were 3.79, 3.19 and 3.29% respectively indicating that the 1 and 3-week-old animals were more susceptible than older ones. High susceptibility may be due to less developed immune system and repair capacity. The same may not be true in all strains of mice because susceptibility to environmental mutagens seems to be genetically controlled[10]. Therefore, the present study suggests that animals of each strain of atleast two age groups (preferably 3 and 9-week-old male mice) could be included for micronucleus assay. The criterion of evaluation of genotoxicity, on one age group of animals (as 8-week-old mice are generally used) does not seem satisfactory for determining clastogenicity of a substance (particularly weak clastogens).

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1. Boller, K. and Schimid, W., *Humangenetik*, 1970, **11**, 35.
2. Matter, B. and Schimid, W., *Mut. Res.*, 1971, **12**, 417.
3. Schimid, W., *Agents Actions*, 1973, **3**, 77.
4. VonLedebur, M. and Schimid, W., *Mut. Res.*, 1973, **19**, 109.
5. Heddle, J. A., *Mut. Res.*, 1973, **18**, 187.
6. Wild, D., *Mut. Res.*, 1978, **56**, 319.
7. Bruce, W. R. and Heddle, J. A., *Can. J. Genet. Cytol.*, 1979, **21**, 319.
8. Tsuchimoto, T. and Matter, B. E., *Arch Toxicol.*, 1979, **42**, 239.
9. Jenssen, D. and Ramel, C., *Mut. Res.*, 1980, **75**, 191.
10. Hayashi, M., Sofuni, T. and Ishidate, M. Jr., *Mut. Res.*, 1982, **105**, 253.

SOME OBSERVATIONS ON PRESERVATION OF FUNGAL CULTURES BY SERIAL SUBCULTURING AND LIQUID PARAFFIN

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THE Biology Division of this Establishment maintains a collection of cultures responsible for biodeterioration of materials for research, quality control and biodeterioration testing[1]. These cultures are normally maintained by continuous growth involving subculturing which is the simplest method of maintenance for living fungi. This method is labour-intensive and time-consuming when a large number of cultures of different requirements are involved. The main disadvantages of this method are: (i) danger of variation in physiological and morphological characteristics, (ii) problem of contamination by air-borne spores or mite carried infections[2], (iii) requires constant specialist supervision to ensure purity of the original culture. The interval between transfers depends mainly on various physiological requirements of the particular fungus and to some extent on external conditions especially on storage temperature. It may vary from a few weeks to 6–12 months. Storage at a lower temperature at about 5°C can extend the transfer interval of most of the fungi[3,4]. Methods of preservation of a particular culture/strain collection depends on several factors viz type of cultures, purpose of preservation, duration of storage, source of isolation, available resources and so on. It is well known that the storage life of cultures can be increased by several methods by reducing its metabolic activity to a minimum. Therefore, an attempt was made to find out the effect of extended transfer period on drying and the viability of a few fungal cultures maintained at 6 ± 2°C with and without liquid paraffin.

Twenty-one cultures viz *Actinomyces* sp. (Culture No. 1), *Aspergillus amstelodami* (2), *A. fumigatus* (3), *Chaetomium globosum* (6), *Penicillium citrinum* (8), *Polystictus hirustus* (11), *Macrophomina phaseoli* (25), *Diplodia* sp. (28), *Memnoniella echinata* (30), *Oospora* sp. (32), *Verticillium glaucum* (34), *Byssochlamys* sp. (35), *Botryodiplodia theobromae* (36), *Cephalothecium* sp. (38), *Haplaria* sp. (40), *Aspergillus oryzae* (42), *Paecilomyces* sp. (43), *Curvularia* sp. (64), *Penicillium* sp. (69), *Trichoderma* sp. (70) and *Aspergillus sydowi* (76)

were studied for 24 months. Two sets of these cultures were prepared in cotton-plugged test tubes (size 18×150 mm). In one set sporulated cultures grown on potato dextrose agar (PDA) medium were covered by sterilized liquid paraffin prepared by autoclaving at 1.05 kg/cm^2 for 2 hr and then heating in an oven at 170°C for 1 hr to remove the entrapped moisture. The purpose of liquid paraffin layering was to avoid the possibility of moisture evaporation from the culture media and decrease the metabolic activity thus minimising the growth of fungi. The second set was maintained without liquid paraffin to serve as control. Both the sets were kept in a cold room at 6 ± 2°C after treating the cotton plugs with wax.

Viability and growth characteristics of these cultures were checked periodically on PDA slants at 30 ± 1°C. The liquid paraffin cultures were revived by removing a small amount of fungal colony with a needle and draining away as much liquid paraffin as possible from the inoculum.

Periodic observations for viability of the cultures and the drying up of the media were recorded. Considerable drying up of the control set was noticed after 18 months. Except six cultures (Nos. 8, 32, 36, 64, 69 and 70) all the remaining cultures showed remarkable drying (over 50%). After 24 months of storage almost all cultures were dried but remained viable though their subculturing became difficult. The variation in the drying up of medium may be due to the variation in the growth characteristics of these fungi; as all other parameters viz tube size, temperature and humidity of storage, medium volume were kept constant. All the cultures kept under liquid paraffin remained viable without drying even after 24 months of preservation. Smith[5] also observed drying of fungal cultures after 9 months of storage and survival of many cultures up to 1 to 2 years when stored without liquid paraffin. He attributed the variation in drying to the medium in which the fungus was grown.

The above results indicate that these fungal cultures can be conveniently maintained by subculturing at an interval of 18 months if maintained at 6 ± 2°C with wax sealing of the cotton plugs without liquid paraffin layer. Their period can be further extended by preventing the drying of the medium by liquid paraffin layering. Though the viability of the cultures was studied only up to 24 months under liquid paraffin, these cultures may remain viable for still longer periods. Some workers have successfully used this method for preservation of fungal cultures for various periods of time[6-13].

The function of paraffin oil is to prevent dehydration of cultures and to slow down metabolic activity and fungal growth through reduced oxygen consumption[6,14]. It also prevents mite infestation which is a scourage to all fungal culture collections[6,15-17]. A wide range of fungi can be preserved by this method but it must be tested before applying, since some cultures deteriorate under mineral oils[18,19].

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1. McGowan, V. F. and Skerman, V. B. D., *World directory of collections of cultures of microorganisms*, 2nd edn., University of Queensland, Brisbane, 1982.
2. Dayal, H. M., Saxena, B. N. and Nigam, S. S., *Indian J. Exp. Biol.*, 1964, **2**, 62.
3. Fennell, D. I., Raper, K. B. and Flickinger, M. H., *Mycologia*, 1950, **42**, 135.
4. Haynes, W. C., Wickerham, L. J. and Hesseltine, C. W., *Appl. Microbiol.*, 1955, **3**, 361.
5. Smith, D., *Maintenance of microorganisms*, Academic Press, London, 1984, p. 83.
6. Buell, C. B. and Weston, W. H., *Am. J. Bot.*, 1947, **34**, 555.
7. Dade, H. A., In: *Herb handbook*, CMI, 1960, p. 78.
8. Fennell, D. I., *Bot. Rev.*, 1960, **26**, 79.
9. Little, G. N. and Gordon, M. A., *Mycologia*, 1967, **59**, 733.
10. Smith, D. H., Lewis, F. H. and Fergus, C. L., *Plant Dis. Rep.*, 1970, **54**, 217.
11. Onions, A. H. S., *Methods Microbiol.*, 1971, **4**, 113.
12. Onions, A. H. S., In: *Proc. Second Int. Conf. on Culture Collections*, University of Queensland, Brisbane, World Federation of Culture Collections, 1977.
13. Sherf, A. F., *Phytopathology*, 1943, **33**, 330.
14. Edwards, G. A., Buell, C. B. and Weston, W. H., *Am. J. Bot.*, 1947, **34**, 551.
15. Norris, D., *J. Aust. Inst. Agric. Sci.*, 1944, **10**, 77.
16. Hartsell, S. E., *Appl. Microbiol.*, 1956, **4**, 350.
17. Wernham, C. C., *Mycologia*, 1946, **38**, 691.
18. Reischer, H. S., *Mycologia*, 1949, **41**, 177.
19. Smith, D. and Onions, A. H. S., *Trans. Br. Mycol. Soc.*, 1983, **81**, 535.