※NUCLEOTIDE DEGRADATION AND PRODUCTION OF HYPOXANTHINE IN SOME INDIAN MARINE AND FRESHWATER FISHES

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Changes in nucleotides and production of hypoxanthine in rohu (*Labeo rohita*), mrigal (*Cihhrina mrigala*) and common carp (*Cyprins carpio*) during storage at 2 – 4°C were examined. Differences were observed between common carp and others. Production of hypoxanthine in pomfret (*Stromateus argenteus*), cat fish (*Arius macronotacanthus*), shark (*Scoliodon* spp.), seer fish (*Scomberomorus guttatus*), ray fish (*Dasyatis imbricata*) and prawns (*Parapenaeopsis stylifera*) was examined during storage at 2-4°C and -28°C. At 2-4°C hypoxanthine increased regularly but at -28°C there was no increase during storage over 28 weeks.

INTRODUCTION

Adenosine-5-triphosphate (ATP) and the products of degradation of fish muscle post-mortem have received considerable attention in recent years. The accumulation of hypoxanthine (HYP) has attracted particular attention as its content is an index of quality/freshness of fish (Kassemsarn *et al.*, 1963; Jones, 1965; Burt, Stroud and Jones 1969; Dingle and Hines, 1971). The HYP test has the advantage over other objective tests such as trimethylamine (TMA), total volatile base (TVB) *etc.*, since it is sensitive in the early stage of storage in ice before significant bacterial activity sets in. It could be useful in determining the pre-freezing quality of fish (Hiltz *et al.*, 1966). It is considered particularly useful in the case

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of irradiated fish (Spinelli, Pelroy and Miyauchi, 1969) and freshwater fish (Bligh, 1971). However, wide variations have been reported in the rate of accumulation of HYP in the different fish species examined (Fraser, Simpson and Dyer, 1968; Fraser, Pilts and Dyer, 1968; Dingle and Hines, 1971). It is necessary therefore to know the changes occurring in all species of fish if the test is to be employed in practice. No attempt has been made so far in India in carrying out systematic studies of this nature though reports relating to nucleotides in fish muscle post-mortem have been published (Cherian and Nair, 1969; Rao, Rangaswamy and Lahiri, 1969). Hence the present studies were undertaken on some species of marine and freshwater fishes available in Bombay. The studies on the freshwater fishes were aimed at elucidating the stepwise breakdown occurring in the nucleotides soon after death of the fish while those on marine fish were meant primarily to evaluate the accumulation of HYP during storage of the fish in chilled and frozen condition. The results are reported and discussed in the present paper.

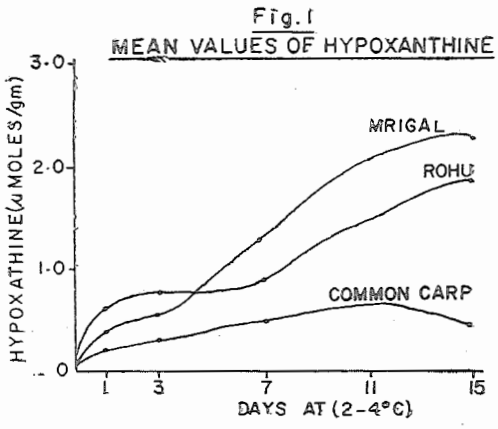
Materials And Methods

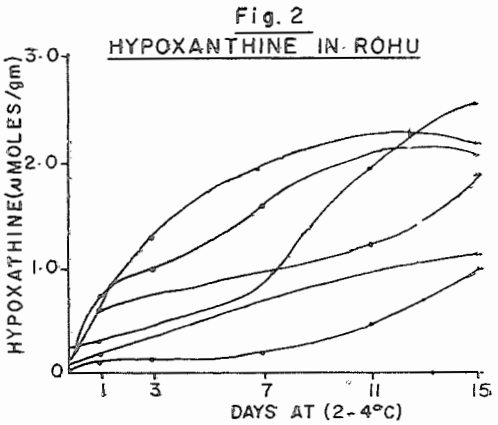
The freshwater fishes common carp (*Cyprinus carpio*), rohu (*Labeo rohita*) and mrigal (*Cirrhina mrigala*) were obtained from a lake in Thana, near Bombay. These were held alive in aquarium tank and used for the experiments after resting them for a week. The marine fish pomfret (*Stromateus argenteus*), cat fish (*Arius macronotacanthus*), sharks (*Scoliodon palasorrah, Scoliodon sorrakowah*), ray fish (*Dasyatis imbricata*), seer fish (*Scomberomorus guttatus*) and prawns (*Parapenaeopsis stylifera*), were obtained from the catches of the Institute's fishing vessel 'MFV Harpodon'. The fish had usually remained at the ambient temperature (about 30°C) for nearly 4 hours prior to being examined in the laboratory.

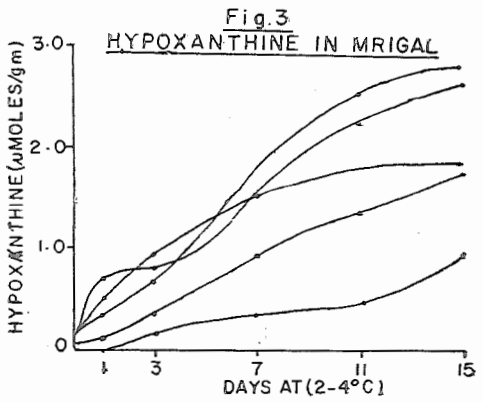
The nucleotides and other biochemicals used were of E. Merck and Fluka. All other chemicals employed in the analysis were of A. R. grade. The xanthine oxidase enzyme was prepared from buffalo's milk in the laboratory employing the method of Dixon and Kodama (1926).

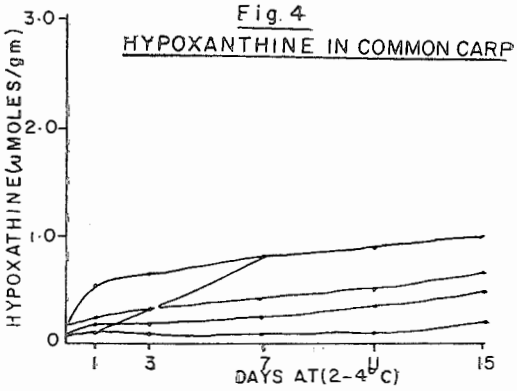
The freshwater fishes were killed by a blow on the head, immediately eviscerated and stored in refrigerator (2-4°C) in polyethylene bags. The marine fishes were eviscerated and stored in crushed ice. For the observation during frozen storage the fish were packed in polyethylene bags and kept in a freezer (-28°C). Samples of muscles were taken at regular intervals from the dorso-lateral portion of the fish starting from behind the head and moving back up to the region of the tail. In the case of prawns all the muscle from two to three specimens were blended together and a portion of it was used for the preparation of the extracts. Dark muscle was separated completely from white muscle before the extraction as hypoxanthine is known to accumulate at different rates in dark muscle as compared with white muscle.

The fish were sampled as described by Dugal (1967). Perchloric acid extracts were prepared by the method outlined by Jones and Murray (1964). 5 g. of the white muscle was homogenised with 5ml. of 10% trichlor acetic acid (TCA), centrifuged, and the supernatent made up to 20 ml. with 1% TCA.









Hypoxanthine was estimated by the method outlined by Kalckar (1947) as modified by Jones and Murray (1964). In this method hypoxanthine is oxidised to uric acid and the absorption at 290mμ due to formation of uric acid was determined using Zeiss PQ Spectrophotometer.

The batchwise ion exchange method of Jones and Murray (1964) was employed for the estimation of inosine and nucleotides.

Results and Discussion

The mean values of hypoxanthine accumulated during 15 days' storage of rohu, mrigal and common carp at refrigeration temperature (2–4°C) are shown in Fig. 1. The rate of accumulation is similar in rohu and mrigal and is significantly greater than in common carp. Values of hypoxanthine in individual fish are shown in Fig. 2-4, which clearly indicate the range of variation occurring among individuals of the same species.

The changes in the adenine nucleotides occurring in these three fishes are shown in fig. 5. In both rohu and mrigal the adenine nucleotides reach 0 level after 15 days but in common carp the level remains quite high after the same period.

Changes occurring in the inosine

monophosphate (IMP) concentration during storage at 2 - 4°C for the three species are shown in fig. 6. In rohu the maximum value is reached after a week and it remains fairly high *i.e.* 2μmoles/g. muscle, on the 15th day; in mrigal the initial value is higher than in rohu and falls during storage to 2μmoles/g. on the 15th day. In common carp the initial value is higher than in rohu and mrigal but falls rapidly to 1μmole/g. by the 15th day.

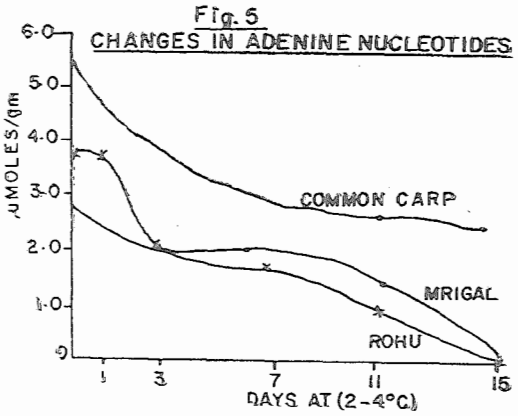
Changes in the inosine concentration during storage are shown in fig. 7. The increase in inosine is rapid in the case of the common carp compared with the rate of increase in rohu and mrigal.

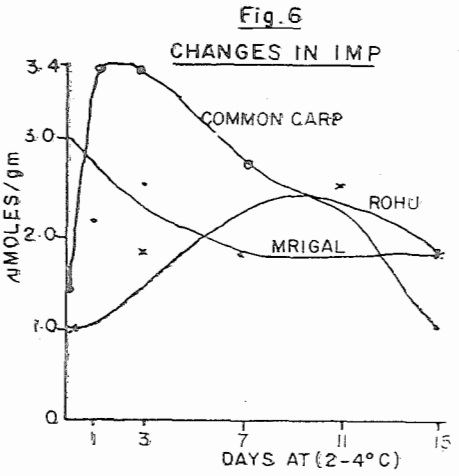
Fig. 8 shows the comparative activity of the enzyme 5'nucleotidase in common carp and rohu. In common carp the activity is much greater compared with that in rohu.

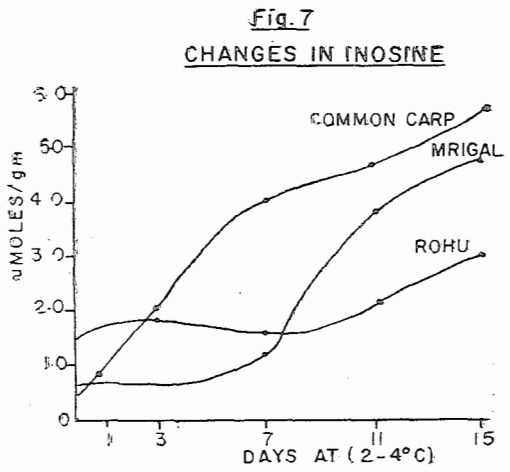
The HYP produced in the marine fishes, sharks, pomfret, cat fish and ray fish, during storage in ice for 15 days is shown in fig. 9. The initial values of HYP are higher than in the freshwater fishes, probably because some time had elapsed between death of the fish and the sampling carried out in the laboratory before commencement of storage in ice. It is noteworthy that sharks showed higher initial values than the other fishes. The ray fish however did not show any rise in HYP concentration even up to the 15th day of storage. In all other fishes except ray fish there was increase in HYP during storage in ice. The HYP concentration in pomfret, seer fish and prawns during frozen storage over a 28 week period is shown in fig. 10. In all the three cases, after an initial increase in the first few days there was no subsequent rise throughout the storage period.

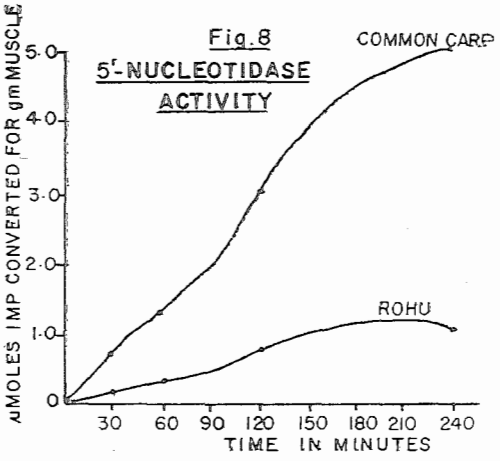
There is practically little HYP detectable in the freshly killed carps but it increases regularly during storage at 2-4°C in all the three species. There is considerable variation among the individuals of the same species However, the mean values of HYP accumulation during storage indicate clearly that the rate of accumulation is higher in rohu and mrigal than in common carp. The changes in the concentration of the adenine nucleotides during storage followed a closely similar pattern in rohu and mrigal differed from common carp in which a higher level was maintained throughout the 15 days' storage whereas in rohu and mrigal the adenine nucleotides could not be detected by the 15th day (fig. 5). Other studies carried out in this laboratory (Nayak, 1975 unpublished observation) indicate that the rate of breakdown of glycogen is slower in common carp compared with that in rohu and mrigal. In view of the close association of glycolysis with the ATP cleavage mechanism the comparatively higher level of adenine nucleotides persisting longer in common carp can probably be explained.

Inosine monophosphate (IMP) increases rapidly in common carp in the first three days reaching values higher than those in rohu or mrigal, and also falls rapidly reaching a value lower than that of rohu and mrigal by the 15th day; in rohu IMP increases slowly but maintains a higher level during the 7th to the 15th day. In mrigal the highest value is reached soon after death but the level falls regu-





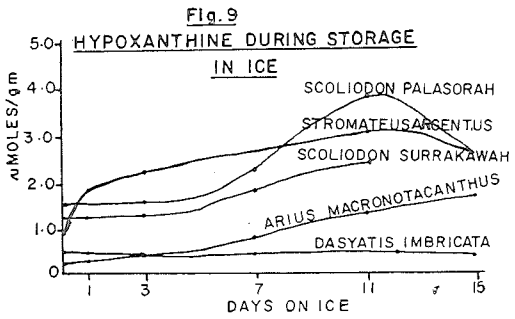


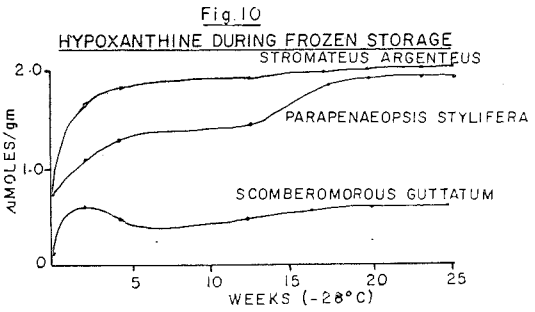


larly and is lower than in rohu during the second week of storage. The higher activity of the enzyme 5'nucleotidase of the common carp muscle compared with that in rohu muscle (fig. 8) explains the rapid reduction in the IMP level in the former. The higher level of inosine and the lower level of hypoxanthine in common carp could be due to lower activity of ribosidase in the common carp.

The differences found between common carp and the two other carps are particularly interesting because while the latter are native Indian major carps the former carp is exotic in origin. The observation that IMP level remains higher in rohu for a longer period than in the other carps is interesting since IMP is known to be a flavour producing compound and rohu is considered tastier among the carps.

In the case of marine fishes stored in ice HYP increases steadily in pomfret, shark and cat fish in this order. In shark initial values are rather high but in the ray, also an elasmobranch, the initial value is quite low and there is no increase





at all during storage. A further study of the nucleotides in the elasmobranchs may be interesting from the comparative aspect. While HYP estimation could be useful as an index of duration of storage/quality/freshness both in marine and freshwater fish kept in ice, for frozen fish, as there is no increase in HYP during the long storage period, the estimation would be of no practical value, except as an indication of pre-freezing quality.

REFERENCES

Bligh, E. G. 1971. *Fish Inspection and Quality Control.*, Ed. R. Kreuser. Fishing News (Books) Ltd., London, U. K. p. 81.

Burt, J. R., G. D. Stroud and N. R. Jones. 1969. *Freezing and Irradiation Preservation of Fish.*, Ed. R. Kreuser. Fishing News (Books) Ltd. London, U. K. p. 367.

Cherian, S. and M. R. Nair. 1969. *Fish. Technol.*, **6**, 1 : 36.

Dingle J. R. and J. A. Hines. 1971. *J. Fish. Res. Bd Can.*, **28** : 1125.

Dixon, M. and K. Kodama. 1926. *Biochem. J*, **20** : 1104.

Dugal L. C. 1967. *J. Fish. Res. Bd Can.*, **24** : 2229.

Fraser, D., S. G. Simpson and W. J. Dyer. 1968. *J. Fish. Res. Bd Can.*, **25** : 817.

Fraser, D., D. P. Pilts and W. J. Dyer. 1968. *J. Fish. Res. Bd Can.*, **25** :239.

Hiltz, D. F., W. J. Dyer, S. Nowlan and J. R. Dingle. 1971. *Fish Inspection and Quality Control.*, Ed. R. Kreuser. Fishing News (Books) Ltd., London, U. K. P. 191.

Hughes, R. B. and N. R. Jones. 1966. *J. Sci. Fd. Agric.*, **17** : 431.

Jones, N. R. 1956. *Technology of Fish Utilisation.*, Ed. R. Kreuser. Fishing News (Books) Ltd., London, U. K. p. 179.

Jones, N. R. and J. Murray. 1964. *J. Sci. Fd Agric.*, **15** : 763.

Kalckar, H. M. 1947. *J. Biol. Chem.*, **167** : 429.

Kassemsarn, B. O., B. Sans Perez, J. Murray and N. R. Jones. 1963. *J. Food Sci.*, **28** : 28.

Nayak, S. 1975. *Personal communication.*

Rao, S. V. S., J. R. Rangaswamy and N. L. Lahiri. 1969. *J. Fish. Res. Bd Can.*, **26** : 704.

Spinelli J., G. Pelroy and D. Miyauchi. 1969. *Freezing and Irradiation of Fish.*, Ed. R. Kreuser. Fishing News (Books) Ltd , London, U. K. p. 425.