CHAPTER V

CHANGES IN THE GLYCOGEN AND LACTIC ACID CONCENTRATIONS OF THE MUSCLE OF SOME FRESHWATER TELEOSTS DURING STORAGE AT -4°C

INTRODUCTION

The recent surge of interest in the biochemical changes occurring in fish muscle postmortem can be attributed to the suitability of fish and fish products in reforming the economics of the country. From multidirectional utilization of fish as protein rich diet, stemmed directly a wide variety of preservation techniques, including storage of fish at low temperatures.

Greater interest has been shown in the immediate biochemical changes that accompany fish muscle postmortem. Of the various factors that are found to influence the course of biochemical changes in the quality of fish muscle during frozen storage, the condition of fish prior to freezing appears to be the most important (Love and Haroldson, 1958; Nishimato and Tanaka, 1960; Dyer and Dingle, 1961; Love, 1962a,b).

The postmortem glycolytic changes, dealing with anaerobic phase of the intermediary metabolism of carbohydrate.
whereby glycogen is converted to lactic acid, have been extensively studied in marine fishes, such as haddock (MacPherson, 1932), frigate mackerel (Amano et al., 1953), red snapper (Noguchi and Yamamoto, 1955), cod (Fraser et al., 1961, 1967), sockeye salmon and starry flounder (Tomlinson et al., 1961), mullet, Bombay duck and grouper (Nasir and Magar, 1963), plaice (Fujii et al., 1966), halibut (Spinalli, 1967). Further, glycogen and lactic acid concentrations in different portions of the muscle of marine fishes, and their levels in relation to the methods and rapidity of killing after capture were recorded in mackerel (Amano et al., 1953). Tauchiya and Kumii (1960) demonstrated the accumulation of lactic acid in the muscle postmortem of tuna. A similar study with concomitant finding was conducted by Tomlinson et al. (1962). The fact that glycolysis can proceed slowly in fish muscle at temperature as low as -10°C was demonstrated by Sharp (1934) who also found that the maximum rate of glycolysis occurred between -3.2°C and -3.7°C. Partmann (1961) while following the pH changes in carp muscle arrived at more or less similar conclusions. Tomlinson et al. (1963) suggested, on the basis of lactic acid accumulation, that glycolysis clearly proceeds at temperatures down to and including -20°C and -30°C.
A few reports have also appeared on the enzyme systems involved in fish muscle glycolysis (Burt, 1961; Nagayama, 1961a, b, c and d).

The present account deals with the changes in the glycogen and lactic acid levels of some commercially important freshwater teleosts, namely, *O. puntatus* Bloch, (Linn.), *C. batrachus* and *I. foerschli* (Bloch), during storage at -4°C. The fishes were stored for a total period of 16 days. Preliminary experiments conducted at this laboratory have indicated that during this length of time at low temperature, most fish species remain within the edibility limit and well beyond the advanced bacterial putrefaction.

**MATERIALS AND METHODS**

The various fish species selected for this study were brought to the laboratory from the local fish market in fresh condition. For each species, specimens of a particular size range were selected. These were wrapped in polythene bags and kept at -4°C. For each sampling, at least three specimens were taken out from the stock after the interval of every four days. The study was continued for a total period of sixteen days. Methods of muscle
Fig. 17. Changes in the muscle glycogen (---) and lactic acid (---) concentration of *C. punctatus* during storage at -4°C.
Fig. 18. Changes in the muscle glycogen (---) and lactic acid (---) concentration of *C. batracum* during storage at -4°C.
PERIOD OF STORAGE, DAYS
sampling and processing were the same as used for other studies (page 32). The estimations of glycogen and lactic acid were carried out according to the methods as previously described under 'Procedure and Methodology'.

RESULTS AND DISCUSSION

The glycogen concentration in the muscle of *G. mungilis* in fresh condition was found to be 104.406 mg/100 g (Table 16). This value declined steadily with the period of storage (Fig. 17). The fall in the glycogen level after a storage of 16 days was about 69%. This was found statistically significant (*p* < 0.001). In contrast to glycogen, the concentration of lactic acid registered a marked increase during the period of storage (Table 17; Fig. 17). From a value of 106.18 mg/100 g, in the fresh state, the lactic acid level was observed to rise to 272.99 mg/100 g when the storage period was extended to 16 days. Thus, an increase of about 156% was noted which was found significant at 0.001 level of probability.

In *G. latrunculis*, the glycogen concentration (234.68 mg/100 g) in fresh muscle registered a significant (*p* < 0.001) fall of about 47% during the total period of storage (Table 18; Fig. 18). The lactate concentration, however,
Fig. 19. Changes in the muscle glycojen (---) and lactic acid ( - - - ) concentration of *H. foetida* during storage at -4°C.
maintained a reciprocal progression as evidenced by a marked \( p < 0.001 \) increase in its concentration during 16 days of storage (Table 19; Fig. 18). This increase was about 60% of the fresh state.

The glycogen and lactic acid concentrations in the muscle of \( J. \) fossilis was found to follow pattern of changes similar to those observed for \( C. \) punctatus and \( C. \) batrachus (Table 20-21; Fig. 19). A fall of about 50% in the glycogen and a rise of about 60% in the lactic acid level seemed to occur during 16 days of storage. These changes in both the constituent were significant at 0.001 level of probability.

Thus, the pattern of changes in the two metabolites were basically similar in the three species studied, though interspecific differences were evident with regard to their initial levels at fresh condition and the percentage of changes during frozen storage. For instance, the initial level of muscle glycogen was highest in \( C. \) batrachus and lowest in \( C. \) punctatus. The lactic acid concentration in the fresh condition, on the other hand, was minimum in \( C. \) batrachus and maximum in \( C. \) punctatus. Similarly, the percentage of fall in the glycogen content was maximum in \( C. \) punctatus and minimum in \( C. \) batrachus. The percentage of rise in the lactic acid concentration, on the other hand,
was greatest in *O. mykata*us, while in the two cat-fishes
the percentages of rise were almost identical.

Although stoichiometric relationship has not been
shown to exist in glycogen and lactic acid proportionality
in fish muscle (MacPherson, 1932; Partmann, 1965), the two
fractions maintained an inverse quantitative relationship,
in different proportions, as has been evident from the
present observations. This indicates that presumably during
storage all the glycogen which has been degraded may not get
transformed to lactic acid or the total occurrence of lactic
acid may not be derived from glycogen. Obviously, the
degradation of glycogen during the course of postmortem
changes may also produce some other intermediate compounds,
as has been discussed in the section dealing with pyruvic
acid (page 47), or metabolites of Embden-Meyerhof pathway.

A corollary to the present finding on the changes in
the glycogen level of fishes was evident in the works of
MacPherson (1932), Harp (1934), Noguchi and Yamamoto (1955),
Fraser et al. (1961), Nasir and Hagar (1963).

Also, the pattern of changes observed in the lactic
acid concentration of the three freshwater species examined
seemed in agreement with the observations of MacPherson (1932)

However, the observed decline in the glycogen level of fish during storage may be more precisely explained on the basis of the fact that during cold storage of fish post-mortem, many lysolytic reactions are drastically accelerated, resulting in the disruption of the cellular structure. This leads to the activation of relevant enzymes, perhaps as a result of increased availability of substrate or due to the release of enzymes from cell organelles (Hiltz and Dyer, 1973).

It has been established that the glycogen present in fish postmortem is generally degraded by the action of phosphorylase (Uno et al., 1957) and amylase (Andreev, 1953). These lead to the formation of glucose-1-phosphate, maltose and glucose. Moreover, stimulation in the activity of phosphorylase is enhanced by the transformation of phosphorylase \( 'b' \) inactive form into \( 'a' \) active form which is supposed to occur in pre-rigor storage of the muscle (Cori, 1956). During the later period of storage, as has been found in the present study, the rate of glycogen degradation is little slower, presumably due to a change in pH which may inhibit the phosphorylase activity. Similar facts have been
pointed out by other workers (Cori, 1936; Danforth, 1965). It is known that the pi and other by-products in fish post-mortem may actually block the transformation of phosphorylase 'b' inactive form into 'a' active form (Cori, 1956) or the susceptibility of glycogen to phosphorylase is slowed (Lawrie et al., 1959) or the availability of glycogen to phosphorylase activation is reduced (Guo et al., 1957). The activity of amylase, attacking glycogen hydrolytically, has been reported to increase due to autolytic changes in fish muscle postmortem (Burt, 1966).

A continuous rise in the level of lactic acid during storage, as evidenced by the observations on the three freshwater species, may be due to the increased rate of conversion of pyruvic acid to lactic acid by lactic dehydrogenase which is instrumental in the above reaction. The disappearance of pyruvic acid in the early stages of iced fish (Jones, 1959) and an increased activity of lactic dehydrogenase (Tappel, 1966) may enhance the increased production of lactic acid. Some of the keto-acids are also fed into citric acid cycle (Jones, 1962), leading to the formation of compounds other than lactic acid.
SUMMARY

Glycogen and lactic acid levels were measured in the muscle of three common freshwater teleosts, namely, *C. punctatus* Bloch, *C. batrachus* (Linn.) and *H. fossilis* (Bloch), during storage at 

The two constituents were found to maintain an inverse quantitative relationship in the muscle of these species. A general decline in the muscle glycogen, which was accompanied by a rise in the lactic acid was, however, noted with the length of storage. The pattern of changes in the levels of the two metabolites were basically similar in the three species, though inter-specific differences occurred in their initial levels at fresh condition, as also in the percentage of changes during frozen storage. The significance of the observed changes has been discussed.