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 sporuation 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*[1], *Pipistrellus mimus mimus*[2], and *Chalinolobus gouldi*[3] 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 μ. 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.*[4] and techniques used were as given by Lillie and Fullmer[5].

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 dorsolateral aspect near its distal end.

Histologically each gland is a compound tubuloalveolar 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 cytopalsm 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.

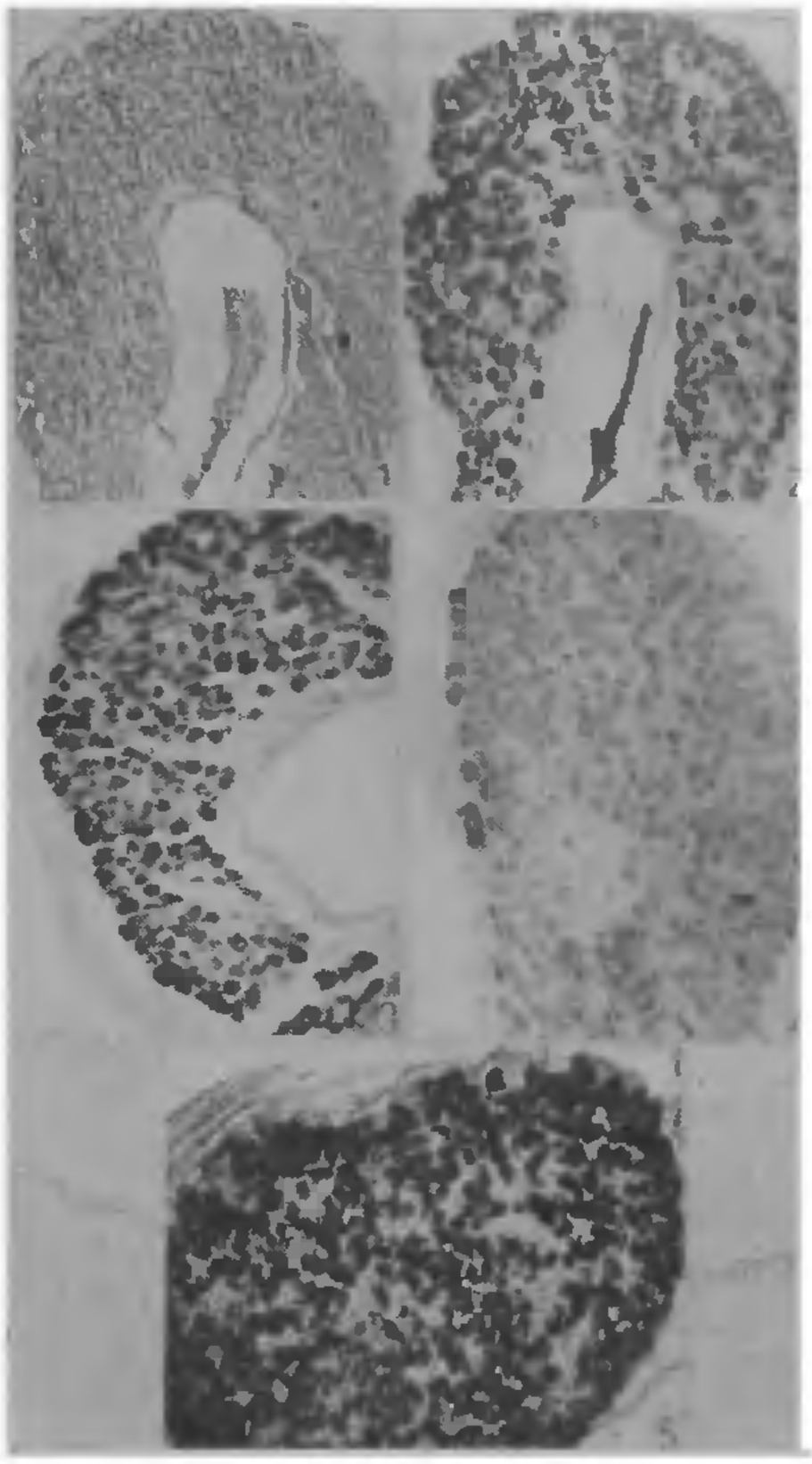
However, the PAS staining is partially blocked by prior phenylhydrazine treatment thereby indicating the presence of both neutral and acidic mucins. The presence of these could also be demonstrated by the sequential staining with alcian blue (AB) (pH 2·5)–PAS which shows a bluish purple stain (Fig. 3). 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 $MgCl_2$ confirmed the presence of sulfomucins.

TABLE I

Histochemical reactions of mucosubstances in mucous cells of Bartholin's gland of bat

Histochemical reaction	Mucous cells of non-pregnant bat	Mucous cells of pregnant bat
PAS	++++ P	+++++ P
Phenylhydrazine–PAS	++ P	+++ P
Saliva digestion–PAS	++++ P	+++++ P
AB pH 1·0	++ B	+++ B
AB pH 1·0–PAS	+++ BP	++++ BP
AB pH 2·5	++++ B	+++++ B
AB pH 2·5–PAS	++++ BP	+++++ BP
AF–AB pH 2·5	+++ BP	++++ BP
Azure A pH 1·5	++ WM	+++ WM
Azure A pH 3·0	+++ WM	++++ WM
Azure A pH 4·5	++++ WM	+++++ WM
AB (Mg^{++} 0·1 M)	++++ B	+++++ B
AB (Mg^{++} 0·2 M)	++++ B	++++ B
AB (Mg^{++} 0·4 M)	++ B	+++ B
AB (Mg^{++} 0·5 M)	..	..
Methylation 37° AB pH 2·5	++ B	++ B
Methylation 37° —saponification AB pH 2·5	++++ B	+++++ B
Methylation 60° AB pH 2·5	..	..
Methylation 60°—saponification AB pH 2·5	++ B	+++ B
Acid hydrolysis–AB pH 2·5	++ B	++ B
Neuraminidase–AB pH 2·5	++ B	++ B
Hyaluronidase–AB pH 2·5	++++ B	+++++ B

Key to symbols used : +·+ = weak; +·++ = moderate; ++++ = intense; +++·+ = very intense; P = pink; B = blue; BP = blue-purple, WM = wet metachromasia; PAS = Periodic acid Schiff; AB = Alcian Blue; AF = Aldehyde fuchsin.



FIGS. 1–5. Fig. 1, T.S. of Bartholin's gland of non-pregnant bat showing large number of alveoli, Note the secretion in main central duct of gland. × 150. Fig. 2. Section of Bartholin's gland of a non-pregnant bat stained with PAS. Note moderately stained mucous cells and PAS positive secretion in the duct. × 150. Fig. 3. Section of Bartholin's gland of non-pregnant bat stained with AB pH 2·5–PAS. Mucous cells are positive to both stains, imparting bluish-purple colour. × 150. Fig. 4. Section of Bartholin's gland of non-pregnant bat stained with AB pH 1·0. Note the weak alcianophilia of mucous cells, × 150. Fig. 5. Section of Bartholin's gland of pregnant bat stained with AB pH 2·5–PAS. Note that the mucous cells are intensely positive to both stains, imparting bluish-purple colour, × 150.

The alcianophilia observed with AB at pH 2·5 is partially lost following acid hydrolysis and mild methylation which is subsequently restored after saponification with 1% potassium hydroxide in 70% alcohol for 20 minutes. This indicates the presence of carboxymucins. The carboxymucins are identified as sialomucins since they show metachromasia with Azure A at high pH of 3·0 and this is destroyed by neuraminidase digestion. Prior treatment of sections

with hyaluronidase shows no alteration in the staining intensity with alcian blue at pH 2·5 nor is the metachromasia lost with Azure A at high pH. Thus the mucous cells elaborate neutral, sulfo- and sialomucins.

During pregnancy the mucous cells show the same histochemical reactions as those of the non-pregnant bat, but the intensity of the reactions with PAS, AB (pH 1·0 and 2·5) and AF is increased than that observed during the non-pregnant condition. This indicates an increase in the concentration of mucins (Fig. 5).

Amongst the various bats studied, Bartholin's gland, which corresponds to the Cowper's gland of male, has been observed as separate entities only in two Pipistrellid bats *Pipistrellus dormeri*[1] and *Pipistrellus mimus mimus*[2] and in *Chalinolobus gouldi*[3]. The histological structure of tubulealveolar gland of *Pipistrellus dormeri* is similar to that reported in human beings[6,7].

The presence of sialomucins has been reported in the bulbourethral gland of mice, rats and guineapig and in the vestibulares majores of human foetuses by Halbhuber and Gunther[9] and in cattles by Friess[9]. Sialomucins are also reported in the Bartholin's gland of this bat together with neutral and sulfomucins.

The epithelium of the greater vestibular glands of cat is found to be rich in mucin shortly before estrus and during the later half of pregnancy but declining during lactation[10]. Marked changes in the histological structure correlated with the reproductive cycle has been reported in the Bartholin's gland of hyaena[11]. In human beings, Bloom and Fawcett reported that this gland undergoes involution after the attainment of puberty. Marked changes in the mucin content is observed in the bat studied here, the amount increasing progressively during pregnancy.

In human beings the Bartholin's glands pour out clean thin mucous secretion to lubricate the vulva at coitus[12,13]. Similarly, the mucin elaborated by the Bartholin's glands of bat may probably act as a natural lubricant of the vagina at coitus during sexual excitement, and during parturition.

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INTERFERTILITY STUDY OF *TRAMETES FLOCCOSA* BRES.

The type of mating system was considered by Nobles[7] as an important character for solving taxonomic problems of Polyporaceae and it is now widely accepted by the mycologists that fungi showing different types of interfertility cannot belong in the same genus[3,4,9]. Although the type of interfertility of several Basidiomycetes have been determined[2–8], *Trametes floccosa* Bres. has not been studied, so far, from this point of view. The present paper communicates the result of interfertility test of *T. floccosa*, a wood-rotting polypore of India.

The sporophore of *T. floccosa* was collected from Santiniketan, West Bengal, India, on a living tree of *Ficus religiosa* L. Twenty-five monosporous cultures were made from the spores of this sporophore following the usual dilution method. When each of the 25 monosporous cultures showed good growth they were checked carefully for clamp connections. The absence of clamp connections was taken as confirmation of their monokaryotic character. Finally 20 monokaryotic cultures were taken into consideration and were paired among themselves in all possible combinations on 2·5% malt agar slants. The culture tubes were then incubated at room temperature (28 ± 2° C) for about a fortnight and the inoculum from the line of contact between the paired mycelia was examined under a microscope. In some pairings clamps were noticed while in others no clamps were formed.