MMTV serum used in our experiment contained antibodies against C-type viruses, a murine leukemia virus preparation derived from AKR mice (provided by Dr Iwai of the Radiation Center of Osaka Prefecture) was similarly assayed. As shown in curve 5, no increase in the enzyme activity bound was observed.

Enzyme immunoassay, as described here, is highly specific for MMTV and reproducible. The assay can detect as little as 3 ng/ml of MMTV and makes it possible to monitor easily the kinetics of MMTV production in tissue culture systems. Sheffield et al.[2] reported that radioimmunoassay can measure 0.05 ng/assay tube of the purified gs antigen (gp 55) from MMTV and 0.5 ng/assay tube of whole virus. The lowest value to be determined by our method is converted into 1.5 ng/assay tube of whole virus. This means that enzyme immunoassay is almost as sensitive as radioimmunoassay. In addition, it was reported that enzyme immunoassay became more sensitive when the IgG possessing antibody activity was concentrated from the antiserum by immunoaffinity chromatography[15]. The use of concentrated monospecific antibodies in the assay system of each polypeptide of MMTV is currently under development.

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Histochemical localization of alkaline and acid phosphatase activities in the skin of *Mystus (Mystus) vittatus* Bl. (Siluriformes)

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Summary. An attempt has been made to localize alkaline and acid phosphatase activities in the skin of *Mystus vittatus* by using histochemical techniques. The alkaline phosphatase activity is found in metabolically active cells such as basal columnar cells, mucous cells and polygonal support cells. The acid phosphatase activity is intense in the outermost squamous support cells and in the basal columnar cells. These activities have been correlated with some physiological functions of the epidermis.

There have been several histochemical studies to establish the correlation between alkaline and acid phosphatase activities and the functional state of the skin of fishes[2-5]. These hydrolytic enzymes play a significant role in the metabolic activities of various cellular components of fish skin. The present attempt is intended to correlate alkaline and acid phosphatase activities with the functions of various cellular components in the skin of *Mystus vittatus*, the structural organization of which has been described by the present authors[6].

Material and methods. The fish specimens were collected locally and kept in aquaria for acclimatization. Skin fragments of 4×8 mm were taken from a lateral site, fixed in cold acetone and cut using 52-54 Paraffin wax. The alkaline phosphatase activity was localized by the calcium cobalt method of Gomori[7] and the modified coupling azo dye method of Pearse[8], and the acid phosphatase activity by the lead nitrate test and the standard azo dye method described by Pearse[8]. Control sections were incubated without using the substrates.

Results. The calcium cobalt method gave a strong brownish black colour reaction with basement membrane, basal columnar cells, small mucous cells, inner polygonal support cells and the *subcutis*, thereby showing alkaline phosphatase activity. These results were confirmed by the modified azo dye technique of Pearse[8] using salt 9, which gave a reddish brown colour reaction. The lead nitrate test of Gomori[7] for acid phosphatase activity gave a strong black colour reaction in basal columnar cells and outermost squamous support cells, and a weak reaction in small mucous cells. The standard coupling azo dye technique of Pearse[8] confirmed acid phosphatase activity in basal columnar and squamous support cells by a strong reddish brown colour in the cytoplasm and a deep blue colour in the nuclei.

Discussion. Alkaline phosphatase activity has been considered to indicate various functions for example, active cell division[9-11]; sites of active transport across the cell membrane[12], and it is closely associated with the osseous layer of the scale as well as sulphated acid mucopolysaccharides and calcium which play and active role in calcification[3] and in the synthesis of mucopolysaccharides[3,13,14]. In *Mystus vittatus* the alkaline phosphatase activity is found in metabolically active cells and extends from the basal layer of the epidermis to the surface, as found in whiting[5]. This activity is possibly connected with active cell division and synthesis of mucopolysaccharides. The replacement of lost cells of the epidermis and secretion of mucopolysaccharides in non-scaly fishes is important for protective functions. Intense activity of alkaline phosphatases in basal columnar

cells, small mucous cells and inner polygonal support cells supports this view. This activity is also seen in the basement membrane of *Mystus vittatus* which supports the view that it may be associated with the fibrous proteins[15] and the passage of metabolites across the cell membranes[16,17]. Insignificant or negative reaction to alkaline phosphatase activity in large mucous cells and dermis account for their poor metabolic activities in the present study.

Acid phosphatase activity has been regarded as having a lysosomal distribution with an important role in hydrolytic and catabolic wasting processes[18-20] and in the cytolysis of keratinized cells[3]. In whiting the acid phosphatase activity is restricted to a narrow zone at the extreme exterior of the epidermis[5].

Since keratinization is not found in the present study and the acid phosphatase activity is confined to the outermost squamous support cells and basal columnar cells, it may have some relation to the hydrolytic and catabolic wasting process.

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Increase in muscle fibres in the lateralis muscle (white portion) of Mugilidae (Pisces, Teleostei)[1]

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Summary. A cycle of postlarval growth is described in the white portion of the lateralis muscle of Mugilidae. The cycle was identified histochemically by the myosin-ATPase reaction.

The postnatal growth of striated muscle in vertebrates is not due exclusively to hypertrophy but also to an increase in the number of muscle fibres. In mammals the increase in fibre number is quite evident shortly after birth[2-8] (as an extension of embryonic tissue differentiation[9]) and during periods of muscle regeneration[10-15]. Muscle growth due to an increase in fibre number in the adult animal is more controversial, even though it has been described in the radialis muscle of rat[16] and, more recently, in the sartorius muscle in man[17,18]. In birds, a postnatal increase in fibre number has been reported in the anterior latissimus dorsi muscle of the adult chicken not only after denervation but also after simple stretching[19]. In fish, postlarval growth has been studied very little; however, it appears to be ascribable not only to hypertrophy but also to hyperplasia, as has been reported in the cod and eel[20-22]. During our histochemical research on muscle fibre types[23-26], while looking for annual variation in the white, pink and red portions of lateralis muscle fibres in fish, we noted a growth cycle in the white muscle of Mugilidae, which is reported in the present paper.

Our results are based on experiments conducted from November 1978 to March 1980 on 2-year-old Mugilidae of the following species of different length: *Mugil saliens* (9-12 cm), *M. capito* (11-15 cm), *M. chelo* (7-8 cm) and *M. auratus* (15-18.5 cm). The growth rates of these fishes differ markedly, being greatest in *M. auratus* and least in *M. chelo*. The fish, collected in the northern Adriatic and kept in aquaria containing sea water at room temperature (18-25 °C), were killed weekly by decapitation under tricaine methanesulfonate (MS-222) anaesthesia. The lateralis muscle samples, taken from regions underneath the 1st and the 2nd dorsal fin, were rapidly frozen in isopentane at −80 °C. 10-µm serial transverse sections were cut in a cryostat and stained for succinate dehydrogenase (SDH)[27], lactate dehydrogenase (LDH)[27], menadione α-glycerophosphate dehydrogenase (M α-GPDH)[27] and myosin adenosine triphosphatase (myosin-ATPase) after preincubation for 1-1.5-2 min in 0.1 M acetate buffer at pH 4.0, 4.2, 4.3, 4.6 and in 0.1 M 2-amino-2-methyl-1-propanol at pH 10.1, 10.2, 10.35 with NaOH[28-30]. Between November and August, in all species studied, the white portion of the lateralis muscle (in contrast to the red and pink ones) was composed of a single fibre type (fast-twitch) 'large diameter' (80-100 µm), which had a high myosin-ATPase activity, and which was alkali-stable and acid-labile (figure 1, a), SDH negative and weakly positive to M α-GPDH and LDH. At the beginning of September, with the exception of *M. chelo*, 'small diameter' fibres (18-20 µm) began to appear, which were intimately connected with, and located between, the large diameter fibres, and whose high myosin-ATPase activity was not only alkali-, but also acid-stable. These fibres constituted approximately 20-30% of the total number of white muscle fibres and were unreactive to the other