MATERIAL AND METHODS
MATERIAL:

List of the material has been furnished in Chapter II.

METHODS:

Field techniques:

Study of extant ferns: The primary survey of the distribution of ferns was done by making a list of the ferns growing in each different locality (vide Page 8). Also, the habitat of the individual fern species was recorded and in many cases their growing conditions were photographed.

Most of the ferns of Darjeeling usually produce sorus either during spring (March-May) or after the rains (August-October). So, field trips were conducted during spring and autumn of 1979 and 1980. Material available in reproductive stage were collected.

Herbarium made out of such collections were identified taking the help from the Botanical Survey of India, Howrah, and are being preserved in the Department of Botany, Bose Institute.

Collection of peat samples:

Darjeeling hills

Peat samples were collected from Lopchu (Text-fig. 3)
and Barasenchal (Text-fig. 4) in Darjeeling hills. Both the deposits were identical in nature and formed in a pond surrounded by hills. During the rainy season it remains submerged (under ± 0.5 m water). The depositional environment in both the cases was more or less similar and it was free from external influences like tidal currents or erosions from the surrounding elevations of the hills.

The samples were collected with the help of a Hiller type of sampler. In this sampler an outer loose jacket with a projecting lip remains stationary while an inner chamber turns so that a longitudinal slit of the jacket and the chamber coincide. After the sampler is pushed inside the deposit it is rotated in a clockwise direction and the sample gets inside the chamber. An anticlockwise rotation helps to close the longitudinal slit. Then the sampler is taken out. The chamber is made open and the samples collected were taken out. Collections were made using two holes alternately. Before opening the chamber, the surface of the sampler was properly cleaned to avoid contaminations. The entire sample was taken at a regular interval of 10 cm. Direction of each sample was marked. At the same time each numbered sample was wrapped in cellophane papers separately. The samples were preserved in a desiccator in a moist condition.

Bengal basin (Kantalia):

The peat was collected from a profile exposed during
the excavation of a new pond. Initially the surface of the peat strata was cleaned with a knife. The thickness of the peat layer was measured and the material was collected at an interval of 7.4 cm. Profile diagram is represented in Text-fig. 2. Materials were preserved similarly as has been stated with the Darjeeling deposits.

Laboratory techniques:

Processing of the spores from the herbarium material:
Sori from the selected materials was crushed on a finely meshed (60-100 mm) screen spread out on a funnel kept in a centrifuge tube.

Acetolysis mixture (anhydrous acetic acid and concentrated sulphuric acid 9:1) was added to the powder collected in the tube and stirred; then heated to a boiling point in a water bath, frequently stirred and was maintained in such condition till the liquid became light brown. The mixture was centrifuged at 2000 rpm and decanted. A little water was carefully added, thoroughly shaken and then a few drops of acetone were added. This mixture was filtered twice through a finely meshed net and centrifuged. Half of the sediment was taken for chlorination and the other half was kept as it was.

About 5 ml of glacial acetic acid, one or two drops of conc. sodium chlorate (freshly prepared) solution and a
few drops of conc. HCl was added to effect chlorination. This process immediately bleached the spores. Chlorination was avoided in case of thin walled grains. The material was thoroughly washed with distilled water by repeated centrifuging. Then the chlorinated and non-chlorinated grains were mixed, a few drops of 50% glycerine was added to the mixture and centrifuged. In the precipitate 50% glycerine was added, stirred and gently heated in water bath for about five minutes to recover the size of the grains. The material was centrifuged for a longer time (about ten minutes) and then the glycerine was decanted.

The centrifuged tubes were kept upside down for a while on a filter paper to decant the last drops of the water and it was incubated at 40°C till the material becomes dry. To prepare slides minute piece of glycerine jelly was taken on a platinum needle, touched on the surface of the spore bearing sediment with jelly cube and transferred to a clean slide.

The jelly containing material was melted on a flame and spreaded. A circular cover glass was placed on the slide and sealed off with solid paraffin (melting point 68°-72°C) under the cover glass.

Preparation of fossil samples: The samples were prepared in the conventional method (Faegri and Iversen, 1960 and Chanda, 1962) which in short is thus: Samples to be analyzed were first scrutinized as such with a lens in order to see...
whether it contained macrofossils. The wet sample was carefully transferred to a porcelain pot, KOH soln. (10%) was added in cold, thoroughly mixed and then allowed to boil for 5-10 min. to allow disintegration. During boiling distilled water was added from time to time to maintain a constant concentration of KOH. The mixture was cooled, sieved through a mesh (diameter 0.3 mm) to eliminate the bigger particles. The mixture was then washed again and again by adding distilled water and centrifuging until the fluid became clear. The material with sandy clay was subsequently transferred to polythene beakers and treated with HF (40%) for two or three days. The last trace of acid product (silicon tetrafluoride is volatile) was removed by repeated washing and centrifuging. Fully washed material was treated with glacial acetic acid, centrifuged, decanted, and then treated with acetolysis mixture in water bath between 80°-100°C, and occasionally stirred with glass rod. The mixture was centrifuged, supernatant was decanted. The residue was washed with distilled water by centrifuging. Residue was finally taken in pure glycerol and heated to 80°C. Thus, the material in glycerol was cooled and preserved in small specimen tubes for microscopic analysis.

The material in the specimen tube was stirred with a small glass rod, one drop was mounted in a slide with a square cover glass. Excess material was removed by blotting. The slide was then ready for microscopical work.
Microscopical study was made by moving the mechanical stage from one end to the other, the direction of the movement was marked. An Leitz Wetzlar (Ortholux) binocular microscope was used for routine study. For such study 42 (NA = 6.5), eyepiece 10X magnification was used. Occasionally, X1000 (oil immersion) was used for a detailed study whenever necessary.

Significant spore types from different deposits were preserved permanently by the method of single grain preparation (Faegri and Iversen, 1950).

The numerical data of each representative fern spore was recorded. Frequency percentage of representation of individual spore type was determined from the total count and represented in spore diagrams. For the present study a total of +200 grains were counted per sample from each deposit.

The photomicrographs were taken with an Zeiss Wetzlar microscope (100 X N.A. 1.30; 40X = 0.65; eyepiece 10X). Magnification of the photomicrographs arranged in different plates were X2000 unless otherwise mentioned.

Some complicated components of the exinous parts of some spores were drawn using camera lucida and they have been arranged in the text-figure with magnification. For the measurement of monolete spores polar view of the grains showing the aperture was selected. For the trilete spores, the polar view...
of the grains was selected and the polar diameter was measured (from a corner of the triangular spore to the middle of the opposite amb) and have been represented in millimicron (μm).