exine, a report which is contrary to the earlier one[2]. Brewbaker[3] studied a number of Compositae and concluded that three-celled pollen grain (at the anthesis) is a characteristic feature of the family Compositae.

A linear tetrad of megaspores is formed consequent upon the two meiotic divisions in the megaspore mother cell (Fig. 1). Embryo sac is of the polygonum type (Fig. 2). Antipodal cells in the newly formed embryo sac are either two or three in number. In some cases the antipodal cells increase in number (Figs. 4, 5) while in others nuclear divisions and fusions result in multinucleate and polyploid cells (Fig. 3). This disproves the opinion so generally held by Harling[4] and Eliasson[5] that the tribe Heliantheae is characterised by two or three secondarily multinucleate, but not dividing antipodal cells.

Fertilisation is porogamous. Endosperm development is of the nuclear type. The primary endosperm nucleus undergoes a few divisions resulting in free nuclei which migrate to the periphery of the embryo sac where they divide further (Fig. 7). Eventually the endosperm enters the cellular phase and fills the entire embryo sac with cellular tissue. By the time a globular embryo is formed the endosperm cells undergo divisions such that a narrow layer of small peripheral cells is formed (Fig. 8). These peripheral endosperm cells resemble the endothelial cells in their appearance and in their glandular nature, but differ from them in being longitudinally elongated. The entire endosperm excepting this peripheral layer is consumed by the growing embryo. Similar condition of formation of peripheral layer in Heliantheae is reported earlier by Kapil and Sethi[6] in *Tridax trilobata*, Padmanabhan[7] in *Tridax procumbens* and Pullaiah[1] in *Tithonia rotundifolia*. The primary endosperm nucleus in a few cases even after formation of two-celled embryo, remains undivided (Fig. 6)

The zygote undergoes transverse division resulting in a terminal cell, *ca* and a basal cell, *cb* (Fig. 9). The former undergoes a vertical division and the latter divides transversely resulting in a 'T' shaped four-celled proembryo. The derivatives of the basal cell *cb* are termed as *m* and *ci* (Fig. 10). The cell *ci* divides by a transverse wall and produces two cells *n* and *n'* (Fig. 11). The cell *n'* after undergoing one more transverse division produces two cells *O* and *p* (Fig. 12). The cell *p* undergoes two or three transverse divisions and produces an uniseriate suspensor (Figs. 13, 14).

The four-celled proembryo is 'T' shaped and both the cells *ca* and *cb* contribute to the development of the embryo proper (Figs. 9–14). Further development of the embryo follows the Senecio variation of Asterad type.

This work was carried out at the Department of Botany, Andhra University. The author is thankful to Prof. H. Maheswari Devi for guidance.

October 9, 1980,

1. Pullaiah, T., *Phytomorphology*, 1978, **28**, 437.
2. Vaidya, P. B., *J. Biol. Sci.*, Bombay, 1961, **4**, 11.
3. Brewbaker, J. L., *Am. J. Bot.*, 1967, **54**, 1069.
4. Harling, G., *Acta Hortic. Bergiani*, 1962, **20**, 63.
5. Eliasson, U., *Op. Soc. Bot. Lund.*, 1974, **36**, 1.
6. Kapil, R. N. and Sethi, S. B., *Phytomorphology*, 1962, **12**, 235.
7. Padmanabhan, D., *Phytomorphology*, 1962, **12**, 356.

A NOVEL IMMUNOGENIC STRAIN : *MYCOBACTERIUM HABANA* AGAINST *MYCOBACTERIUM ULCERANS* (BURULI ULCER) INFECTION IN MICE

N. B. Singh, I. S. Mathur, H. P. Gupta and A. Srivastava

Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India

'Buruli ulcer', is a local ulcerative disease of humans caused by *Mycobacterium ulcerans*. Its response to known antitubercular regimens is very poor[1,2], and at present no prophylaxis is available. Bacillus Calmette and Guerin (BCG), the only vaccine strain of mycobacteria has shown little protection against this ailment[3]. Search for immunogenic strain(s) amongst mycobacteria and/or other eubacteria, is of great interest. In a general screening programme for search of immunogenic strains against experimental tuberculosis of mice, a strain of atypical mycobacteria designated *M. habana* (TMC 5135) was found to afford high degree of protection against *M. tuberculosis* $H_{37}Rv$ challenge in mice[4] and guinea pigs[5]. This strain of *M. habana* was followed in further studies for its immunogenic spectrum against various models. One such model available with us was *M. ulcerans*-mouse model. When *M. ulcerans* is given to the mouse through intravenous route, it produces fulminating type of disease with high mortality rate and in chronic conditions ulceration of the tail is the most common symptom. *M. habana* was tested in this model in two successive experiments.

Expt. No. 1 : Five groups of mice comprising of 20 animals each were taken. In the first and second groups *M. habana* alone was administered subcutaneously in 4 and 1 mg/mouse doses respectively. In the third and fourth groups, the same two doses were

TABLE I

Effect of vaccination with M. habana *against a low challenge by* M. ulcerans*

Vaccinating dose		Median Survival time** (days)	Survival at day 30 of *M. ulcerans* challenge			Survival at day 60 of *M. ulcerans* challenge		
M. habana (mg)	IFA (ml)		No./Total	Protection %	P^T	No./Total	Protection %	P^T
4·0	..	>60·0	15/15	47	0·05	12/15	56	<0·01
1·0	..	55·0	17/17	47	0·01	7/17	17	NS
4·0	0·1	>60·0	14/15	40	0·05	12/15	56	<0·01
1·0	0·1	55·0	15/17	35	NS	6/17	11	NS
Nil	Nil	33·0	9/17	..	..	4/17	..	..

*Intravenous challenge, 0·3 mg (wet weight)/morse.
**Experiment terminated on day 60 of challenge.
The *P* values were calculated by the adjusted Chi-square method.

TABLE II

Effect of vaccination with M. habana *against a high challenge by* M. ulcerans*

Sl. No.	Vaccinating dose: M. *habana* (mg)	Vaccinating dose: I.F.A. (ml)	No. of Mice	Survival time (days): Median	Survival time (days): Mean ± SE	Statistical analysis**: 't'	Statistical analysis**: Df	Statistical analysis**: *P*
1.	4·0	..	18	17·5	19·9±1·8	5·1	34	<0·001
2.	1·0	..	20	14·0	15·0±1·2	2·8	36	<0·01
3.	4·0	0·1	18	23·0	21·4±1·6	6·3	34	<0·001
4.	1·0	0·1	18	15·5	15·7±0·9	4·1	34	<0·001
5.	Nil	Nil	18	11·0	11·2±0·5	..	..	..

*Intravenous challenge, 1·5 mg (wet weight)/mouse.
**Comparison made with Sl. No. 5 (control), using student's 't' test.

combined with 0·1 ml/mouse of incomplete Freund's adjuvant (IFA). The last group was kept as unvaccinated control. The animals were challenged intravenously after 28 days of vaccination with *M. ulcerans* (0·3 mg/animal).

Expt. No. 2 : Since the 0·3 mg/animal challenge dose of *M. ulcerans* did not kill all the animals of the control group in Expt. No. 1, even upto 60 days of challenge, this was increased 5 times to 1·5 mg/animal. All the other experimental conditions were identical to those in Expt. No. 1.

The parameters of study included the weekly record of body weight and morbidity and daily record of mortality, necropsy score and impression smear examination for the presence of acid fast bacilli of the visceral organs. The main criteria of protection were considered to be prolongation of survival time of vaccinated animals *vis-a-vis* that of control animals and the per cent protection obtained on days 30 and 60 of *M. ulcerans* challenge. This was calculated by subtracting the percentage of survivors of control group from that of vaccinated group.

The results are presented in Tables I and II. In all the vaccinated groups the lesions were much less in severity and the number of AFB were also less in comparison to control groups. There was significant protection in 4 mg/mouse dose in both the experiments. However, the 1 mg/mouse dose was found to have significant protection in the second experiment, but was non-significant in the first experiment. The protection has been tested against low and high doses of challenge to measure the degree of protection

against acute and chronic type of disease. The inclusion of IFA does not seem to enhance the immunogenicity in both the experiments. The parameters used to study have elucidated that *M. habana* has afforded protection against *M. ulcerans* infection in mice. The study has paved the way for future research in the direction of vaccine and chemotherapy of this infection.

The authors are grateful to the Director, Dr. Nitya Nand, for his keen interest in the work. The technical assistance of Shri P. S. Pangati is duly acknowledged.

April 2, 1981.

1. Oluwasanmi, J. O., Solanke, T. F., Oluriu, E. O., Itayemi, S. O., Alabi, G. O. and Lucas, A. O., *Am. J. Trop. Med. Hyg.*, 1976, 25, 122.
2. Revill, W. D. L., Pike, M. C., Morrow, R. H. and Ateng, J., *Lancet*, 1973, 11, 873.
3. Smith, P. G., Revill, W D. L., Lukwago, E. and Rykuslun, Y. P., *Trans. Roy. Soc. Trop. Med. Hyg.*, 1976, 70, 449.
4. Gupta, H. P., Singh, N. B., Mathur, I. S. and Gupta, S. K., *Indian J. Exp. Biol.*, 1979, 17, 1190.
5. Singh, N. B., Mathur, I. S., Gupta, H. P. and Gupta, S. K., (In press).

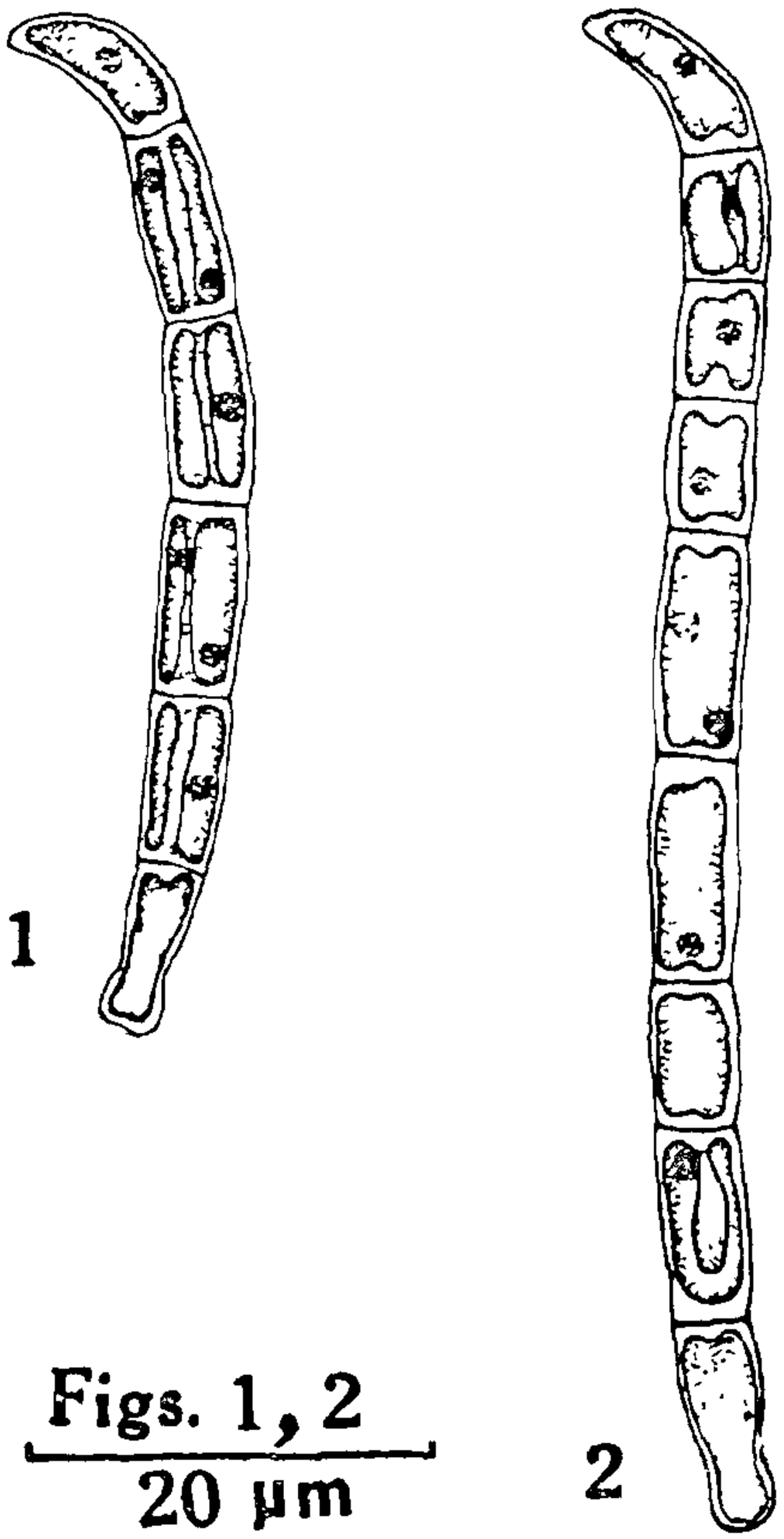
URONEMA AFRICANUM BORGE FROM ANDAMAN ISLANDS—A NEW ADDITION TO INDIAN FLORA

BRAJ NANDAN PRASAD AND P. K. MISRA
Department of Botany, University of Lucknow
Lucknow 226 007, India

THE genus *Uronema* Lagerheim is represented in India by five species only[1–3], *viz.*, *U. confervicolum* Lagerheim, *U. elongatum* Hodgetts, *U. gigas* Visher, *U. indicum* Ghose and *U. terrestre* Mitra. The present paper records a sixth species *U. africanum* Borge[2] from Port Blair (South Andaman Island). It is intended to record the taxon as an addition to the Indian flora and to briefly describe the Indian plant.

Uronema africanum Borge (Figs. 1–2)

Filaments 2–11 cells long, basal cell with attaching disc slightly constricted towards base; apical cell attenuated with pointed end and curved at a 45° angle. Cells cylindrical, 3·5–5·0 μm broad and 6–12·5 μm long, basal cells 4–9 μm long. Each cell with one parietal chloroplast filling the whole cell and containing 1 or 2 pyrenoids. At some intercalary positions, filaments are slightly constricted at septa. Zoospores not seen.



FIGS. 1–2. *Uronema africanum* Borge. Fig. 1. A young filament. Fig. 2. A mature filament showing basal and apical cells.

Habitat :

Epiphytic on *Cladophora* sp. and *Oedogonium* sp. in a freshwater pond at Sipighat (Port Blair). Coll. No. 516F. Date 10–10–1979.

The present specimen differs slightly from Borge's plants[2] in possessing slightly less curved apical cells. However, the overall morphology of the plants is similar to his description.