Table 1. *Enzyme activities of some of the promising strains of Pseudomonas putida and E. carotovora.*
(enzyme activity in I.u/ml)

Strain No.	Age (hr)	pH	*P. putida*	*E. carotovora*
NBPP-13	40	8.96	1.250	—
NBPP-16	42	8.76	1.336	—
NBPP-18	42	8.80	2.016	—
NBPP-20	42	8.81	1.393	—
NBPP-31	16	7.61	—	1.350
NBEC-1	16	8.19	—	1.276
NBEC-20	16	8.10	—	1.466
NBEC-25	16	7.85	—	1.228
NBEC-24	16	7.97	—	1.283

pH during this period was 7.34 and 9.14 respectively.

In *E. carotovora*, one strain of NBEC-20 showed enzyme activity up to 1.47 I.u./ml. (table 1). Here also, the pH went up to the alkaline side, 8.1 in 16 hr, but the enzyme activity was lost on prolonged cultivation (beyond 24 hr). On the other hand, in *P. putida* there was no loss of enzyme activity up to a maximum period of 46 hr. In view of this the fermentation was terminated by 16–20 hr and harvested. The common observation in both organisms was that the pH profile showed a tendency to rise to the alkaline side. There was no correlation of extreme pH increase and enzyme production in both strains.

The advantage of the present medium for primary screening of the bacterial strains for L-asparaginase production over the TGY medium includes the utility of the former for diverse genera like *Pseudomonas* and *Erwinia*. Secondly, the present medium is composed of simple salts with low quantities of rich nutrients like yeast extract and tryptone unlike that of TGY medium where both ingredients are in much larger quantities. The latter properties could serve as a model if scale-up studies for large scale production of L-asparaginase are to be carried out.

A somewhat similar medium for L-asparaginase production by *P. boreopolis* was reported by Smirnova *et al*[4] where in the enzyme activity was 0.47 I.u./ml. But the present medium with *P. putida* gave much higher enzyme activities—0.6 to 2.0 I.u./ml, the carbon source in both cases was glucose.

By employing the rich medium of TGY, L-asparaginase activity with *E. carotovora* was earlier reported[5] to be 2 I.u. whereas with the present relatively simple medium, 1.47 I.u. of enzyme activity could be obtained with the same organism.

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MORPHOLOGICAL VARIATIONS IN *EPIDERMOPHYTON FLOCCOSUM*

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PLEOMORPHISM, described as the variation in phenotypic expression of micro-organisms, is a phenomenon encountered commonly in dermatophytes[1,2]. This has been attributed to the difference in the culture media or fungal strains themselves[2]. Although several reports are available on the pleomorphism in fungi, it has not been adequately explained. In our work on dermatophytes, on strain of *Epidermophyton floccosum* (BMS 14) showing considerable variations morphologically

upon subculture was encountered. This report deals with some preliminary observations on this fungus with special reference to the macroscopic and the microscopic changes during pleomorphism.

E. floccosum, isolated from a clinical type, *t. cruris*, was maintained on SGA agar without antibiotics and subcultured at monthly intervals. They were designated as A, B, C and D, and showed marked variation on SGA slope (figure 1). The onset of the variation was marked by discrete white spots on the culture and this increased in proportion, on subculture. Microscopic observations showed differences in the growth pattern, nature of mycelium and spore-types of the four subcultures. Thus, the buff-coloured, thin, radiating mycelium with abundant chlamydospores in the primary culture changed into white, highly branched mycelium devoid of spores. The transitional stage in the second sub-culture showed light-yellow branched mycelium with abundant macroconidia and few chlamydospores (table 1). Increase in pleomorphism in this fungus was gradual culminating in the total loss of essential characteristics necessary for taxonomic identification. It was also observed that larger the number of subcultures, greater was the rate of pleomorphism.

Morphological features are accepted universally as the main criteria for giving a fungus its taxonomic position[2]. Dermatophytes, a special group of human and animal-pathogenic fungi, have been found to be more frequently susceptible to pleomorphism than the other groups of fungi. Attention on this aspect has been drawn from several reports in the literature. The effect and the influence of environmental factors such as temperature, water[3], increased aeration[4], carbon dioxide[5,6], salt concentration[7-9] on the variation or on the reversal of the pleomorphic mutant to *status quo ante* for taxonomic evaluation[7] have been suggested and explained. Sodium chloride has been shown to influence the conidial production *in vitro* and the restriction of growth rate at higher percentage (5%) of NaCl. It is possible that in human hosts, where the microclimate on the skin produces high salt concentration due to sweat, the growth rate is also restricted; but once the fungus is cultured on agar media, the chlamydospores present in abundance during the initial stages slowly give way to conidia. This is reflected in the present study, wherein the second subculture has shown a large number of macroconidia than chlamydospores. A good correlation between the morphological changes and the macromolecular composition (protein, sugar and lipids) of the fungus is also observed (unpublished data) during the subcultures. In order to preserve the morphology of the fungus and to prevent high pleomorphism, it has been found that sodium chloride (1.5%) added to SGA agar serves as a good alternative, as suggested earlier[7].

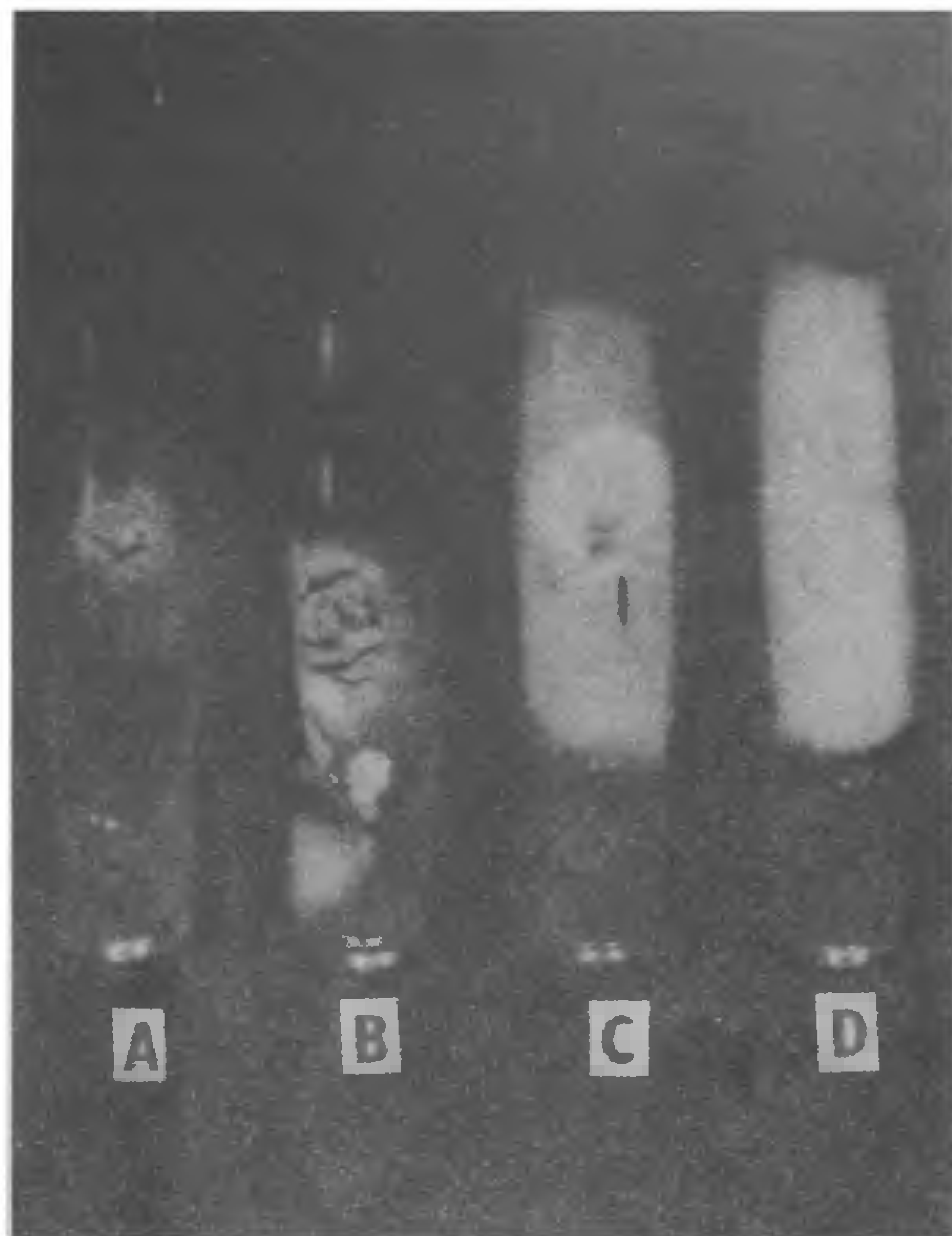

Figure 1. Morphological variants of *E. floccosum*. A: Primary isolation from patient. B: First subculture. C: Second subculture. D: Third subculture. Note: The small white patches in B show the initiation of pleomorphism.

Table 1 *Morphology of variants of E. floccosum*

	A (Primary isolation)	B 1st sub-culture	C 2nd sub-culture	D 3rd sub-culture
Colour	Yellowsih green	Buff	Creamy white	White
Texture	thin, sparse	powdery	floccose	floccose
Mycelium	radiating	branched	branched	branched
Macro-conidia	few	moderate	high	sparse
Chlamydo-spore	abundant	moderate	few	nil

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CYTOGENETIC EFFECTS OF CLOFIBRATE AND ITS CALCIUM SALT DERIVATIVE IN SWISS MALE MICE

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DRUGS are one of the largest groups of chemicals to which man is exposed and there is an ever-growing need to evaluate them for mutagenic activity. In the present study, clofibrate (ethyl *p*-chlorophenoxyisobutyrate), an anticholesterol drug, and its calcium derivative were studied for their mutagenic effect using spermatocyte meiotic chromosome analysis as there is very little information available regarding this aspect. There are a few reports that clofibrate and its derivatives which are potent peroxisome proliferators are carcinogenic[1]. In the present study the meiotic chromosomes were scored from each group of animals for each type of aberrations at 7, 15 and 30 days of treatment to examine the effect on early and late spermatogenic cycle.

Swiss male mice (5–6 weeks old) obtained from Biological Evans, Hyderabad were divided into groups containing 6 animals each and were orally administered with clofibrate 100, 200 and 400 mg/kg body wt for the time indicated. Animals were sacrificed 24 hr after the scheduled treatment. Control animals were given 4 % gum acacia, in which the drug suspension was made. The metaphase chromosomes of spermatocytes of the testicular cells were prepared according to literature method[2]. Metaphases (500–600 per group) were screened for numerical (euploids and hyperploidy and hypoploidy) and structural (autosomal and xy univalencies and translocations) aberrations as described earlier [3–5]. Statistical analysis was carried out using X^2 test[6].

The analysis of spermatocyte I chromosomes at diakinesis of 1st meiotic metaphase showed direct morphological evidence of genetic alteration in the cells. Results obtained are presented in tables 1 and 2. At 7 days the incidence of total aberrations with clofibrate was 19.97 %, 25.57 % and 27.78 % with 100, 200 and 400 mg/body wt respectively as compared with 11.62 % in controls. However, the calcium salt of chlorophenoxyisobutyrate derivative gave rise to 17.71 %, 19.59 % and 25.48 % with the above corresponding doses. Individual types of anomalies such as euploids, aneuploids and univalents were significant but the translocations were not significant at any dose.

As the duration of the treatment increased from 7 to 15 days the percentage of aberrations also increased and they were 26.64, 31.94 and 33.83 with clofibrate and 19.43, 20.93 and 23.93 with its calcium derivative at 100, 200 and 400 mg/body wt dosage as compared with 13.22 % in controls. All the above values were significantly higher than the controls. As observed in 7-day treatment, the translocations were not significant but other individual abnormalities were significant. Only one dose of 100 mg of clofibrate and its calcium derivative were administered for 30 days as the other two higher doses were found to be lethal or toxic (the animals were sluggish and unhealthy looking) when administered for longer periods. Further treatment of 100 mg/body wt of the test compounds for 30 days gave rise to 28.32 % and 23.95 % of total aberrations with clofibrate and its calcium salt respectively. The aberration frequency was significant as compared to control frequency of 13.11 %. Except translocations all other individual types of aberrations were highly significant than controls.

The above data clearly suggest that the increase in the dosage and duration of the treatment enhanced the rate of aberrations. It is also clear that the parent drug clofibrate is more potent in causing chromosomal aberrations than its new calcium derivative.