Figure 18.

Inner surface of ovipositor sheath.

p peg.

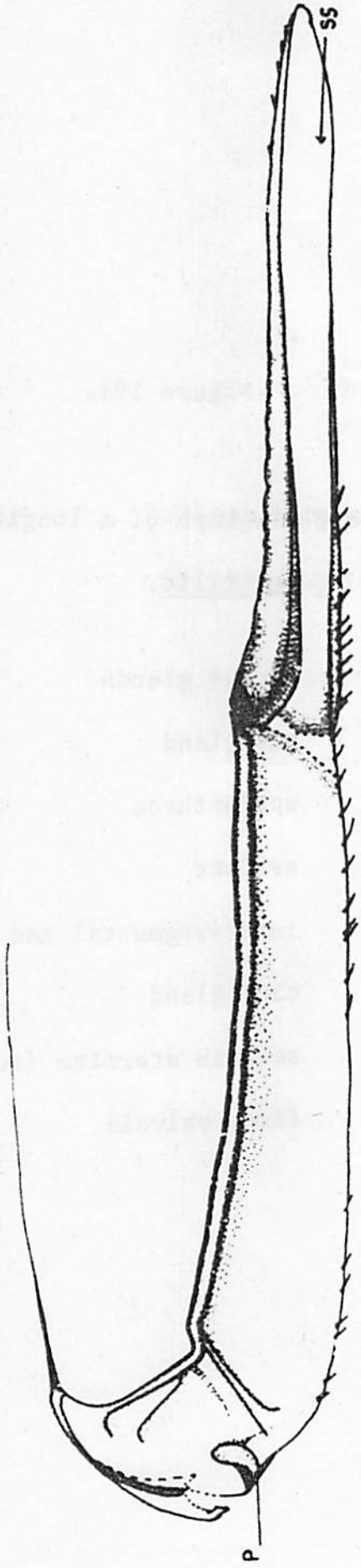

SS
P

Figure 19a.

Tracing made from a photograph of a longitudinal section of a female pupa of *S. noctilio*.

mu gl	mucous glands
oil	oil gland
sp	spermatheca
od	oviduct
is	inter-segmental sac
cg	club gland
VII s	seventh stermite (sub-genital plate)
I vl	first valvula

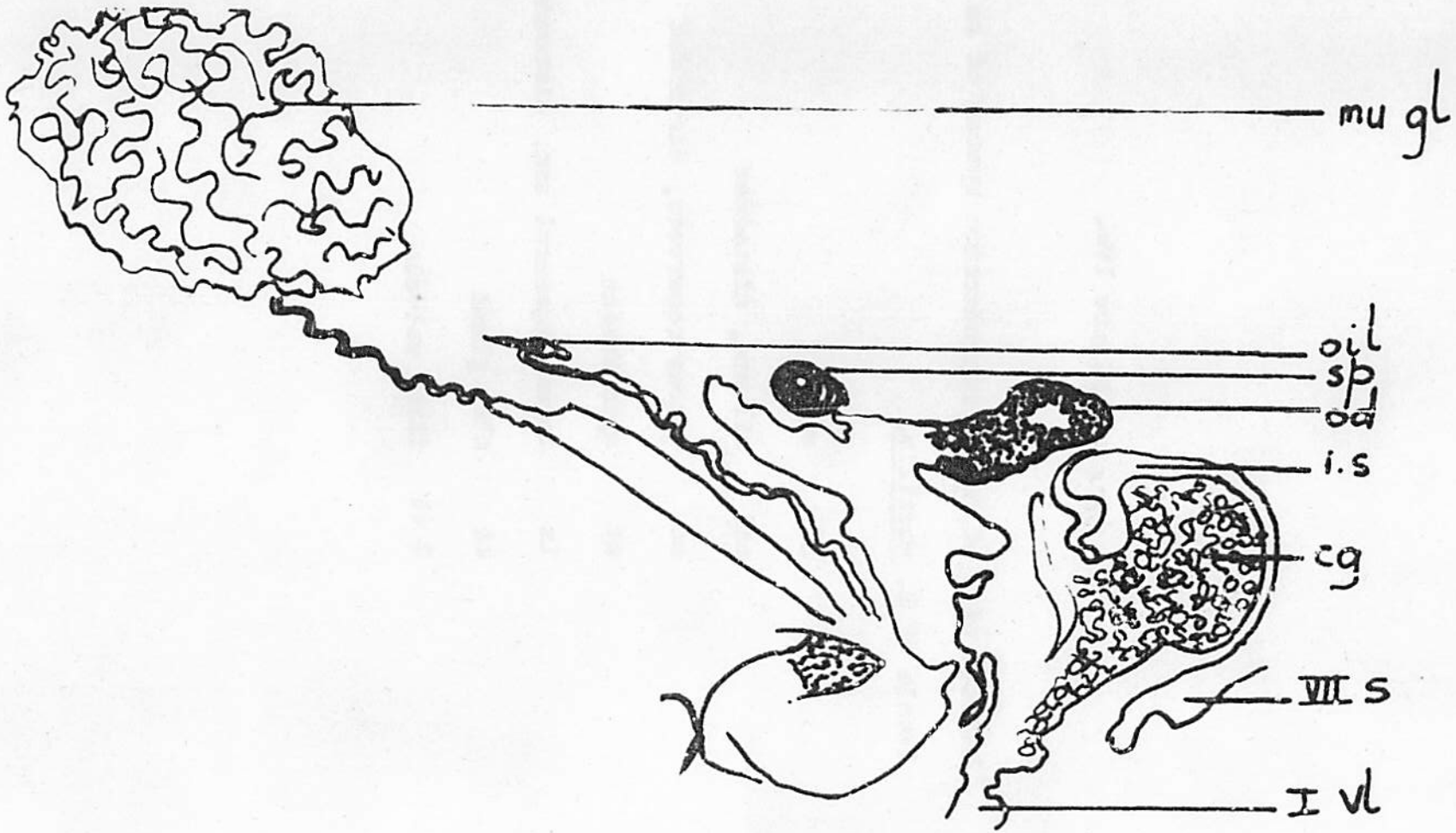

mu gl
oil
sp
od
i.s
cg
VII s
I vl

Figure 19b.

Lateral view of female reproductive system of an adult female of S. vectilio.

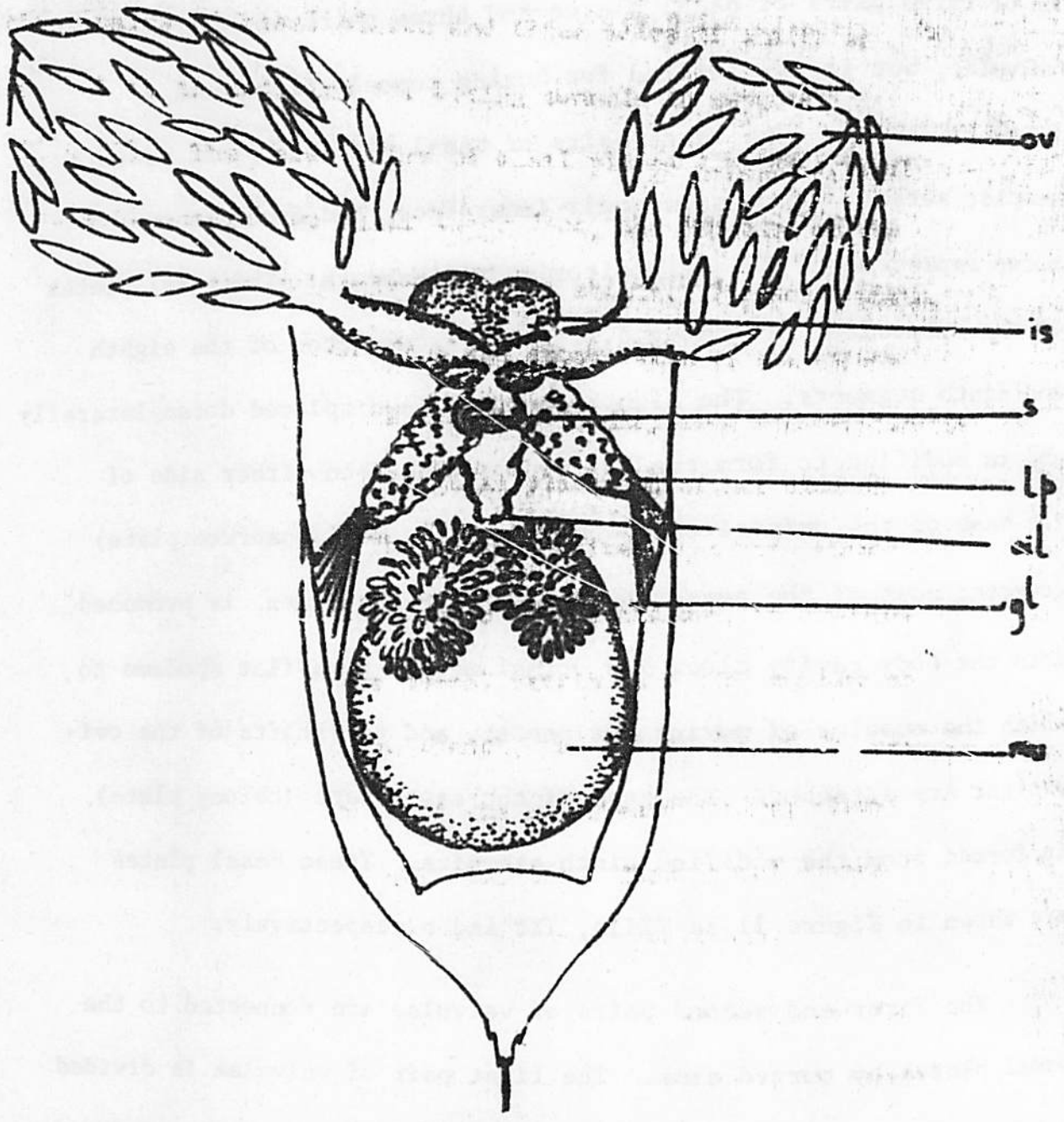
ov	ovary
oil	oil sac, distended
ms	mucous reservoir, distended
sp	spermatheca
is	inter-segmental sac, distended
cg	club gland
I vl	first valvula


ov
oil
mu
ep
ts
cg
vl

Figure 19c.

Dorsal view of female reproductive system of adult *S. noctilio*.

ov	ovary
s	spermatheca
lp	lateral pouch
oil	oil sac
gl	mucous gland
mu	mucous reservoir



9c

ing the infection of the inter-segmental sacs.

As in all Hymenoptera, the ovipositor of S. noctilio is made up of three pairs of basal plates and three pairs of shafts or valvulae, but it is modified for boring tunnels in sapwood. (Figs. 11 & 12). The three pairs of basal plates support several compact sets of muscles on their inner face, and function as the motor apparatus of the ovipositor and its sheath. The basal plates of the ovipositor are derived from the exoskeleton of the eighth and ninth segments. The eighth sternite is displaced dorso-laterally and is modified to form the triangular plates on either side of the base of the ovipositor. The ninth tergite, (quadrate plate) covering most of the posterior region of the abdomen, is produced into the body cavity along its dorsal margin as a flat apodeme to which the muscles of moving the sheath, and the shafts of the ovipositor are attached. The base of the saw-sheath (oblong plate) is formed from the modified ninth sternite. These basal plates are shown in Figure 11 as VIIIs, IXt and b respectively.

The first and second pairs of valvulae are connected to the basal plates by curved arms. The first pair of valvulae is divided proximally into two arms. (Fig. 12). The outer arm curves outwards and upwards to articulate with the apex of the triangular plate. The inner arm curves upwards into the body cavity. Its swollen base or club organ is covered by a pouch of the inter-segmental

membrane, known as the inter-segmental sac, described in the introduction. Figure 14 illustrates the anatomy of the club organ, showing details of the unicellular glands within the club with long, wide ducts opening into the inter-segmental sac.

The second pair of valvulae is fused dorsally and curled under along its edges to form the two channels within which the first pair of valvulae slide back and forth as the female drills in the wood. (Fig. 15). Figure 16 shows the first valvulae of the left side pulled some distance out of the second valvulae. The curved arms of the second valvulae are continuous with the base of the saw-sheath. (Fig. 17). The saw-sheath and base encloses the drilling shafts of the ovipositor. (Fig. 18).

There are three pairs of accessory glands associated with the female reproductive system. Two pairs of these glands have ducts opening into the ovipositor. They are the paired bunches of finger-like glands on the dorsal surface of the mucilage reservoir, and the median sac-like gland, which is not depicted by Francke-Grosmann (1939) in her diagram of S. juvencus. It is suggested from these studies that the mucous reservoir and its glands are homologous with the poison sac and glands of the honeybee which also opens by a narrow neck into the swellings of the second pair of valvulae. According to Snodgrass (1956) the neck of the poison sac is kept open by folds in the chitinous lining

which form interrupted rings. Similar ridges can be seen lining the duct of the mucous reservoir in sections of the female pupa of S. noctilio in Figure 19b.

The oil sac lies loosely attached to the anterior wall of the mucous duct. It opens into the ovipositor at the point where the first pair of valvulae diverge. It appears that the oil sac of S. noctilio and the alkaline gland of the honey bee are homologous structures. During the pupal stage the looped thick walled oil sac of S. noctilio has an extremely narrow lumen. Snodgrass (1956) describes the alkaline gland of the honey bee as a thick walled convoluted tube opening directly into the base of the "bulb".

Figure 19a and Figure 19b show the differences in the relative sizes of the reservoirs of these two pairs of accessory glands of S. noctilio during the pupal and adult stage. The mucous reservoir of the adult is an enormous, spherical sac, whereas in the pupa it is difficult to detect it connecting the conspicuous finger-glands and the clearly-defined mucous duct. The oil sac changes from a thick-walled looped tube with a narrow lumen in the pupa to a turgid, distended conical sac in the mature adult.

A pair of lateral pouches appears to arise from the membranous posterior wall of the vagina of S. noctilio. These pouches extend dorsolaterally towards the point of articulation of 8S and 9T.

Figure 20.

Spermatophores of S. noctilio obtained from the upper vas deferens.

Mag. 100x

480x

1400x

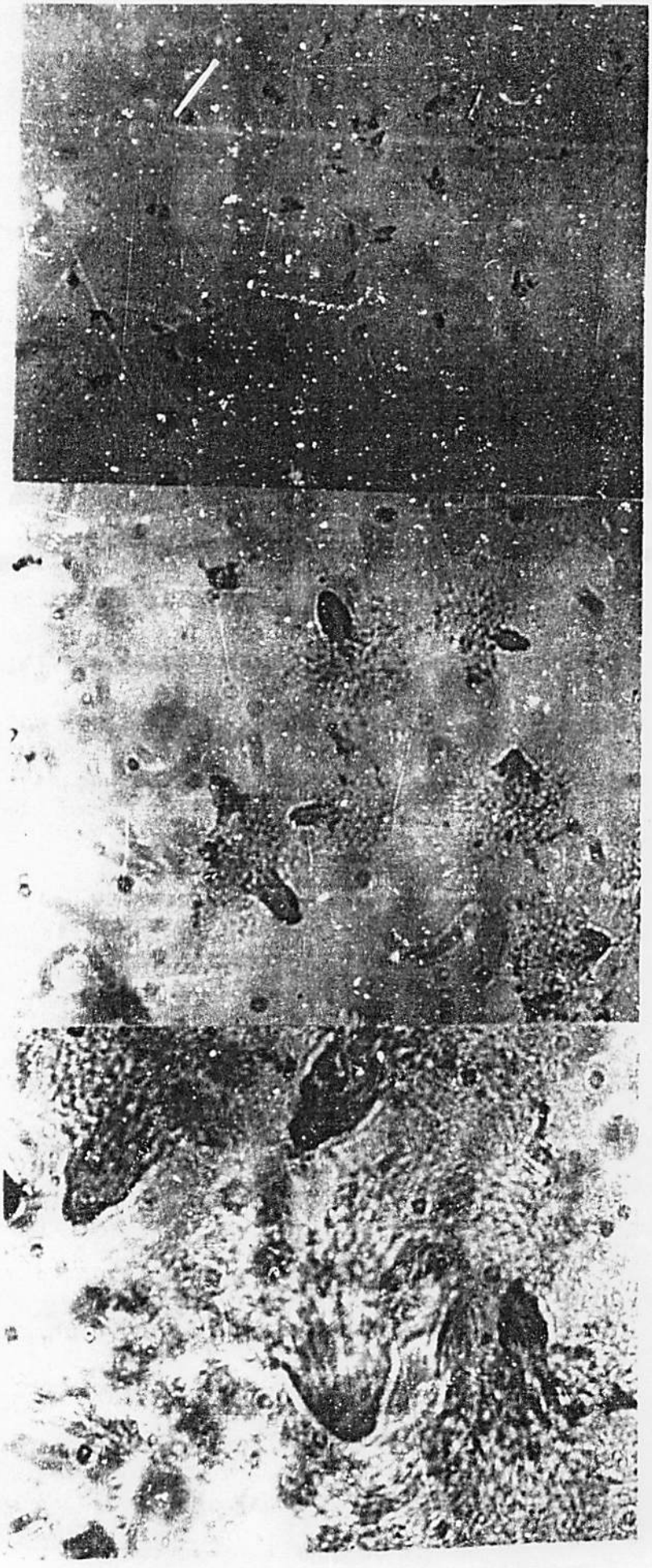


FIG. 20

They are attached by strong muscles to the dorsal apodema of 9T and to the inner surface of 8S. (Fig. 19c). The surface of the pouches is covered in a mass of white cells which are thought to be homologous with the lubricating glands of the honey bee. Kochevnikov (1900) who discovered these glands in the honey bee, assumed that their secretions lubricated the basal plates of the sting. The glands are unicellular, each opening by a duct into a pouch of the membrane between 8T and 9T.

Apart from the accessory glands already described, the female reproductive system is made up of large paired ovaries occupying most of first six segments of the abdomen. The short paired oviducts and the even shorter common oviduct lead into the vagina through a T-shaped opening. Chitinous rings support the walls of the oviduct. The anterior and dorsal surfaces of the vagina are thick and firm, whereas the posterior wall is thin and membranous. The narrow coiled spermatheca is situated on the dorsal wall of the vagina. During the pupal stage, the spermatheca has virtually no lumen and appears in sections as a tightly coiled ball (Fig. 19b). Saline squashes of the spermatheca of the mated female contain spermatophores, innumerable discarded tails and spermatozoa.

Spermatophores similar to those found in the spermatheca can be obtained by pricking the uncoiled section of the vas deferens of the adult male.

After 30 seconds fixation in osmium vapour, the heads of the spermatozoa appear as dark specks clustered inside the dome-shaped gelatinous capsule of the spermatophore. The tails are spotted with dark granules. (Fig. 20).

V. SECRETIONS OF THE ACCESSORY GLANDS

(i) Oil.

The transparent colourless contents of the median glandular sac formed discrete drops on the glass slide. When frozen, the oily secretion became cloudy. Innumerable needle-like, crystals visible in a beam of polarised transmitted light, appeared to be the cause of this cloudiness. Some crystals remained when the oil regained room temperature and the cloudiness disappeared.

Droplets of the secretion were coloured by Oil Blue N and Sudan IV showing that they contained lipids. Threads pulled from a lump of mucus did not stick to slides which has been smeared with the oily secretion. The secretion gave an acidic reaction when tested with drops of 0.2% Nile Blue. When thin layer chromatograms of the oily secretion were treated with iodine vapours to reveal lipids, five distinct spots, became visible, with an additional faint spot near the solvent front. The spot lying second from the origin was much larger and darker than the others, indicating that there is a single major component in the oil.

As this major component appeared to have the same rf as cholesterol, further slides were developed in $SbCl_3$ to test for steroids. Only the standard reacted with this reagent, so

probably there are no steroids in the oily secretion.

Chromatograms were also developed in 2'7' Di-chlorofluso-cein to test for non-polar lipids. Under U/V light, four fluore-scent green spots appeared indicating the presence of both sat-urated and unsaturated non-polar lipids. In chromatograms freshly sprayed with Bromo-Thymol-Blue, five yellow spots appeared against a place blue background, showing that the oily secretion contains at least five fatty acids.

In laboratory tests, threads of mucus harden within minutes on exposure to the air. Adult females of Sirex noctilio oviposit over a period of a fortnight without any apparent trouble from hardening mucus. Dissected shafts of the ovipositor glisten with oil and threads of mucus did not stick to glass slides which had been smeared in oil. Possibly the oily secretion acts as a non-stick lubricant for the moving shafts of the ovipositor.

It seems likely that this film of oil keeps the inner sur-faces of the ovipositor free from contamination. Wax packets kept for a month in drops of the oil secretion showed no signs of surface contamination. When transferred from the oil to plates of agar, several packets developed surface contaminants, mainly yeasts.

The presence of at least five fatty acids in this secre-

tion suggests that it might supply nutrients for the fungus inoculated into the sapwood as well as for any crops of fungus associated with the ovipositor. Gaut (pers. comm.) has shown that in the presence of unsaturated fatty acids supplied experimentally as nutrients, the rate of growth of cultures of Amylosterum spp. increases as the C-chain length of the acids increases from 10 to 18. However, the intensity of the spots of the chromatograms revealed in iodine vapour, indicates that the fatty acids in the oily secretion of the woodwasp are probably saturated, see Stahl (1965) p. 150.

(ii) Mucus.

The mucus is clear and colourless. It turns deep amber in dead specimens. The firm ball of mucus can be drawn out into sticky threads which become brittle within a few minutes.

These threads can take up water rapidly and become sticky again. A ball of mucus takes up water slowly, however, Thus, two hours after an equal volume of borate buffer pH 10 had been added to it, it was still firm, although all the buffer was absorbed overnight.

A thin smear of mucus on a slide disappeared within twenty minutes in 0.1N KOH. The smooth shiny surface of the gel became fluffy after 2 hours in 8M urea, indicating that hydrogen bonds

were being broken down. The gel dispersed rapidly with some frothing in 6N HCl.

When tested with Millon's reagent, which reacts with Tyrosine, the mucus gave a positive reaction by turning orange. These results indicated that proteins might also be present.

The PAS test for carbohydrates was inconclusive. Once the fixed mucus had been oxidised with periodic acid, it reacted with fuchsin to produce a dense purple-pink colour, possibly indicating that carbohydrate was present. As the reaction with periodic acid was not blocked by acetylation, it was not possible to confirm this result.

When testing for lipids with a saturated solution of Sudan Black B in 70% alcohol, the mucus became black. Nevertheless, this colouring did not wash out in an acetone rinse, and thus the result could not be attributed to the presence of lipids. It seemed likely that the secretion contained protein capable of staining with Sudan black B.

Using both the standard method of Pearce and the method of Kramer and Windrum, listed by Pearce, the mucus was tested with toluidine Blue for metachromasia. The mucus was not fixed. In both cases the mucus became blue (O-metachromasia). According to Pearce acid mucopolysaccharides should turn pink.

When the mucus was treated with 0.0004% methylene blue buffered at pH 2.6 it was able to bind the dye even at this pH, showing that it was strongly basophilic.

When tested with Alcian Blue, the mucus turned clear blue-green, which is the colour reaction shown by acid mucopolysaccharides.

Both mucus that had been incubated with hyaluronidase, and a control piece that had been submerged in 0.85% saline, turned blue when stained for twenty minutes in 0.5% Toluidine Blue. Half an hour later, the colour in the blob of mucus treated with hyaluronidase had vanished. Hence the hyaluronidase had affected the reaction between the mucus and toluidine blue. Possibly, this could be explained as the hydrolysis of some of the hyaluronic acid in the mucus by the hyaluronidase.

Although unsatisfactory results were obtained using PAS and Toluidine Blue and inconclusive results were available from the test with hyaluronidase, the results obtained using Alcian blue, Methylene blue, Sudan Black B and Millon's reagent, support the provisional identification of the mucus as acid mucopolysaccharide protein complex which is corroborated by the results from the chromatograms of the acid hydrolysate.

The paper chromatograms of the acid hydrolysate of the mucus

were developed with four reagents. Attempts were not made to determine precise Rf values, nor to identify components unequivocally, but the chemical nature of the compounds in the hydrolysate was investigated and standards were used to verify the colour reactions produced by the reagents.

Two bands in the chromatograms of the hydrolysate developed with phenol, water, reacted with ninhydrin, showing the presence of amine groups in the hydrolysate, and corroborating the indication of protein obtained with Millon's reagent.

When the solvent system methyl ethyl ketone: Proprionic acid:Water (60+20+20) was used, eight bands in the chromatograms reacted with ninhydrin.

When chromatograms of the hydrolysate were developed in phenol, water, and subsequently were dipped in $AgNO_3$ for detecting sugars, five black bands were revealed.

A single orange band appeared on experimental chromatograms sprayed with Acetylacetone - Dimethylaminobenzaldehyde reagent, showing the presence of hexosamines in the hydrolysate.

Three bands in the experimental chromatograms gave the same colour reactions with p-anisidine HCl as the standards glucuronic and galacturonic acid.

Taken together, these results indicate that the mucus is a protein-carbohydrate conjugate. A further test for sulphate groups was carried out on the hydrolysate. After adding a few drops of $BaCl_2$ to the hydrolysate, a beam of light was transmitted through it. The fine white precipitate confirmed the presence of sulphate groups.

The presence of proteins, uronic acids, hexosamines, oligosaccharides and sulphate groups in the mucus, is consistent with its being an acid micopolysaccharide-protein complex.

King (1966) mentioned that the germination of the arthrospores must be rapid as two days after oviposition she could find no trace of them near the eggs. It has been assumed that the mucus is a source of nutrient for the fungal oidia inoculated into the sapwood by woodwasps. Tests carried out in Section VI substantitate this assumption.

VI. CULTURING

(a) Cultures from the larval gut.

Attempts to culture Amylostereum from the hind gut of female larvae were unsuccessful. Clarke (1933) mentions that he dissected three female larvae under sterile water when removing sections of the gut for culturing whereas the larvae in this study were cut up dry. As the larval cuticle is not sterile, his results must be considered suspect. His cultures could easily have been contaminated with scrapings of fungus from the tunnel.

Yeasts were cultured from fore, mid and hind-gut of both male and female larvae, in all but a five of the larvae. Diplodia and Trichoderma were obtained from these remaining larvae, indicating that these yeasts and fungi are not digested by the gut fluids.

(b) Cultures from the hypo-pleural organ.

Pure cultures of Amylostereum were obtained from three excised organs. These organs, together with the underlying agar and the mycelium were cleared and mounted. The mycelium could be seen growing out of the pits. There were numerous cystidia near the margin of the cultures, confirming the identity of the fungus (see Talbot, 1964).

In two instances the excised organs were over run with Trichoderma, and the cultures were discarded. Although a third organ

was also contaminated, threads of a white mycelium with clamp connexions could also be detected.

(c) Cultures made from larval tunnels.

Of the 108 slivers of wood shaved off the surface of larval tunnels, only seven produced pure cultures of the symbiotic fungus. The rest were contaminated with either Diplodia or Trichoderma. Sub-cultures could have been made from the stray threads of basidiomycete fungus sprouting from the surface of the wood, however, King (1964) had already made many isolates of the symbiotic fungus from the tunnels.

(ii) a). The dense white mycelium lining one of the pupal chambers created the impression that the inter-segmental sacs could readily be infected by the invasion of fungus from the surrounding growth. Cultures were taken from the wood in an attempt to identify the fungus. These were over-run with contaminants but a stained and mounted sliver of wood showed a basidiomycete fungus growing among the contaminants.

Further information was obtained from the cast pre-pupal skin found in another pupal chamber.

On one side of the skin, a series of dark patches were apparent in the characteristically elliptically shaped region of the hypo-pleural organ. Unlike the usual glistening ridge of wax packets, these dark patches had a crumbly appearance, and were separated from each other by regularly arranged transverse folds reminiscent of collapsed septa. Threads of a clamp fungus were seperated from fragments taken from these patches. Examination of the stained mount made of this culture showed that the mycelial threads originated from the dark patches. Scattered over this skin were numerous tiny scrapings of wood which were encrustered with the mycelium a basidiomycete fungus. See Fig. 22a. Cultures made from these scrapings developed cystidia, indicating that the fungus was Amylostereum sp.

(ii) b. Ovipositor and inter-segmental sacs.

No fungus could be cultured from the inter-segmental sacs of any pupae. The youngest adults used during these experiments were newly moulted and therefore soft and brown. Attempts at culturing the symbiotic fungus from the inter-segmental sacs were unsuccessful. The symbiotic fungus could sometimes be cultures from the external

surface of the body. In one case the shafts of the ovipositor gave a positive result.

Adults which had blackened but not started boring out of the pupal chamber gave similar results, except that the fungus was not obtained in cultures made from the shafts of the ovipositor.

In these females, the abdomen was packed with fat body, the white inter-segmental sacs contained a few droplets of oil, and the accessory glands showed slight secretory activity.

Detailed results were obtained from a female which had started boring. Separate cultures were made of the inter-segmental sacs, the club glands, the tip of the abdomen, the ovipositor sheath, the distal ends of the ovipositor, the bases of the first pair of valvulae, the surface of the frass and the wood lining the pupal chamber. Five days later, the only development was the contaminants growing from the wood. By the 12th day a tuft of fungal threads with damp connexions could be seen growing from the tips of the valvulae. Cultures of the symbiotic fungus were obtained from the tip of the abdomen. After three weeks there was still no sign of any growth from the other parts of the ovipositor and sacs.

Cultures made from the sacs and ovipositor shafts of females which were chewing through the reddish bark, from females with their heads visible within the emergence holes and from females