A thaliana (Col-O) also yielded 'zero-erucic' type mutants as reported recently[4,5]; these mutants, like Canola type *B napus*, showed little or no C 20:1 or C 22:1 in the seed lipid. Obviously, the Col-O mutants and the Bus-O natural variant represent alteration of genes at different *loci* determining fatty acid chain elongation at two different sites in the same pathway. Another natural variant Co-4 showed a high C 16:0 (20%) content; this type of high C 16:0 (19%) content was also shown by a mutant of *A. thaliana* (Col-O) but the mutation simultaneously reduced C 18:1 content in the seed lipid by 50% (ref. 5). A similar type of mutant due to alteration of a single recessive gene that raised C 16:0 but lowered C 18:1 level in seed oil was also described in *B. campestris*[12]. The biochemical basis of this mutation is not known. Since the natural variant Co-4, unlike these mutants, did not show any significant change of C 18:1 content from the average value (about 18%), this ecotype can also be very useful in segregation analysis of its genetic traits in the studies on inheritance of saturated fatty acids (C 16 : 0 and C 18 : 0) in *A. thaliana* oil seeds. Quantity of a fatty acid in seed oil appears to be a stable genetic trait of any individual strain[13] that can be utilized for alteration of fatty acid composition through breeding. It must be noted that biochemical basis of the regulation of fatty acid composition of oil is largely unknown. Voluminous earlier work on the mode of inheritance of C 22 : 1 content in *B. campestris* and *B. napus*[9, 11] suggested that it is controlled by genes in additive fashion, as dominance is incomplete and epistasis is absent. Recent studies with fatty acid biosynthesis and chain length elongation mutants of *A. thaliana* (Col-O) also showed that the genes controlling fatty acid composition in seeds are recessive and quantity of a fatty acid in lipid is largely influenced by gene dose effect[4, 5]. So far, genetic studies on fatty acid composition of oil in *A. thaliana* have been restricted only to Col-O strain (wild type) and its mutants. Our study showed that there are at least two *A. thaliana* ecotypes (Bus-O and Co-4) whose fatty acid composition varies distinctly from that of Col-O. These variants may be useful in molecular genetic studies on inheritance of lipid fatty acid composition.

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Effect of substance P on the melanophores of a fish and frog *in vitro*

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We have investigated the effect of substance P on the isolated scale melanophores of a freshwater fish *Cirrhinus mrigala* and the isolated skin melanophores of Indian bull frog *Rana tigrina*. Substance P induced dispersion in the fish melanophores in a concentration-dependent manner. Frog melanophores too responded with dispersion to applied substance P, but the responses were not related to its concentration. Denervation of fish melanophores and re-suspension of dispersed melanophores in saline indicates that the effect of substance P on the fish melanophores is direct and irreversible. On the frog melanophores, the effect of substance P may be of indirect nature via release of other neurotransmitter substances.

SUBSTANCE P, a sialogogic undecapeptide which was first discovered by Euler and Gaddum[1], is a neurotransmitter and ubiquitously present in the various

organs of invertebrates and vertebrates[2–5]. The effect of substance P has been described on many tissues of different animals[2–6]. However, its effect on the melanophores of any animal group has not, to our knowledge, been described so far, although, the effects of classical and other neurotransmitter substances, hormones, etc., have been described on fish, amphibian and reptilian melanophores[7–10]. We undertook this study to investigate the effect of substance P on the isolated scale melanophores of a teleostean fish *Cirrhinus mrigala* and isolated web melanophores of the Indian bull frog *Rana tigrina*.

The fishes *Cirrhinus mrigala* (Ham.) of either sex, 10–12 cm in length and 8–10 g in weight were procured from local fish farms and transported to the laboratory alive. The fishes were acclimatized in the laboratory for at least 48 h, with normal day and night cycle of the prevailing season at the room temperature between 15 and 25°C (January to March). Scales were removed from dorsal region below the head and the lateral side of the fishes and immediately immersed in 0.7% NaCl solution. This saline medium was found most suitable for isolated scale melanophore preparation of this fish species[11]. The scales were equilibrated in saline medium for 15 min with frequent shaking. After equilibration 3–5 scales were incubated in various concentrations of substance P for ten min in small glass petri dishes with a total volume of 10 ml. Fresh stock solutions of substance P were made using the glass distilled water and further diluted in saline medium. The final concentration of substance P in a petri dish is expressed in molar concentration. Control and treated scales were placed on a glass slide with dermal side down along with incubation medium and covered with a cover glass. Individual melanophores were measured with an ocular micrometer (Erma, Japan) in low power microscope and mean melanophore size index (MSI) was calculated according to the method of Bhattacharya *et al.*[12]. Dose response curve for different concentrations of substance P was then plotted. The increase and decrease in MSI from the control value represent dispersion and aggregation of the melanophores respectively. When the antagonists were employed, the scales were first incubated in it for ten min followed by addition of substance P and further ten min incubation. In the present study, both dermal and epidermal melanophores were studied together. Whenever they responded differently, they have been mentioned separately.

For denervation of fish melanophores by cooling treatment the following procedure was adopted. After removing the scales from fishes, they were immersed in 0.7% saline as usual. After equilibration period the scales were transferred to a glass beaker containing 100 ml of fish ringer solution as described earlier[13]. The air was bubbled through the solution and the beaker was cooled at 4°C for 18–24 h. After the cooling treatment, the beaker was placed at room temperature for 30 min. Then scales were again transferred to 0.7% saline and equilibrated for 10–15 min and further experiments with substance P were carried out as described above.

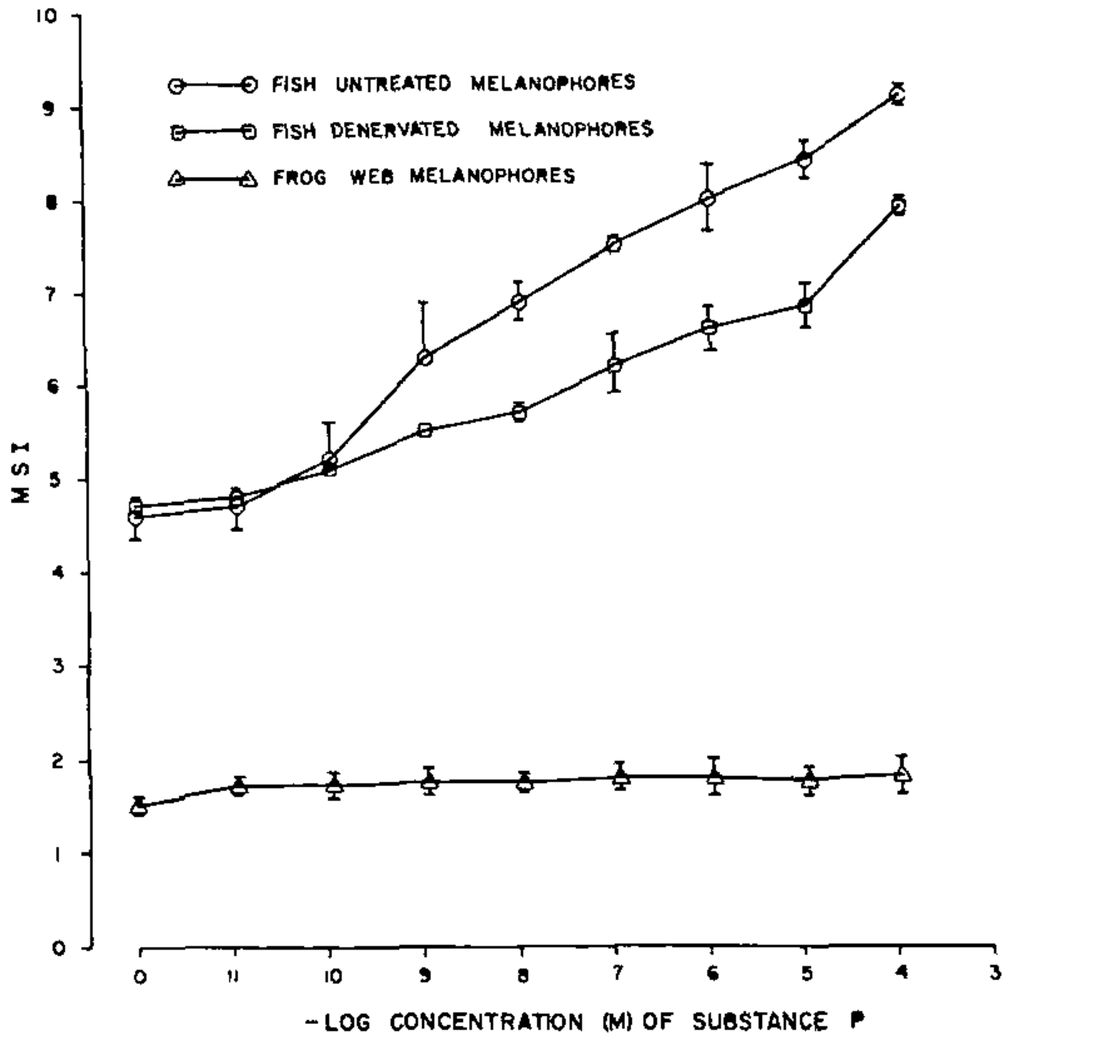

Figure 1. Log dose–response curves of substance P on untreated and denervated melanophores of *C. mrigala* and frog web melanophores Abscissa. molar concentrations of substance P Ordinate responses of the melanophores as melanophore size index (MSI) Each point is the mean of seven and six experiments on untreated and denervated melanophores respectively from different fishes. $n = 6$, on the frog web melanophores. Vertical bar indicates SE.

Frogs (*Rana tigrina*) of either sex (75–120 g in weight) obtained from an animal dealer of Lucknow were acclimatized in the laboratory for 48 h. The frogs were pithed with the help of a pithing needle. The webs between the fingers of both hind limbs were cut out carefully with sharp scissors. These webs were cut into smaller pieces of 2–3 mm^2 size within 0.8% saline medium. The skin pieces were equilibrated in saline medium for 15 min and further procedure was the same as described above for the fish scales.

Drugs used were substance P (Sigma, USA), phentolamine HCl (Ciba-Giegy, Basel, Switzerland), propranolol HCl (ICI, Madras, India).

Substance P in a dose range of 10^{-11} M to 10^{-4} M elicited dispersion in the isolated scale melanophores of *C. mrigala*. The dispersion induced by substance P in the melanophores of *C. mrigala* was dose dependent – higher the dose larger the response (Figure 1). The dispersion caused by substance P was detectable in both dermal and epidermal melanophores of the fish. However, in the dermal melanophores it was more pronounced than epidermal ones. In the epidermal melanophores, the response of substance P was not consistent and never increased beyond 11%, while in the dermal melanophores the increase was up to 95.94% in the highest dose of substance P treated melanophores.

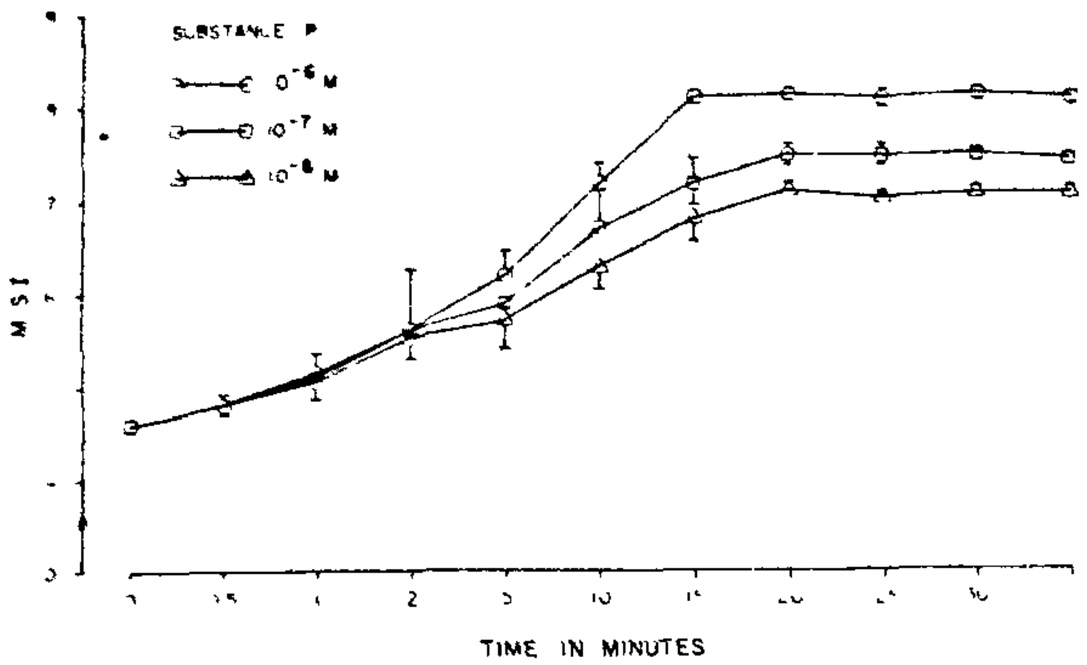

Figure 2. Effect of incubation time on the response of *C. mrigala* melanophores to three concentrations of substance P. Abscissa incubation time in minutes Ordinate responses of the melanophores as MSI Each point is the mean of five experiments on different fishes Vertical bar indicates SE.

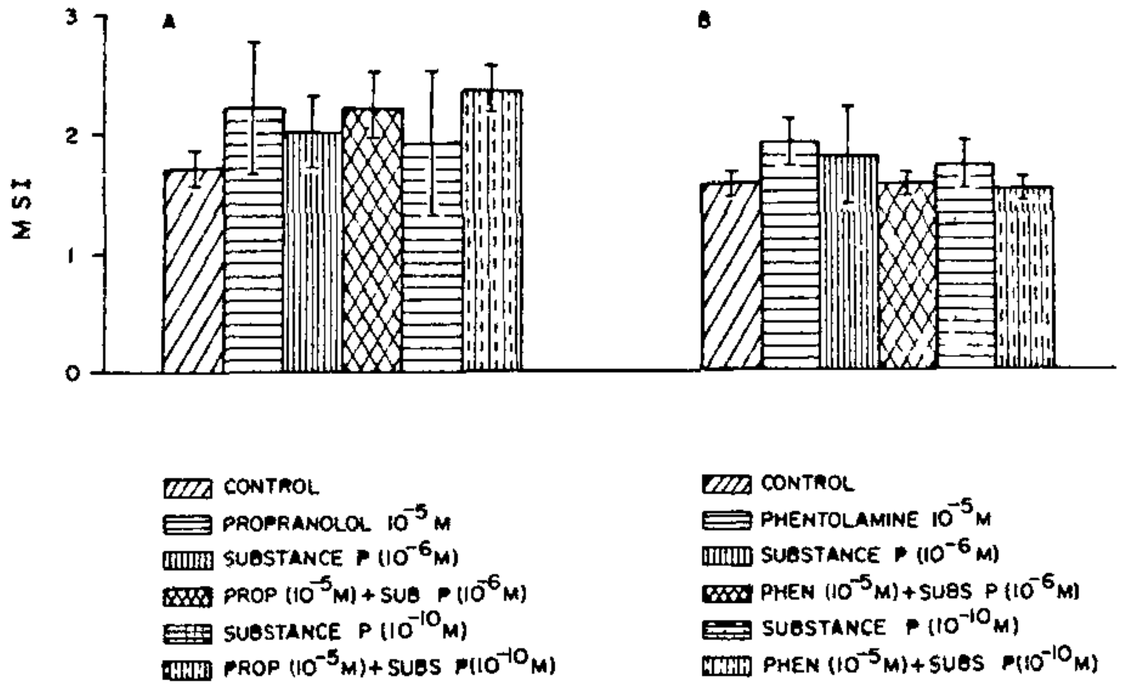

Figure 3. Effect of propranolol (*a*) and phentolamine (*b*) on the dispersal responses of the frog web melanophores to substance P. Each bar represents mean ± SE from six experiments on different frogs

The latent period to induce dispersion in the melanophores by substance P was 10–15 s. To detect the effect of incubation time on the response of fish melanophores to substance P, three concentrations were selected and the effects of 0.5 to 60 min of incubation in substance P were observed (Figure 2). It was found that up to 15 min there was marked increase in the dispersal response of the melanophores which was further increased a little up to 20 min. Further increase in the incubation period up to 60 min did not result in any change in the dispersal state of the melanophores (Figure 2).

To find out the nature of dispersion of *C. mrigala*, melanophores induced by substance P, i.e. whether it is reversible or not. The scales with dispersed melanophores under the influence of substance P were transferred to saline medium for up to 60 min with frequent change of saline and shaking of petri dishes to aerate the cells. The melanophores were again observed with the same time interval as in Figure 2. It was found that incubation of the scales in saline medium which were treated in substance P and dispersed up to the maximum extent were never returned to the original level or the control value of MSI, therefore, it seems that the dispersion induced by substance P in the fish melanophores is irreversible. This experiment was repeated for all the concentrations of substance P which were employed and shown in Figures 1 and 2. It was found that denervation of the melanophores by the cooling treatment of the scales resulted in a decrease in the sensitivity of the melanophores to substance P (Figure 1). However, nature of the response, i.e. dispersion of the melanophores, was not changed. This indicates that the effect of substance P on melanophores may be direct in nature and the involvement of specific substance P receptors cannot be ruled out.

In the mammalian smooth muscles, specific substance P receptors have been reported and characterized[14]. In these muscles, substance P induced an excitatory effect. While on the fish gut, response to substance P has also been reported to be of excitatory nature, but the types of substance P receptors involved in fish gut excitatory responses seem different from the mammalian species[15]. However, the characterization of substance P receptors on the fish melanophores is yet to be done.

Substance P in the dose range of 5×10^{-11} M to 5×10^{-4} M also induced dispersion in the isolated web melanophores of the frog *R. tigrina*. However, the maximum level of dispersion aroused by substance P was up to 16.45%. It was found that the dispersion of melanophores induced by initial doses of substance P could not increase further by higher concentration (Figure 1). Therefore, it seems likely that the dispersion induced by substance P in the frog melanophores may not be dose-related. To investigate the nature of the response of frog melanophores to substance P, the adrenergic receptor antagonists phentolamine and propranolol were employed. It was observed that phentolamine and propranolol (10^{-5} M) *per se* induced dispersion in the skin melanophores of the frog (Figure 3).

Phentolamine inhibited the dispersion caused by substance P in the frog melanophores while propranolol failed to do the same (Figure 3). These results indicate that the effect of substance P, i.e. dispersion on the frog melanophores may be mediated through alpha adrenoceptors. However, this effect is likely to be indirect in nature, i.e. substance P may release catecholamines from the nerve endings of the melanophores which in turn causes the dispersion of melanophores. On the longitudinal muscle strips of few fish species, it has been demonstrated that the excitatory action of

substance P is mediated partially indirectly by the release of neurotransmitter, like acetylcholine and 5-HT[15]. Similar mechanisms may be involved in the dispersal response of frog melanophores to substance P. The neurotransmitter substance involved, however, may be a catecholamine in the frog (*R. tigrina*) melanophores. Catecholamine-induced dispersal responses in the frog (*R. tigrina*) melanophores are well documented[12, 16–18].

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