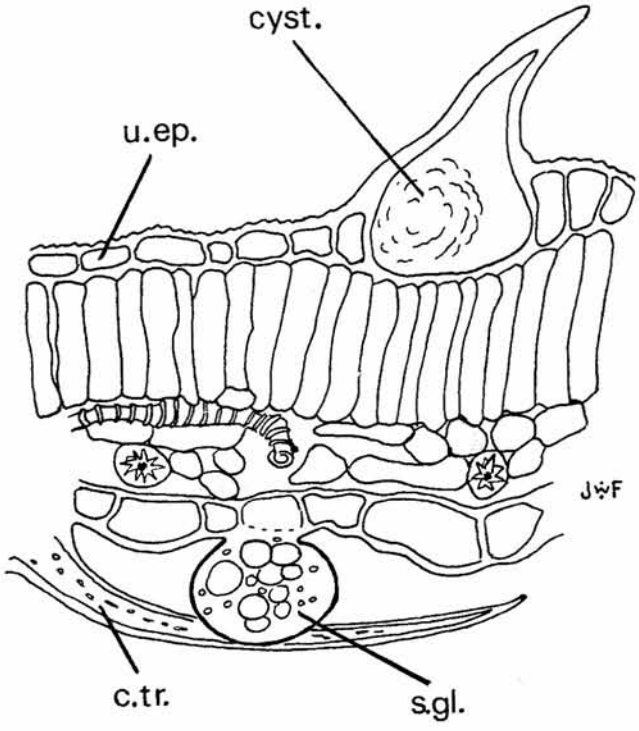

FIGURE 9

Transverse section of leaf; freezing microtome, mounted in lactophenol: c.tr. *covering trichrome;* cyst. *cystolith trichrome;* s.gl. *sessile gland;* u.ep. *upper epidermis (× 350)*

Association of cannabinoids with the glands

Some living cannabis plants (SP5) which had been shown to contain mainly Δ^1THC with traces of cannabidiol, were examined with a stereoscopic light microscope. The resin from the stalked glands of the bracteoles was picked off with a fine needle and transferred to petroleum spirit; that from the large sessile glands of the anthers was also treated in the same way. To remove the resin from the sessile glands of the leaves, the latter were gently pressed against lens paper. This loosened or burst the delicate glands and the resin was immediately absorbed by the paper from which it was extracted with petroleum spirit. All three petroleum spirit extracts were examined separately by gas-liquid chromatography and each shown to contain mainly Δ^1THC with a trace of cannabidiol, by position on the chromatogram and retention time relative to an internal standard (triacontane). With the stamen glands there was a higher proportion of cannabidiol than with the others. These results show directly, for the first time, that cannabinoids are contained in the resinous secretions; they do not however prove quantitatively that all the cannabinoids present in the plant are exclusively contained in the glands.

Accordingly in another variety (UNC.258) whose main component was cannabidiol (CBD) the following three parts were examined: (*a*) large bracts with no glands observable, (*b*) small bract tips, devoid of glands and (*c*) bases of the same bracts as in (*b*) which contained abundant stalked glands. Analysis by GLC gave the following results for the air-dried material:

(*a*) large bracts, no glands	3.0 mg CBD/g
(*b*) small bract tips, no glands	4.9 mg CBD/g
(*c*) small bract bases, numerous glands . .	16.7 mg CBD/g

In another variety (SP4), containing Δ^1THC and CBD, small bracts almost devoid of glands were compared with bracteoles containing abundant stalked glands:

(*d*) small bracts, very few glands	13.9 mg THC/g
	6.4 mg CBD/g
	20.3 mg/g total
(*e*) bracteoles, abundant glands	57.8 mg THC/g
	35.1 mg CBD/g
	92.9 mg/g total

These results indicate that the cannabinoids mainly occur in the glands but they also show that some occur in tissues devoid of glands. It should be noted that the amount of cannabinoids in the bracteoles (freed from young seeds) is remarkably high being about 10 % of the dry weight. The petroleum spirit soluble fraction of these bracteoles was 19.4 % indicating that about half of the resin consists of cannabinoids.

Summary and discussion

1. A new type of resin-containing gland has been described; it differs from the normal stalked glands by the absence of stalk, absence of intra-cellular structure and its position on the lower surface of leaves and bracts of male and female plants, and on the anthers. In contrast the stalked glands are mainly present on the outer (lower) surface of the bracteoles, and the upper (inner) surface of the small bracts subtending the bracteoles.

2. Both the sessile and stalked glands have been shown to contain the cannabinoids typical of the whole plant. This is probably the first time this has been demonstrated directly by GLC methods. However, evidence is produced to show that smaller amounts of the same cannabinoids are contained in tissues devoid of glands. Other workers (1,4) have suggested that the laticifers may contain cannabinoids and this would certainly be worth re-investigating.

3. I have long assumed that close association of the stalked glands with the developing female inflorescence suggest that the cannabinoids may function as a protective from excessive insolation for the delicate ovaries and seeds. However, the presence of sessile glands containing typical cannabinoids in both male and female plants, and on the under surface of leaves, even in the vegetative phase, calls this idea in question.

Materials and methods

Plants

Details of the plants quoted in the text are as follows:

UNC.254—A Thailand strain, which was grown out-of-doors in England in 1971.

UNC.258—A Turkish strain, grown as above.

SP4—A Nepalese strain, grown as above.

SP5—A police seizure of plants, some grown in a house and some under polythene sheeting in a garden, in North London in 1971.

Gas chromatography

A modification of Lerner's method (5) was used.

Microscopy

A Cambridge Stereoscan Mark 2A was used. Fresh plant material was mounted and examined without treatment (figures 2 and 4) or after coating with gold-palladium (figures 1, 3, 5-7). For figure 8, the fresh leaf was treated by Anderson's critical point drying method (6). For figure 9 sections (60 µm) were cut from fresh material, using a freezing microtome, and mounted in lactophenol.

Acknowledgments

I would like to thank Miss J. Fillery, and the Department of Botany, Imperial College, London, for the excellent electron micrography, Miss E. Sayron for help with the gas chromatography, and Dr. O. J. Braenden of the Division of Narcotic Drugs, United Nations, Geneva, for seeds of the UNC.254 and UNC.258 strains.

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