

RESEARCH COMMUNICATIONS

Table 1. Composition of total lipids, nonsaponifiables, hydrocarbons and sterols of body flesh and hepatopancreas of *P. monodon*

Components	Body flesh	Hepatopancreas
Total lipids ^a	1.53	17.9
Nonsaponifiables ^b	40.2	13.9
Hydrocarbons ^b	3.9	3.7
Sterols ^c	Trace	Trace

^a Expressed as % w/w of wet tissue.

^b Expressed as % w/w of total lipids.

^c Trace quantities, below 1.0%.

Table 2. Fatty acid compositions of hepatopancreas and body flesh of *Penaeus monodon*

Components ^{a, b, c}	Hepatopancreas ^d	Body flesh ^d
13 iso	0.73	0.50
14:0	5.16	1.35
15:0	1.88	0.88
15:1	—	0.82
16:0	28.37	18.93
16:1 ω9	7.72	2.70
16:2 ω9	—	1.62
17:1	1.05	1.41
18:0	6.37	12.75
18:1 ω9	17.61	14.22
18:2 ω6	6.52	6.71
20:0	0.90	0.50
18:3 ω3	4.79	2.77
18:4 ω6	0.74	0.20
18:4 ω3	1.06	0.88
20:3 ω6	1.06	0.88
20:4 ω6	5.05	12.97
22:2 ω6	2.95	0.39
20:5 ω3	4.96	10.44
24:0	—	0.46
22:4 ω6	0.79	2.03
22:4 ω3	0.68	1.18
22:5 ω6	0.37	1.13
22:6 ω3	1.23	4.03

^a Short hand notation implies chain length number of the double bonds.

^b The ω-values indicate methyl end chain from centre of the double bond furthest removed from carboxyl end.

^c The data shown are mean of three experiments that are within ± 5% for most of the components.

^d Values expressed as % w/w of total fatty acids.

the saturated fatty acids, the highest level was of palmitic acid (28.4%) in the hepatopancreas but the acid was 19% in the body flesh. Among the unsaturated fatty acids, the major component was oleic acid (17.6%) in the hepatopancreas and it was 24.2% in the body flesh. EPA, the major bioactive fatty acid, was about 5% in the hepatopancreas and 10.4% in the body flesh. Of the other polyenoic acid, mention could be made of DHA, which was only 1.2% in the hepatopancreas, against 4% in the body flesh.

Omega-3 fatty acids, the principal building blocks of marine fish oils, have a number of health-enhancing properties¹³. These fatty acids, especially the EPA, in the diet reduce concentrations of cholesterol and

triglycerides of the plasma by lowering the rate of synthesis of low density lipoprotein and very low density lipoprotein, which are carriers of triglycerides and cholesterol¹⁴, by the liver and vascular tissues. Thus, it is suggested that adult patients with circulatory and other symptoms can be treated medically if EPA is taken regularly in fish-oil capsule form and various heart diseases can be prevented^{15, 16}. Similar studies with DHA indicate that it is effective in skin disorders, relieves inflammatory conditions, aids brain development and also forms a good part of the retina of the eye¹⁷.

1. Bligh, E. G. and Dyer, W. J., *Can. J. Biochem. Physiol.*, 1959, 37, 911-917.
2. Christie, W. W., *Lipid Analysis*, 2nd edn, Pergamon Press, Oxford, 1982, pp. 51-55.
3. Schlenk, H. and Gallerman, I. L., *Anal. Chem.*, 1960, 32, 1412-1414.
4. Ghosh, A. and Dutta, J., *Trans. Bose Res. Inst.*, 1972, 35, 13-15.
5. Misra, S., Ghosh, A. and Dutta, J., *J. Sci. Food Agric.*, 1983, 35, 59-65.
6. Kates, M., *Techniques of Lipidology*, 2nd edn, Elsevier, Amsterdam, 1986, pp. 122.
7. Ackman, R. G. and Burgher, R. D., *J. Am. Oil Chem. Soc.*, 1965, 42, 38-42.
8. Ackman, R. G., Burgher, R. D. and Jangaard, P. M., *Can. J. Biochem. Physiol.*, 1963, 41, 1627-1641.
9. Misra, S., Dutta, A. K., Dhar, T., Ghosh, A., Chowdhury, A. and Dutta, J., *J. Sci. Food Agric.*, 1983, 34, 1413-1418.
10. Misra, S., Ghosh, M. K., Choudhury, A., Dutta, A. K., Pal, P. K. and Ghosh, A., *J. Sci. Food Agric.*, 1985, 36, 193-196.
11. Dutta, A. K., Pal, P. K., Ghosh, A., Misra, S., Nandi, S. and Choudhury, A., *J. Am. Oil Chem. Soc.*, 1986, 63, 223-225.
12. Misra, S., Misra, S., Choudhury, A. and Ghosh, A., *Food Chem.*, 1986, 22, 252-258.
13. Patlak, M., *Res. Res. Rep.*, 1985, 9, 1-5.
14. Illingworth, D. R., Harris, W. S. and Connor, W. E., *Arteriosclerosis*, 1984, 4, 270-275.
15. Ackman, R. G., *n-3 News*, 1986, 1, 1-4.
16. Ackman, R. G., *Food Technol.*, May 1988, pp. 151-155.
17. Lee, T. H., Hoover, R. L., Williams, J. D., Sperling, R. I., Ravalese, J., Spwi, B. W., Robinson, D. R., Corey, E. J., Lewis, R. A. and Austen, K. F., *New Engl. J. Med.*, 1985, 312, 1217-1224.

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Development of marked basophilia in the liver of *Heteropneustes fossilis* by some selected chemicals

R. R. Upadhyay and Lokesh Upadhyay

Cancer Research Institute, Faizabad 224 001, India
Institute of Medical Sciences, Banaras Hindu University,
Varanasi 221 005, India

Picric acid, *o*-nitrophenol and furadan were ineffective in producing any change in the liver of

Heteropneustes fossilis, whereas Urethane, 2-AAF and carbaryl induced marked basophilia in the liver, indicating that these cells are undergoing tumourigenic process.

DURING our studies on tumour promoters and carcinogens present in our environment¹⁻⁵, it was found that pricking works as a strong tumour-promoting stimulus in the liver of *Heteropneustes fossilis*, as evident from the development of marked basophilia⁶, which is a precondition for hepatocellular carcinoma formation⁷. This effect is similar to basophilia formation in the liver of this fish following initiation with DMBA and promotion with croton oil⁸. Six-month-old fish (weighing 16 g, 6.5 cm in length) were maintained in separate water tanks – having fresh water supply – in six groups of 20 each and given a supplementary diet⁹. Observations were recorded after three weeks.

Fish from group 1 were allowed to remain in a water tank containing a specified quantity of picric acid, while those in groups 2 and 3 were maintained separately in water tanks containing specified amounts

of urethane and α -nitrophenol. The fish in groups 4 and 5 were maintained in tanks containing known concentrations of 2-acetylaminofluorene (2-AAF) and furadan, while those in group 6 were kept in a water tank containing a fixed concentration of methyl carbamate-1-naphthol (carbaryl) (Table 1). The fish were maintained in tanks for eight weeks, after which the experiments were terminated by immersing the fish in cold water. The fish were then sacrificed for histopathological examination. Their livers were fixed in Bouin's solution and paraffin sections (0.5 μ m thick) of these organs were stained by Heidenheim's iron hematoxyline and eosin¹⁰.

During histopathological investigations, picric acid, α -nitrophenol and furadan were found ineffective in introducing any marked cellular change in the liver of *H. fossilis*, whereas in the case of urethane, 2-AAF and carbaryl the liver of fish exhibited marked hyperplasia, thickening of cell walls, loss of cytoplasm and vacuolization, followed by a marked displacement of the nucleus, to the extent that it became eccentric with

Table 1. Carcinogenicity of some chemicals tested in the liver of *H. fossilis* (duration of experiment, 8 weeks)

Chemical	Concentration in water (ppm)	Number of fish/survival	Histopathological changes in liver
Picric acid	0.2	20/19	Cellular structures normal
Urethane	0.001	20/19	Hyperplasia, loss of cytoplasm, displacement of nucleus, basophilia, high degree of mitosis (see text)
α -Nitrophenol	0.001	20/20	Cellular structures normal
2-Acetylaminofluorene (2-AAF)	0.0002	20/20	Hyperplasia, loss of cytoplasm, displacement of nucleus, high degree of mitosis (see text) basophilia
Furadan	0.0001	20/19	Cellular structures normal
Methyl carbamate 1-naphthol (carbaryl)	0.0002	20/20	Hyperplasia, loss of cytoplasm, displacement of nucleus, high degree of mitosis, basophilia (see text)

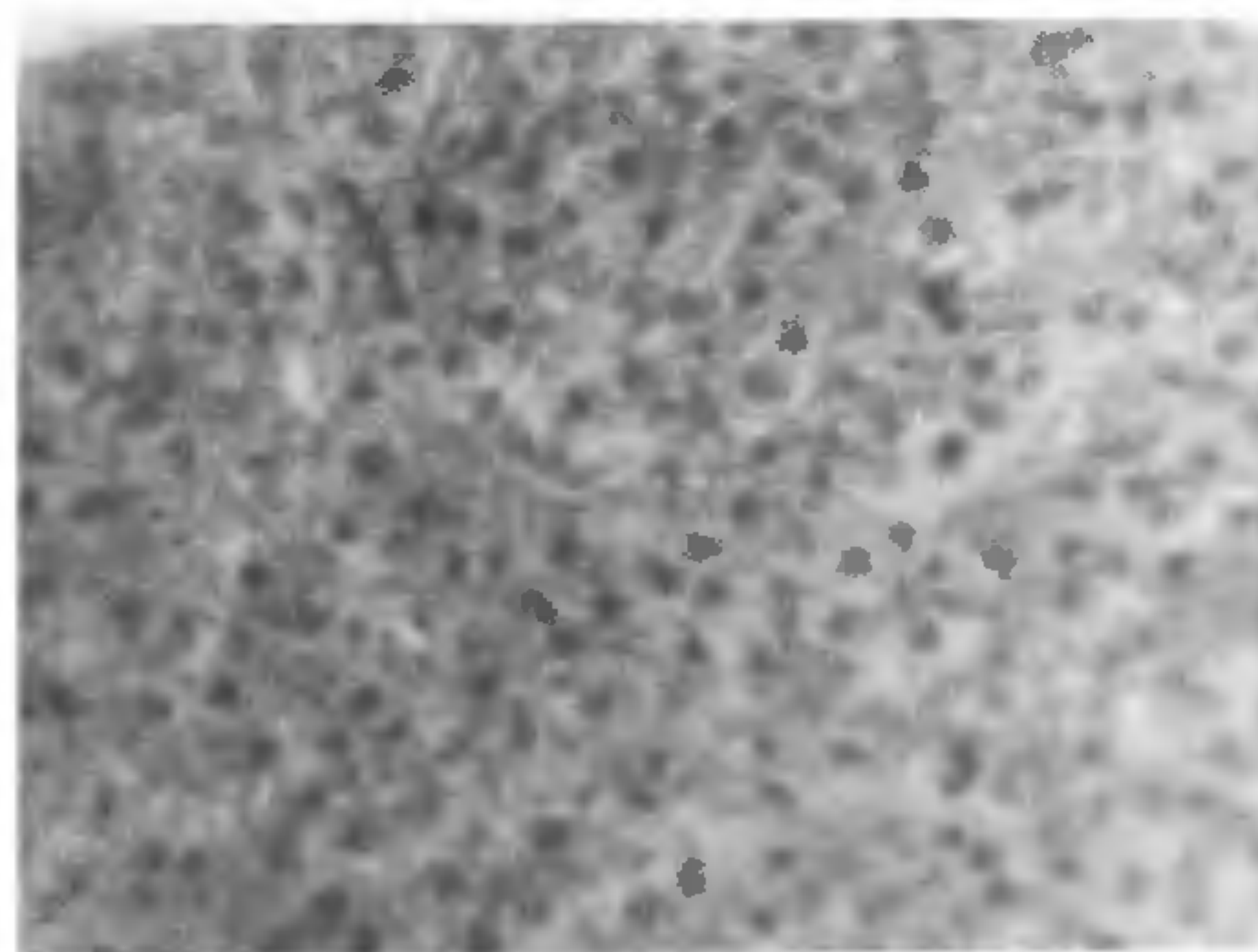


Figure 1. Photomicrograph of the liver of *H. fossilis* (400 \times), showing normal structures.

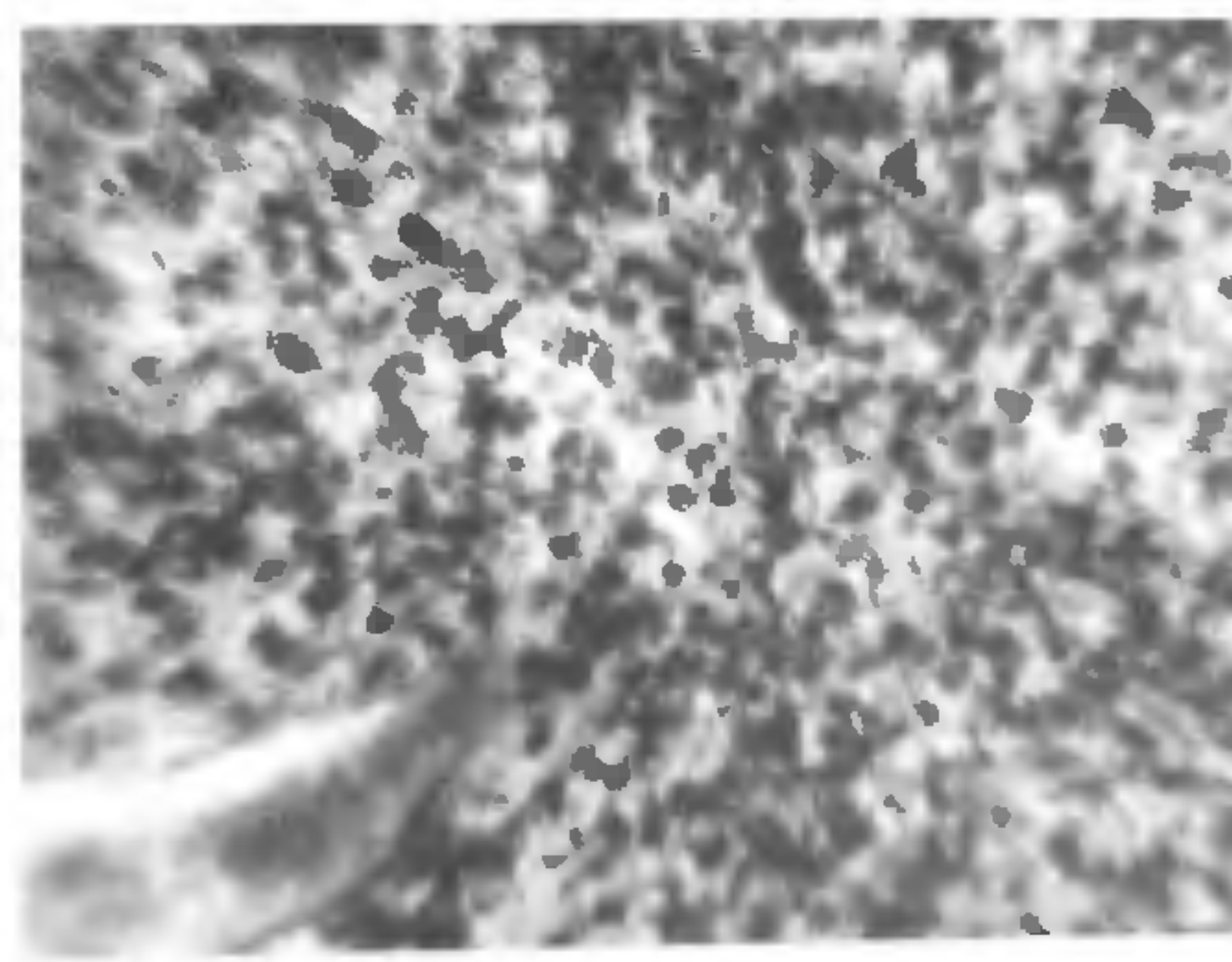


Figure 2. Photomicrograph of the liver of *H. fossilis* treated with urethane, showing changes in the liver (400 \times) structure.

a high degree of mitosis, followed by well-marked basophilia⁸ of the cells (Figures 1 and 2).

Urethane, a well-known multipotential carcinogen in mice, can act as an initiator and promotor of carcinogenesis¹¹⁻¹³ and has been found to exhibit strong carcinogenic effect in the liver of this fish. Similarly, 2-AAF – the first pesticide synthesized, exhibiting carcinogenicity and initiation potential in mammals¹⁴⁻¹⁶ – was found to initiate the formation of strong basophilia coupled with other cellular changes in the liver of this fish, thus extending its carcinogenic domain to fish as well. Similarly, carbaryl, which exhibits carcinogenicity in mammals¹⁷, has been shown to cause cellular changes along with strong basophilia in the liver of *H. fossilis*.

It is thus evident that urethane, 2-AAF and carbaryl exhibit basophilia in the liver of this fish, indicating that liver cells undergo a tumourigenic process, leading eventually to hepatocellular carcinoma formation^{7, 18}.

1. Upadhyay, R. R. and Hecker, E., *Phytochemistry*, 1978, 15, 1070-1072
2. Upadhyay, R. R. and Tilabi, J., *Cancer Lett.*, 1983, 18, 317-320.
3. Upadhyay, R. R., Islampanah, S. and Davoodi, A., *Jap J. Cancer*, 1980, 71, 557-559.
4. Upadhyay, R. R., Bakhtavar, F., Mohseni, H., Sater, A. M., Saleh, N., Tafazuli, A., Dizaji, F. N. and Moenzadeh, G., *Planta Med*, 1980, 38, 151-154.
5. Upadhyay, R. R., Ghanayat, P., Behbehani, A. N. and Sanayan, B. D., *Indian J. Cancer*, 1987, 24, 30-35.
6. Stanton, M. F., *J. Natl. Cancer Inst*, 1965, 34, 117-130.
7. Upadhyay, R. R. and Swaroop, A., *Indian J. Cancer Biol Res.*, 1988, 1, 20-23.
8. Upadhyay, R. R. and Swaroop, A., *Indian J. Cancer Biol Res.*, 1988, 1, 15-19.
9. Swaroop, A. and Upadhyay, R. R., *Adv. Zool*, 1985, 6, 114-115
10. Swaroop, A. PhD thesis, Avadh University, Faizabad, 1984.
11. Berenblum, I. and Haran, N., *Br. J. Cancer*, 1955, 9, 453-455.
12. Berenblum, I. et al., *Biochem. Pharmacol.* 1959, 2, 168-176
13. Tannebaum, A. and Silverstone, H., *Cancer Res.*, 1958, 18, 1225-1231.
14. Wiesberger, E. K. and Wiesberger, J. H., *Adv. Cancer Res*, 1958, 5, 331-441.
15. Armuth, V. and Berenblum, I., *Inst. J. Cancer*, 1977, 20, 292-295
16. Richte, A. C. and Saffiotti, U., *Cancer Res.*, 1955, 15, 84-88.
17. *Evaluation of Carcinogenic Risk of Chemicals to Man*, IARC Monograph, 1976, vol. 12, p. 37.
18. Vesslinovitch, D. S., Mikhatlovitch, S., Rao, K. V. N. and Goldfarb, S., in *Carcinogenesis and Biological Effects of Tumor Promoters* (eds. Hecker, E. et al.), Raven Press, New York, 1982, pp. 127-131.

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In vitro selection for high-temperature tolerance in cultured shoot explants of *Pinus caribaea* Morelet

Seema Bedi

Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX, UK

Present address Department of Agronomy, Punjab Agricultural University, Ludhiana 141 004, India

Selection of tolerant plants is the first step towards the production of plants adapted for growth in stressed environments. An attempt was made to select heat stress tolerant *Pinus caribaea* via tissue culture from explants originating from four provenances. The explant performance was evaluated using data on explant height and extent of leaf necrosis. The population structure of provenances was markedly altered by selection pressure imposed by high temperature. At 35°C, variations in growth were significant between explants within a provenance but not between provenances. At 37°C, variation in growth became significant between provenances also. This variation in performance of provenance explants may be attributed to the native temperature range experienced by a provenance. An improvement in explant performance at 37°C was also observed in those provenances which had been pre-conditioned at 35°C over unconditioned controls.

TEMPERATURE is an important environmental factor determining in part the geographical distribution of plants. The optimal temperature for growth and the range over which it occurs varies between species and genotypes of the same species. A plant's habitat is generally determined by temperature and when ambient temperature is higher than that of the plant's natural range, heat injury is more likely to occur¹.

Tissue culture, largely callus and cell suspension cultures, have been initiated in several angiosperm species for resistance against cold², minerals³, frost⁴, salt⁵. The use of tissue culture technique for high temperature tolerance^{6, 7} is important because even marginally changed tolerance limits are likely to be reflected in an increased species range in managed ecosystems. Previously Bedi⁸ established that 35°C was a supra-optimal temperature for the growth of *P. caribaea* *in vitro*. Explants grown at this temperature showed a poor rate of survival and those which survived showed a significantly lower growth rate than those grown at 25 or 30°C. This investigation seeks to determine the existing variation in tolerance to high temperature and to select tolerant material at the provenance and clonal level, depending upon the extent of diversity in the population.

Shoot explants were obtained from seedlings raised under glasshouse conditions. Seeds from five diverse