their sulphide counterparts at all concentrations against both the fungal species. All the compounds are quite toxic even at low concentrations except compound nos I_b and II_b. As the length of alkyl chain increases, the fungicidal activity of the compounds is also enhanced. These results reveal that all these sulphides and sulphones are very active and their activity appears to be governed by the nature of alkyl chain.

Molluscicidal activities of two of them have been tested against a mollusc–*Lymnea acuminata*. The results (table 2) clearly indicate that the activity is both dose-dependent and time-dependent. The compound no. II_a causes considerable mortality of snails.

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EFFECT OF AUTONOMIC DRUGS ON THE ISOLATED MELANOPHORES OF THE WALL LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

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ABSTRACT

The nature of autonomic receptors present in the isolated skin melanophores of *Hemidactylus flaviviridis* was investigated by measuring the mean melanophore size index (MMSI). It was found that adrenaline and isoprenaline caused the dispersion of the melanophores, whereas phenylepherine caused aggregation. The melanophores of the wall lizard may have alpha as well as beta adrenergic receptors which mediate the aggregation and dispersion respectively. Acetylcholine, atropine and eserine, all caused melanophore aggregation in varying degree. 5-HT and histamine, both, caused a clear aggregation of melanophores.

INTRODUCTION

THE physiology, pharmacology and endocrinology of the melanophores of fish, amphibians and mammalian melanocytes have been extensively studied[1,2]. However, similar studies on the reptilian species are scanty[3]. In *Anolis carolinensis* the only extensively studied reptilian species, Hadley and Goldman[4] described a mosaic population of melanophores, having both alpha and beta adrenergic receptors, and others having only beta receptors. Unfortunately, pharmacological characterization of the receptors in melanophores of other species has not been done, needless to say, of any Indian species. Therefore, we have selected this common wall lizard

Hemidactylus flaviviridis for investigating the nature of receptors present in its skin melanophores.

MATERIALS AND METHODS

Wall lizards, of either sex, 5 to 6 inches in body length were captured and kept in wire-gauzed cages, fed with food and water *ad libidium*. Pieces of skin 4–5 mm were excised from the decapitated *H. flaviviridis* and immediately placed in reptilian saline of 0.8%. These skin pieces were allowed to soak in the experimental saline for about 30 min. Preliminary experiments were conducted using drugs in a wide range of concentrations; however, the two concentrations which produced minimum and maximum response were chosen for further investigations, and are reported here. Prior to addition of any drug, the saline was frequently changed. Drugs were freshly dissolved in 0.8% saline. The responses of control as well as drug treated melanophores were measured according to the method of Bhattacharya *et al*[5] and the values recorded were staged as mean melanophore size index (MMSI). Experiments were conducted under laboratory conditions during March–April. The following drugs were used: adrenaline hydrochloride (Burroughs and Wellcome India Ltd.), isoprenaline hydrochloride (Sigma, USA), phenylepherine hydrochloride (Sigma, USA), 5-hydroxytryptamine creatinine phosphate, (Boehringer Ingelheina), acetylcholine chloride (BDH, England), eserine sulphate (Sigma, USA), atropine sulphate (E Merck, Germany) and histamine diphosphate (Sigma, USA).

RESULTS AND DISCUSSION

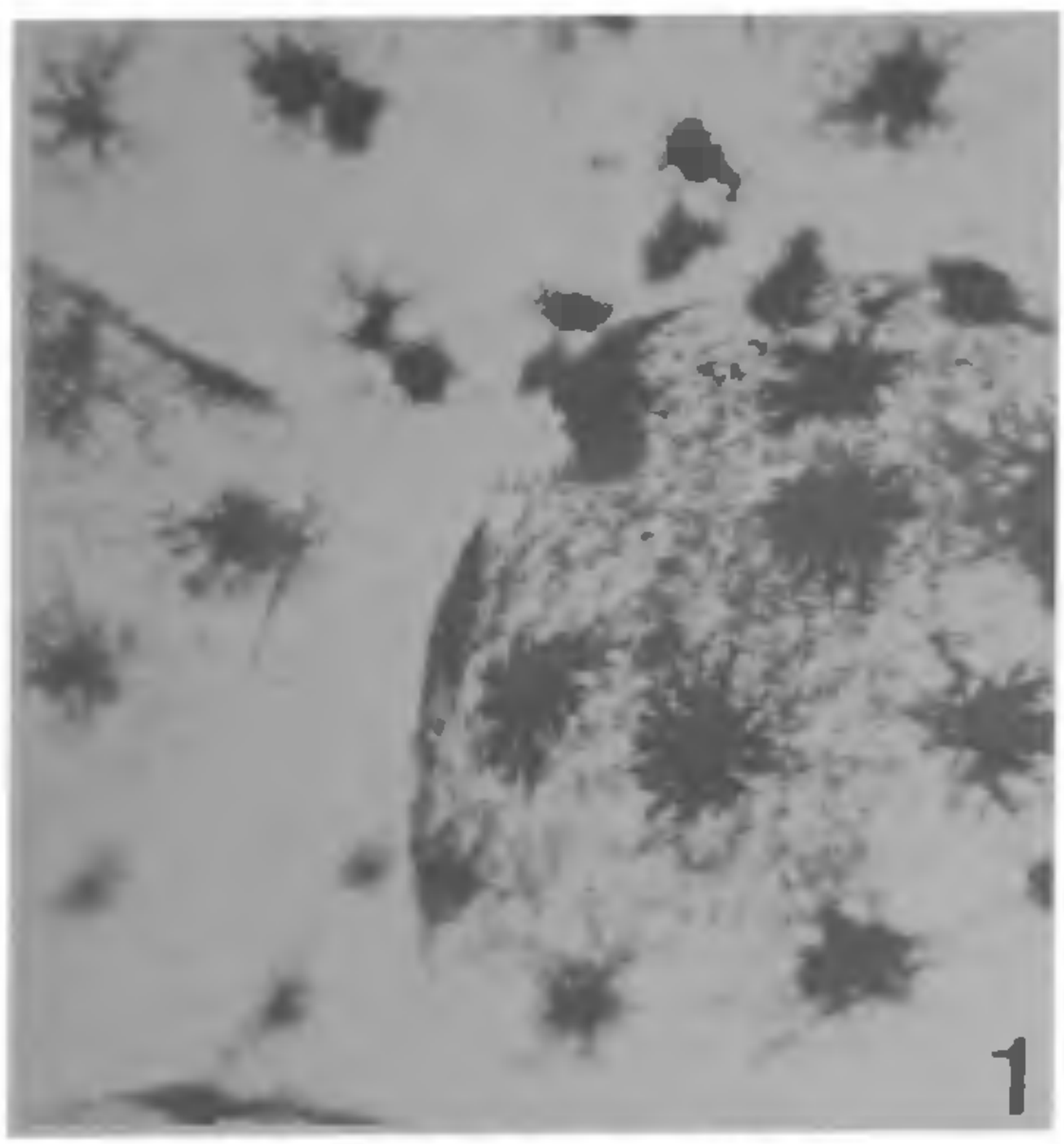
It was found that untreated control melanophores in 0.8% saline had a fairly constant MMSI, which ranged from 3.50 to 3.75 for about 1 hr (figure 1 and table 1). It was observed that adrenaline produced a marked dispersion of all the melanophores. The dispersing effect was more pronounced in a higher concentration of adrenaline (figure 2 table 1). Isoprenaline, in both concentrations exhibited a powerful dispersing effect on the melanophores of *H. flaviviridis* which was highly significant (table 1 and figure 2).

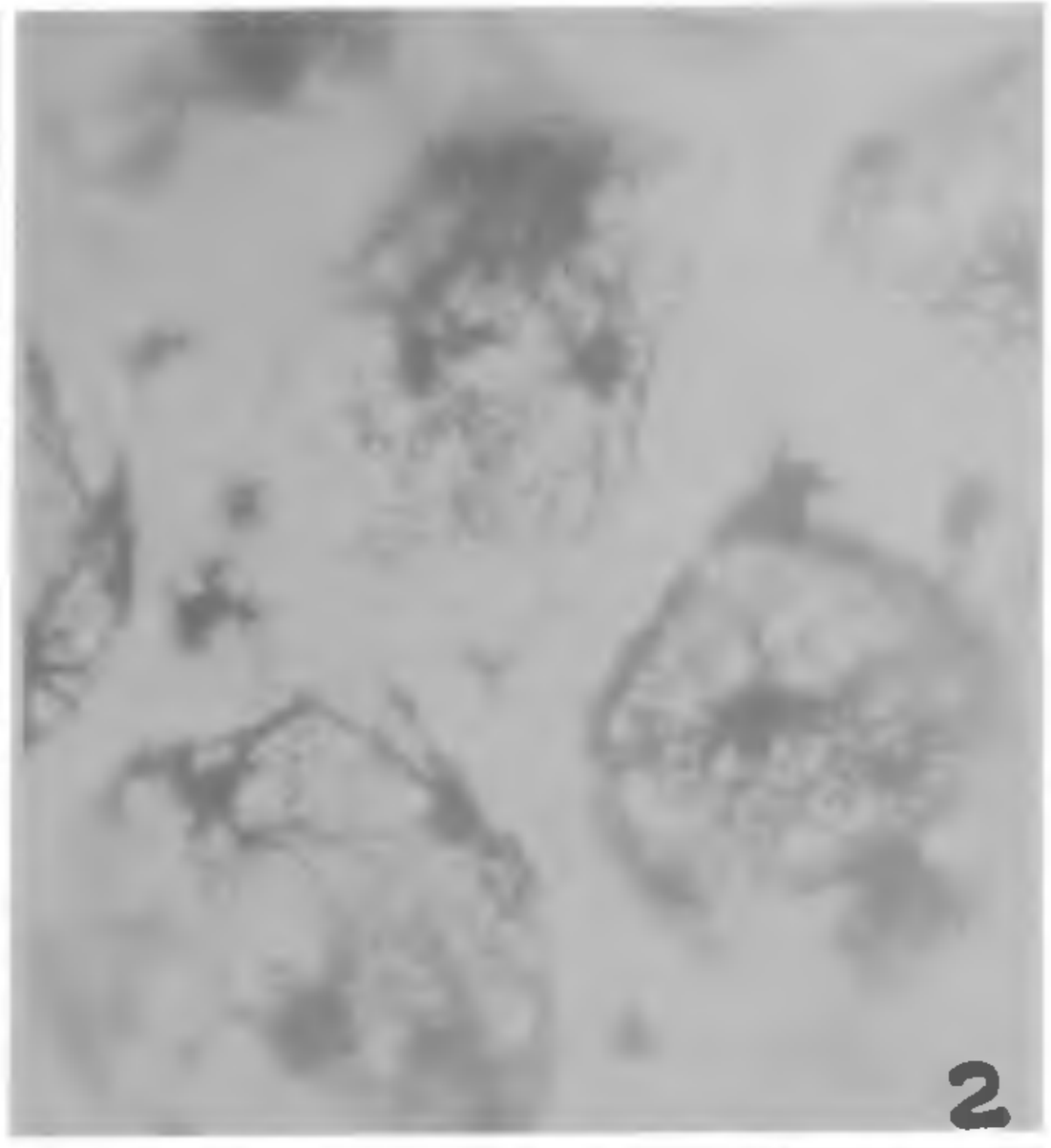
On the other hand phenylepherine which is a specific alpha adrenergic receptor agonist[6] produced a distinct aggregation of all the melanophores (table 1 and figure 3). Serotonin (5-HT) also induced a potent aggregation of all the melanophores. The melanophores became much aggregated at this stage (figure 4). Acetylcholine upto a concentration of 5×10^{-6} g/ml was not able to produce any measurable effect; however, an increase in the dose of acetylcholine to 1×10^{-5} g/ml caused a slight aggregation, which is not statistically significant. Further increase in the dose of acetylcholine upto 5×10^{-5} g/ml caused a clear dispersion. Atropine, *per se* caused aggregation of melanophores in various concentrations. To find out the effect of atropine on the responses to acetylcholine atropine was incubated in a few experiments in the same concentration as mentioned in table 1 and acetylcholine was then added. It was observed that acetylcholine produced no effect. In a wide dose range histamine persistently caused melanophore aggregation of *H. flaviviridis* (table 1).

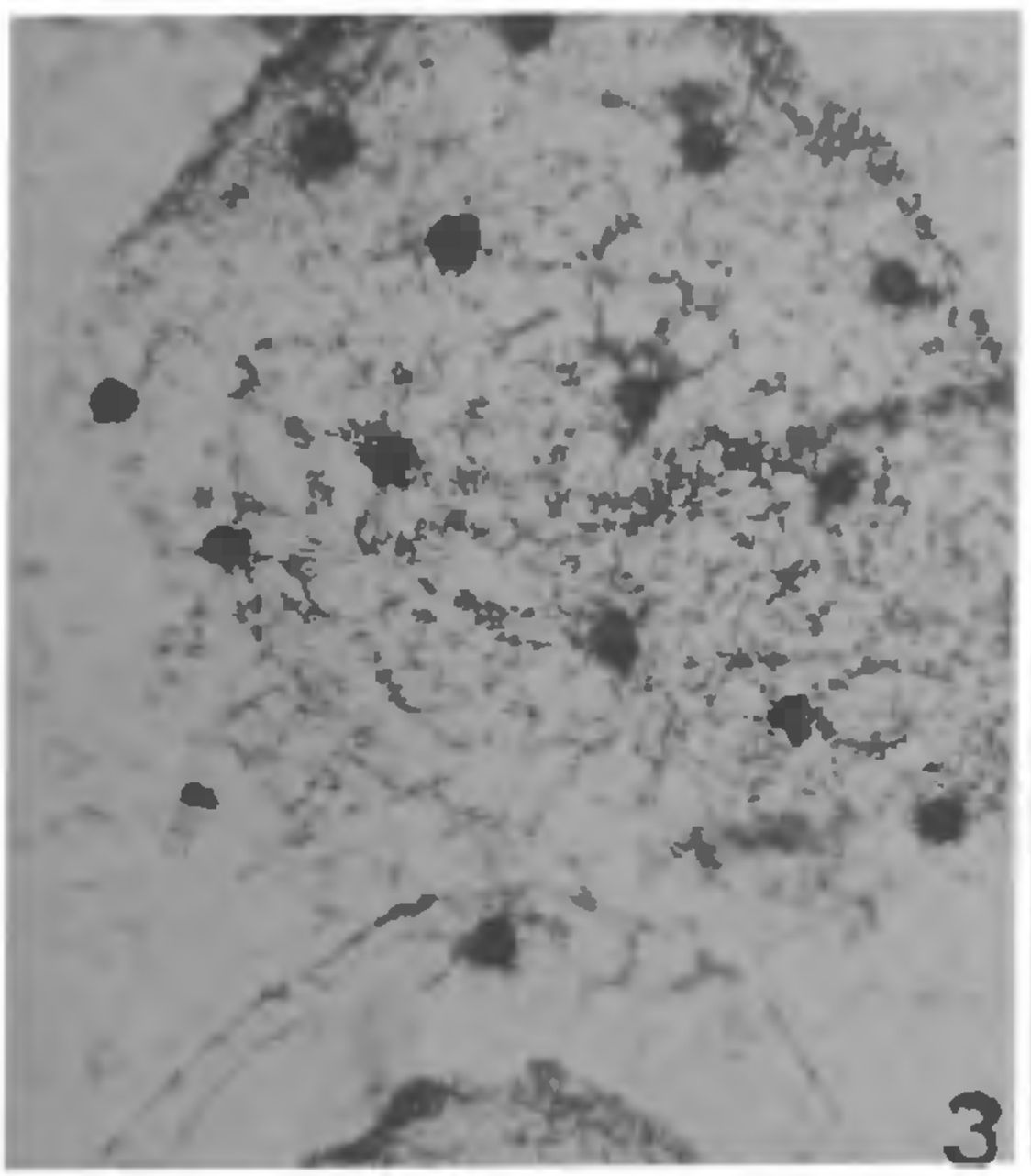
In the present studies, adrenaline caused a dispersal effect on the melanophores, this dispersion affected by adrenaline is of moderate nature. In earlier studies of reptilian species the effect of adrenaline has been found to be either dispersion or aggregation[7–9]. This indicates that the effect of adrenaline is variable from species to species. The dispersal effect of isoprenaline in the present study may be due to the activation of beta adrenergic receptors. In *Anolis*, an American lizard, Hadley and Goldman[4] had also found a similar dispersal effect of isoprenaline in the isolated skin pieces. The effects of phenylepherine suggest the presence of alpha adrenergic receptors in the melanophores of *H. flaviviridis*. The results with adrenaline, isoprenaline and phenylepherine indicate that the melanophores of *H. flaviviridis* possess two types of adrenergic receptors. The beta ones, which are more dominantly present and the alpha ones which may be few in comparison to others. The responses of both the receptors are antagonistic in nature which confirm with the classification of adrenergic receptors[10].

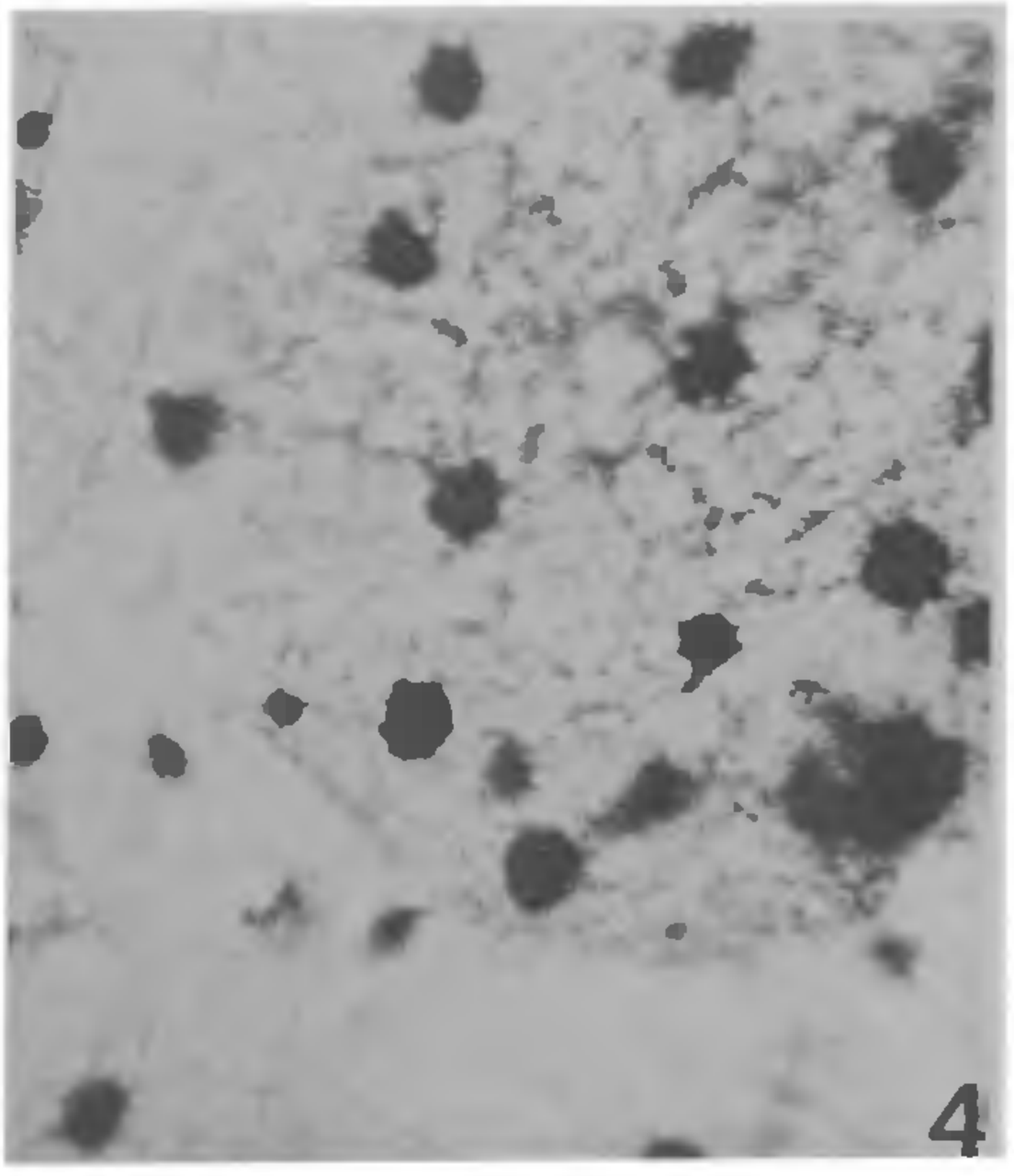
5-HT in the present studies has been a strong pigment-aggregating agent in various concentrations (figure 4, table 1). The data on other reptilian species are lacking on the responses of melanophores to 5-HT.

Acetylcholine has been reported to be ineffective in inducing any responses in *Anolis* melanophores, except in very high concentrations[4]. In the present study acetylcholine varies its effect with different concentrations. At lower concentrations it has produced no effect, but at moderately higher concentration, a dispersion of melanophores was observed. It is well known that a great deal of diversity occurs within this group of lower vertebrates in response to different drugs[3]. Eserine and atropine both caused aggregation of melanophores in the present study, thus the responses of melanophores of *H. flaviviridis* to cholinergic drugs vary greatly.









Figures 1–4. 1. Showing the normal melanophores of *H. flaviviridis* in 0.8% saline, **2.** showing the dispersion of melanophores by 2×10^{-6} g/ml of isoprenaline, **3.** showing the aggregated melanophores in response to 1×10^{-5} g/ml phenylepherine, **4.** showing the aggregated melanophores in response to 1×10^{-5} g/ml of serotonin (5-HT) (magnification × 80)

Table 1 *Effect of Drugs on the* MMSI* *of H. flaviviridis*

No. of Expts.	Experimental Drug	Dose in g/ml	MMSI ± SE	Level of significance
6	Control	0.8% saline	3.700 ± 0.250	—
6	Adrenaline	1×10^{-6}	3.835 ± 0.804	N S.
6	Adrenaline	1×10^{-5}	4.816 ± 1.286	N S.
6	Control	0 8% saline	3.745 ± 0.144	—
8	Isoprenaline	2×10^{-6}	8.256 ± 0.418	$P < 0.001$
8	Isoprenaline	1×10^{-5}	7.972 ± 0.525	P < 0.001
6	Control	0.8% saline	3.851 ± 0 189	—
6	Phenylepherine	1×10^{-6}	1.384 ± 0.513	P < 0.001
6	Phenylepherine	1×10^{-5}	1.225 ± 0.348	$P < 0.001$
3	Control	0.8% saline	3 658 ± 0.053	—
3	Serotonin (5-HT)	1×10^{-6}	0.999 ± 0.080	$P < 0.001$
3	Serotonin (5-HT)	1×10^{-5}	0.724 ± 0 216	$P < 0.001$
6	Control	0.8% saline	3.470 ± 0.364	—
6	Acetylcholine	1×10^{-5}	3.265 ± 0.602	N.S.
6	Acetylcholine	5×10^{-5}	4.013 ± 0.018	N.S.
6	Control	0 8% saline	3.626 ± 0.334	—
6	Eserine	1×10^{-6}	2.347 ± 0.876	N.S.
6	Eserine	1×10^{-5}	2.338 ± 0 889	N.S.
6	Control	0.8% saline	3 918 ± 0 411	—
6	Atropine	1×10^{-5}	2.044 ± 0.302	$P < 0.001$
6	Atropine	5×10^{-5}	2.149 ± 0.438	$P < 0.05$
6	Control	0.8% saline	3.815 ± 0.344	—
6	Histamine	1×10^{-6}	2.516 ± 0.562	N.S.
6	Histamine	1×10^{-5}	2.617 ± 0.554	N.S.

< MMSI: Mean melanophore size index
NS: Not significant statistically.

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DISTRIBUTION OF TRIMETHYLAMINE OXIDE IN SOME MARINE AND FRESHWATER FISH

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ABSTRACT

The distribution of trimethylamine oxide (TMAO) in some marine and freshwater fish species has been reported. Among the marine species, fish belonging to the class elasmobranchs, teleosts and crustacea were studied. It was observed that all marine fish species had appreciable amount of TMAO whereas in the freshwater fish the amount was insignificant. The significance of these results has been discussed.

INTRODUCTION

MARINE fish and very few freshwater fish contain substantial amounts of TMAO and the determination of the degradation product trimethylamine (TMA) has been used as a specific index of bacterial spoilage[1]. Extensive experiments on stored cod or sampled from the market have provided a statistical relationship between TMA and sensory scores[2,3].

Thus the original concentration of TMAO in fish muscle would be of value as it has indirect influence on its quality. The total volatile bases (TVB) and TMAO content of a few Indian fishes were reported earlier[4–7]. However the estimation of TVB carried out by these workers was by microdiffusion method which is considered less accurate and less sensitive than the picrate method[8]. Moreover very few Indian fish species have been studied so far.

The aim of the present investigation was to estimate the amount of TMAO in some Indian fish species using the picrate method. As the variability of TMA is influenced by biological variations in the concentrations of precursors it was thought that TMAO values could be of use to predict the shelf life of stored fish[1,9]. This information could be valuable to the fisheries industry.

MATERIALS AND METHODS

Fresh marine fish were obtained from Sasoon dock and Versowa landing sites of Bombay coast and freshwater fish were caught from a lake at Thana and preserved in ice. All reagents used were of analytical grade.

The fish were beheaded, gutted and 10 g of the white muscle were homogenised in a waring blender with distilled water (2 ml/g meat). Homogenate was centrifuged at 3000 rpm and 8 ml supernatant was mixed with 2 ml of 20% HCHO. This was used for analysis.

One ml of 25% HCl and 0.5 g of Devarda's alloy were added to 5 ml extract and kept in boiling water bath for 15 min. TMAO was reduced to TMA[8]. The solution was cooled and filtered. The filtrate and washings were collected and made upto 100 ml. TMA concentration before and after reduction of TMAO was estimated by the picrate method[10].

Extracts of 1 ml each was taken in stoppered polythene tubes with 3 ml of distilled water, 1 ml 20% HCHO, 10 ml toluene and 3 ml saturated K_2CO_3. After vigorous mixing, 7 ml of toluene layer was transferred to another polythene tube containing 0.3–0.4 g granular anhydrous Na_2SO_4 and mixing repeated. Five ml of this toluene was mixed with 5 ml of 0.02% picric acid in another polythene tube and the colour obtained read at 420 nm using Carl Zeiss PMQII spectrophotometer. Three samples of muscle mince were analysed for each fish and two fish were studied in each species.

RESULTS AND DISCUSSION

The values of TMAO content for elasmobranchs reported in the present study differ significantly from values of some workers[8,11,12]. The highest amount of TMAO reported[11] for some species of elasmobranchs were in the range from 1–1.4 g/100 g of muscle. However these species were not studied in this investigation.

There was a wide variation in the TMAO content of