release of fatty acid into the perifollicular area[3]. However, this still ignores the complexity of the pilosebaceous duct microenvironment with its particular microbial flora and specific metabolites, which may be involved in the inflammatory skin reactions around acne lesions. Therefore, the existence of other casual agents of inflammation is suggested.

Prostaglandins – members of a new hormonal system – can exert marked effects on various important physiologic processes and act as regulators of cell function[4]. During recent years, their involvement in inflammatory skin reactions has been recognized. Prostaglandins participate in the development of various inflammatory skin disorders e.g. primary irritant dermatitis[5], allergic contact dermatitis[6] or erythema induced by UV-light radiation[7]. Our work aims to clarify whether prostaglandins participate as potential terminal inflammatory mediators in acne vulgaris.

P. acnes strains were subcultured under both aerobic and anaerobic conditions on artificial agar for about a week. The strains were then carefully removed from the dishes, immediately transferred to cold ethanol and stored at −20 °C under nitrogen until analysis. An improved extraction procedure was performed. Ethanol was evaporated under reduced pressure, the samples, after addition of water, were acidified to pH 3 and extracted 3–6 times with ethyl acetate. The final ethyl acetate fraction was washed with a small amount of water until neutral reaction and concentrated under nitrogen. The total lipid extracts were further purified by column chromatography and TLC[8–10]. The extracts and eluates were always compared with reference samples of PGE_2.

Our analysis revealed the presence of prostaglandin-like substances of E-type in the lipid fraction of *P. acnes*. Prostaglandin precursor, as arachidonic acid, has been demonstrated in protozoa, algae and mosses and γ-linolenic acid in fungi and flagellates[11], but to our knowledge the occurrence of prostaglandins in microorganisms has not been reported before. Our results support the idea that prostaglandins have a direct effect on the target tissues. According to the current concept, the principal effect of E-prostaglandins appears to be on adenyl cyclase, the enzyme responsible for synthesis of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The lipid fraction of *P. acnes* possesses an important stimulatory effect on ovarian cAMP (unpublished data). Moreover, this fraction manifested also a potent biological activity of PGE type using smooth muscle strips from human oviduct (unpublished data) as well as on gerbil colon (unpublished data). Lately, these compounds has been shown to elicit a PGE-like response in hamster cheek pouch vessels (unpublished data). Consequently, prostaglandin-like substances of E-type produced by *P. acnes* might be released extracellularily and act on the epidermal cell receptors of the follicular orificium.

It is evident that an acne lesion is associated with several interrelated factors, where *P. acnes* metabolites seems to gain a profound clinical significance. Our findings may add a new dimension to the understanding of the inflammatory process in acne vulgaris, and perhaps even to inflammatory reactions caused by other pathogenic bacteria, virus or fungi containing intracellular prostaglandin-like substances.

1 R.E. Kellum, K. Strangfeld and L.F. Ray, Archs Derm. *101*, 41 (1970).
2 A.M. Kligman, V.R. Wheatley and O.H. Mills, Archs Derm. *102*, 267 (1970).
3 S.M. Puhvel and R.M. Reisner, J. Invest. Derm. *58*, 66 (1972).
4 S. Bergström, Science *157*, 382 (1967).
5 J. Søndergaard, M.W. Greaves and H. Paulli-Jørgenson, Archs Derm. *110*, 556 (1974).
6 H. Paulli-Jørgensen and J. Søndergaard, Archs derm. Res. *257*, 143 (1976).
7 G.P. Mathur and V.M. Gandhi, J. Invest. Derm. *58*, 291 (1972).
8 K. Gréen and B. Samuelsson, J. Lipid Res. *5*, 117 (1964).
9 E. Änggård, K. Gréen and B. Samuelsson, J. biol. Chem. *240*, 1932 (1965).
10 W.L. Smith and W.E.M. Lands, Biochemistry *11*, 3276 (1972).
11 D.A. van Dorp, Acta biol. med. germ. *35*, 1041 (1976).

White leaf disease of *Cynodon dactylon* Pers., a mycoplasmal disease in India

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Summary. White leaf disease of Bermuda grass (*Cynodon dactylon* Pers.) has been reported from a few countries to be caused by a mycoplasma-like organism. We have tried to observe the causal agent in white leaf diseased plants of *C. dactylon* collected near Varanasi. Both tetracycline treatment and electron microscopy have revealed the presence of mycoplasma-like bodies in phloem cells of the diseased plants.

White leaf disease of the Bermuda grass (*Cynodon dactylon* Pers.) has been observed in the field for the last several decades (figure 1). It was thought formerly that the white variety is probably a diseased state of the plant, without assigning any scientific reason[2]. A suspected virus causing similar symptoms was also reported on this host by Slykhuis[3]. Chen et al.[4] and Joseph et al.[5] detected mycoplasma-like bodies in the sieve tube of white-leaved Bermuda grasses under electron microscope. They also got positive results with tetracycline treatment. The present studies were made to find out whether the material from Varanasi contained the same organism.

Material and methods. Tetracycline treatment: The diseased plants were uprooted and their roots were thoroughly washed in running distilled water. Tetracycline hydrochloride was dissolved in sterilized distilled water in various concentrations. The roots of the diseased plant samples were dipped in such solutions for 24 h and washed again in running distilled water. Such treated plants, in groups of 5, were planted in earthen pots containing sterilized soil and observed for the remission of disease symptoms.

Penicillin treatment: Treatment with penicillin G was carried out in accordance with the tetracycline treatment. A set of 5 healthy plants were also treated with tetracycline hydrochloride and penicillin G separately which served as controls.

Heat therapy: The roots of the diseased plants, after thorough washing in water, were dipped in sterilized distilled water in groups of 5 in 2 different pots, and kept on water-bath at 50 and 60 °C for 2 h. A control set of 5 diseased plants was also run concurrently at room temperature (25±2 °C).

Fig. 1. Plant of *Cynodon dactylon* Pers. showing white leaf disease symptoms (indicated by arrow) in natural infection. 1/15 of natural size.

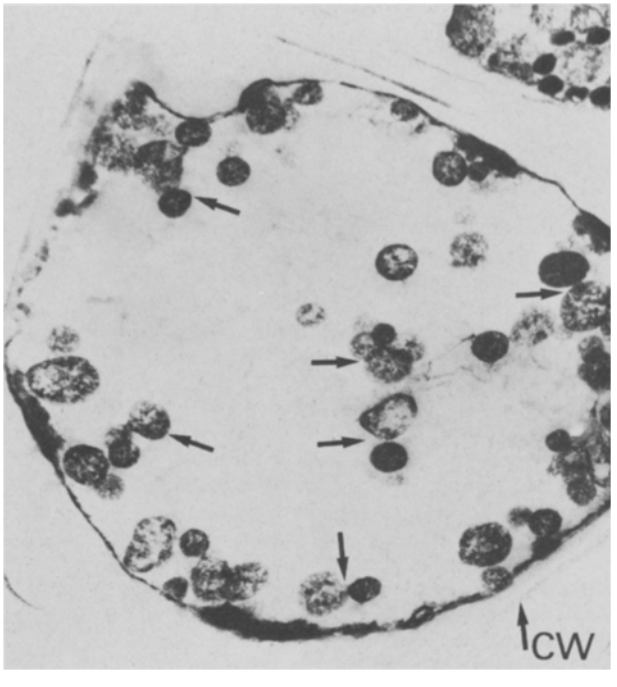

Fig. 2. Electron micrograph of phloem cell of diseased leaf of *Cynodon dactylon* Pers. showing oval to round and budding wallless mycoplasmal bodies (indicated by arrows) and cell wall (CW) of the host cell. × 30,000.

Electron Microscopy: Diseased leaves of *C. dactylon* were cut into small pieces and fixed in aqueous glutaraldehyde. The material was rinsed several times with phosphate buffer (pH 7.0) for 3 h and fixed in 1% osmium tetraoxide (OsO_4) for 3 h. The pH was adjusted to 7.0 with phosphate buffer. The materials were then dehydrated in ascending concentrations of acetone, and finally embedded in epoxy resin. Ultrathin sections were cut, stained with lead citrate and examined under HU-12 electron microscope.

Results and discussion. Diseased plants treated with tetracycline hydrochloride recovered at 1, 2, 3 ppm within 1–2 days, but the symptoms reappeared within 7–8 days. At 5, 10, 20 and 30 ppm the plants prolonged the recovery period (4–5 days), but the disease recurred within 7–8 days. At 40 ppm recovery and reappearance took 5–6 and 9–10 days respectively. At 3 different doses, e.g., 50, 100 and 250 ppm the period required for recovery and reappearance were 7–8 and 10–12 days respectively. Administration of antibiotic at still higher concentrations, e.g., 500, 1250 and 1500 ppm rendered the leaves highly soft and thinner in contrast to the healthy, turgid and comparatively thicker leaves of uninfected plants. Symptoms reappeared after 30–35 days at 500 ppm and 40–41 days at 1000 ppm, and at 1250 and 1500 ppm after 45–46 days. There was no change in control plants.

The electron microscopic observations reveal that round to elongated, and in some cases budding mycoplasmal bodies were present in phloem cells of *C. dactylon* (figure 2). The round to oval cells that lacked cell wall, measure 100–130 nm and are present scattered in the cell. The chloroplasts are apparently not visible in such infected phloem cells.

Heat treatment was ineffective in suppressing the disease symptoms in *C. dactylon*. It is quite likely that this strain of mycoplasma-like organism is heat resistant.

Doi et al.[6] unequivocally reported mycoplasma-like organism as an incitant of aster yellows, which was earlier thought to be caused by a virus. They also reported that tetracycline can inhibit the growth of pathogen in diseased plant. Several other workers (Whitcomb and Davis[7], Davis and Whitcomb[8] and Maramorosch[9]) have corroborated the positive effect of antibiotic on the remission of disease symptoms in plants incited by mycoplasma-like organism. Penicillin was ineffective in the present experiment. The remission of disease symptoms by tetracycline, and the presence of round to elongated wall-less bodies in the phloem cells, corroborate the mycoplasmal etiology of white leaf disease of *C. dactylon* collected near Varanasi. However, the absence of chloroplasts in the infected phloem cells is either partly due to the overgrowth of the mycoplasmal bodies or differential action of fixatives.

1 Biological Laboratories, National Women's University, 630 Nara (Japan).
2 G.A. Watt, in: A dictionary of the economic products of India, vol. 2, p. 689. Cosmo Publ., New Delhi 1972.
3 J.T. Slykhuis, Pl. Prot. Bull. *10*, 1 (1962).
4 C.T. Chen, S. Leo and M.J. Chen, Rep. Taiwan Sug. Exp. Stn *56*, 49 (1972).
5 M.B. Joseph, A. Zelcer and G. Loebenstein, Phytopathology *65*, 640 (1975).
6 Y. Doi, M. Teranaka, K. Yora and M. Asuyama, Ann. Phytopath. Soc., Japan *33*, 259 (1967).
7 R.F. Whitcomb and R.E. Davis, Ann. Rev. Entomol. *15*, 405 (1970).
8 R.E. Davis and R.F. Whitcomb, Ann. Rev. Phytopath. *9*, 119 (1971).
9 K. Maramorosch, Ann. Rev. Microbiol. *28*, 301 (1974).