

callusing at the basal end (Fig. C). Plantlets produced were mostly lean and weak (Fig. D). The growth response was genotypically oriented<sup>8</sup>. Of all the different combinations of various lines, the cv. Aohar of barley gave the best response, whereas the source of the pollen did not matter.



FIGS. A-D. *In vitro* growth of *Hordecale* embryos on synthetic media. Fig. A. 3-week-old culture of an excised embryo (9 days after pollination) on MS + 2, 4-D (2 mg/l) : note the profuse callusing. Fig. B. 3-week-old culture on MS + CH + IAA + kinetin showing sparse proliferation and initiation of roots. Figs. C, D. Three and four-week-old hybrid plantlets.

The *Hordecale*, thus produced, will be employed as a bridge for increasing the genetic variability in barley. In addition to these hybrids, some haploids would be expected<sup>9</sup> and they can be incorporated into the barley improvement programme.

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#### EFFECT OF FAT SOLUBLE VITAMINS ON GROWTH AND SPORULATION OF *PESTALOTIOPSIS*

FUNGAL growth and sporulation is markedly affected by vitamins<sup>1-3</sup>. However, reports on the utilisation of fat-soluble vitamins by fungi are scarce. Therefore, such studies were undertaken on *Pestalotiopsis versicolor* (Speg.) Steyaert, isolated from the leaves of *Gnetum gnemon* L. and *Nepenthes khasiana* Hook., f. and *Pestalotiopsis theae* (Saw.) Steyaert var. *minor* from the leaves of *Callistemon lanceolatus* DC.

Pure isolates were cultured on vitamin-free Asthana and Hawker's medium. The pH of the medium was adjusted at 5.5. Heat sensitive vitamins A, K and E were added to the medium after autoclaving. The liquid medium (50 ml.) was taken in 250 ml conical flasks and vitamins (50 ppm each) were added to the flasks. A mixture of 0.5 ml benzene and 0.5 ml of 80% ethanol was used for dissolving the vitamins. Each flask was inoculated with 1 ml of spore suspension at 22°C ± 2°C for ten days and the mycelial mats were filtered through Whatman Filter paper No. 42, washed and dried at 60°C for 48 hours. The average of three replicates were recorded. Sporulation was measured as suggested by Bilgrami and Verma<sup>3</sup>.

Maximum mycelial dry weight (210 mg) of *Callistemon* isolate (*P. theae* var. *minor*, IMI No. 226396) was recorded when vitamin D-3 was furnished whereas vitamins A and E were favoured most by both the isolates of *P. versicolor*, i.e., IMI Nos. 226392 (from *Gnetum*) and 226376 (from *Nepenthes*) and the mycelial weights were 330 mg and 400 mg respectively. Growth of *Callistemon*-isolate was retarded a little in the presence of all the vitamins as compared to that of control. However, sporulation on all the media was generally excellent except in *Callistemon*-isolate under the effect of vitamin E.



Practically all the fat-soluble vitamins, viz., A, D-3, E and K were used by the present isolates of *Pestalotiopsis* and induced satisfactory sporulation within 3-5 days. In no case the sporulation was inferior to that of control. The results are interesting as the syntheses of these vitamins by fungi are rare.

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#### BARTHOLIN'S GLAND OF THE INDIAN PIPISTRELLID BAT, *PIPISTRELLUS DORMERI* (DOBSON)

INVESTIGATIONS on the reproductive processes of bats are being conducted in this laboratory for the past several years. Amongst the various bats studied, Bartholin's glands, which correspond to the Cowper's glands of the male, are seen as separate entities in *Pipistrellus dormeri*<sup>1</sup>, *Pipistrellus mimus mimus*<sup>2</sup>, and *Chalinolobus gouldi*<sup>3</sup> all belonging to the family Vespertilionidae. A perusal of the literature on Bartholin's gland reveals that there is practically no information on the distribution and histochemical characterisation of mucins elaborated by these glands in Chiroptera. The present study has, therefore, been undertaken to analyse the mucins secreted by these glands and to determine the possible role of these mucins in the physiology of reproduction.

Specimens of *Pipistrellus dormeri* (Dobson) were collected from old ruins of a fort in Chikaldhara, Distt. Amravati. The lower part of the vagina along with the Bartholin's glands were dissected out from 12 specimens of which 4 were nonpregnant and 8 were in various stages of pregnancy. The tissues were immediately immersed in cold (4°C) solution of 2% calcium acetate in 10% formalin. After fixation for 24 hours, the tissues were thoroughly washed by chilled water, dehydrated through graded series of ethanol, embedded in paraffin and sectioned at 5 to 6  $\mu$ . For histological observations the sections were routinely

stained with haematoxylin and eosin and Mallory's triple staining technique. For histochemical characterisation of mucins the sections were stained by well-known histochemical methods. The classification of mucins was according to Spicer *et al.*<sup>4</sup> and techniques used were as given by Lillie and Fullmer<sup>5</sup>.

The Bartholin's glands are pear-shaped structures lying on either side of the vagina and abutting against the wall of the vulval aperture. A single duct arises from each gland and opens into the vagina on the dorso-lateral aspect near its distal end.

Histologically each gland is a compound tubulo-alveolar gland of the mucous type and is surrounded by a well-defined fibrous connective tissue capsule (Fig. 1). The alveoli are separated from each other by thin connective tissue, the fibres of which stains blue in Mallory's triple staining procedure. Blood capillaries and minute ductules, which are lined by squamous to cuboidal cells are present in the connective tissue. A section through the middle of the gland shows a large central cavity lined by a regular epithelium of cuboidal cells. The central cavity continues as the main duct which opens in the vaginal lumen. The alveoli, which are the secretory units of each gland, are lined by columnar cells with basally situated nuclei and basophilic cytoplasm. The lumen of the central duct always contains an eosinophilic secretion whose quantity, however, varies during the different phases of the reproductive cycle. During pregnancy there is an overall increase in the basophilia of the cytoplasm of these mucous cells and the secretion in the duct.

Table I shows the results of histochemical tests employed. In the nonpregnant specimens the cytoplasm of the mucus secreting cells lining each alveolus stains intensely with periodic acid Schiff reagent (PAS) (Fig. 2). The intensity of this stain is not altered by saliva or diastase digestion thereby indicating that glycogen may not be present in appreciable quantities.

However, the PAS staining is partially blocked by prior phenylhydrazine treatment thereby indicating the presence of both neutral and acidic mucins. The presence of these could also be demonstrated by the sequential staining with alcian blue (AB) (pH 2.5)-PAS which shows a bluish purple stain (Fig. 3). The alcianophilia observed with AB at pH 2.5 is higher than that observed with AB at pH 1.0 (Fig. 4) thus indicating the presence of both carboxymucins and sulfomucins. The sulfomucins could also be stained with aldehyde fuchsin (AF) in the aldehyde fuchsin alcian blue (pH 2.5) sequential staining and by the metachromasia observed with Azure A at a low pH of 0.5. Studies on extinction values of basophilia in the presence of graded concentration of MgCl<sub>2</sub> confirmed the presence of sulfomucins.