CHAPTER - 10

HISTOENZYMOLOGY OF THE REGENERATING LACERTILIAN TAIL: 2. GLUTAMATE DEHYDROGENASE (GDH; E.C. 1.4.1.2.) ACTIVITY DURING REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Glutamate dehydrogenase catalyses the reversible reaction between α-Ketoglutarate and glutamate with both NAD as well as NADP serving as the coenzymes. Depending upon the concentration of factors such as ATP, NH_3 as well as the necessities of the tissues concerned, the reaction could proceed in either direction. This reaction as is generally known, could either lead to the formation of amino acids or else their deamination and entry into the TCA cycle oxidation. Interest presently concerns not only with the possibility of correlating the presence of this enzyme with the synthesis of the specific amino acids of the glutamate family, but also to apparently act as an indication of the existence of biosynthetic reactions involved in the aspartate, pyruvate and serine families of amino acids as well. Since it is

generally accepted that in a normal tissue protein catabolism is a rare event unless stimulated by certain abnormal conditions, and as developmental processes are known to be actively associated with protein synthesis, the activity of the enzyme GDH during regeneration could be safely assumed to be directed towards biosynthetic pathways. Though it is an accepted view that increased protein metabolism should be a characteristic feature of regeneration, curiously enough, there are no reports or investigations regarding the quantitative or qualitative aspects of protein anabolism; neigh even the demonstration of enzymes concerned in aminoacid synthesis. In this background, the present investigation on the histochemical distribution and localization of the enzyme GDH in the regenerating tail is undertaken as a preliminary step in this direction. Moreover, a previous investigation on SDH and ICDH during tail regeneration in Mabuya carinata (Shah and Ramachandran, 1976) had suggested α-Ketoglutarate as an important intermediary product and hinted at its possible utility value as a starting point for the synthesis of aminoacids. The enzyme GDH, since it catalyzes

the conversion of α-Ketoglutarate, could also help to substantiate the above contention arrived at earlier.

MATERIAL AND METHOD

Adult Mabuyas maintained on a diet of insects served as the experimental animals. The autotomy of the normal and regenerating tails was carried out as described earlier (Shah and Ramachandran, 1970, 1972). The wound surfaces were blotted to remove blood and tissue fluids from the cut end and the tails were fixed on a microtome chuck of a cryostat maintained at -20°C. Longitudinal and transverse sections of 12-18 μ thickness were cut and incubated for about 45 minutes at room temperature in the incubation medium prepared as per the method described by Pearse (1972). Further processing of the tissue sections was also done as described in the same method. A few sections incubated in a substrate blank medium and a few others treated with water at 80°C before incubation, served as the controls.

OBSERVATIONS

NORMAL TAIL

Of the various tail tissue components, the muscles depicted the highest concentration of the enzyme, the localization of which was both sarcoplasmic as well as mitrochondrial. Three types of fibres could be differentiated with regard to their mitochondrial number and enzyme concentration. All the peripheral fibres in a fasiculus were comparatively smaller in size with high enzyme intensity and mitochondrial number. Of the remaining fibres which were almost of the same size, some revealed fewer mitrochondrial number and lesser enzyme activity whereas others were intermediate to the above two types in enzyme activity and mitochondrial concentration.

Apart from the muscles the only other components which showed any enzyme activity worth noticeable were the stratum germinativum in the integument, marrow cells of the vertebral column and, the nervecord in that order. All the other components of the tail registered a poor to nil activity of the enzyme.

EXPLANATION TO FIGURES

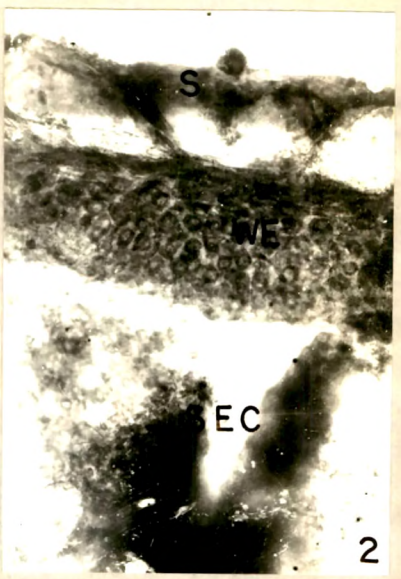
Fig. 1. Photomicrograph of L.S. of wound healing tail showing GDH activity. CEM - Cut end of muscles; NC - Nerve cord; SEC - Subepithelial cells; VC - Vertebral column; WE - Wound epithelium.

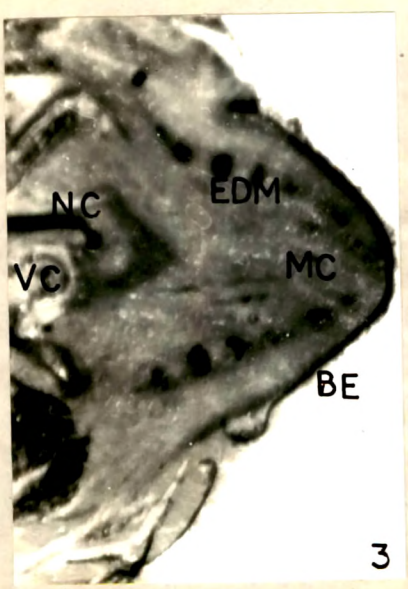
Fig. 2. Wound epithelium (WE) together with the subepithelial cells (SEC) showing GDH activity. S - scale. 125 X.

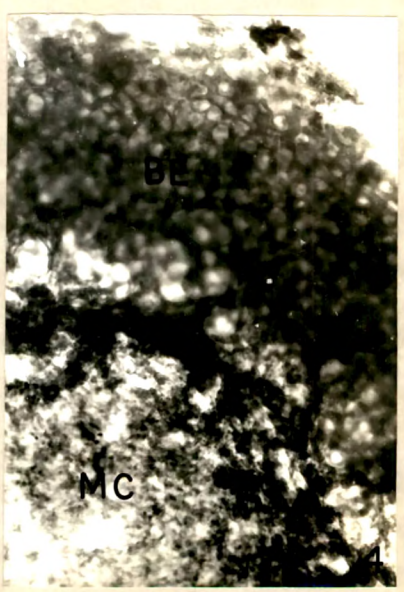
Fig. 3. L.S. of Blastema showing GDH activity. BE - Blastemic epithelium; EMD - Early differentiating nuscles; MC - Mesenchymal cells; NC - Nerve cord; VC - Vertebral column.

Fig. 4. Blastemic epithelium (BE) and the underlying mesenchymal cells (MC) depicting high GDH activity. 125 X.


CEM
NC
VC
SEC
WE
1


S
EC
2


NC
EDM
VC
MC
BE
3

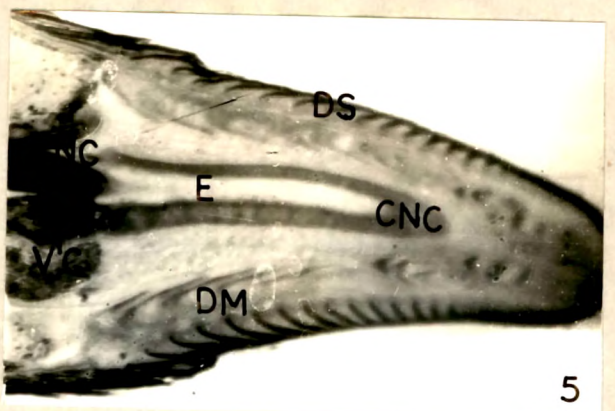

BE
MC
4

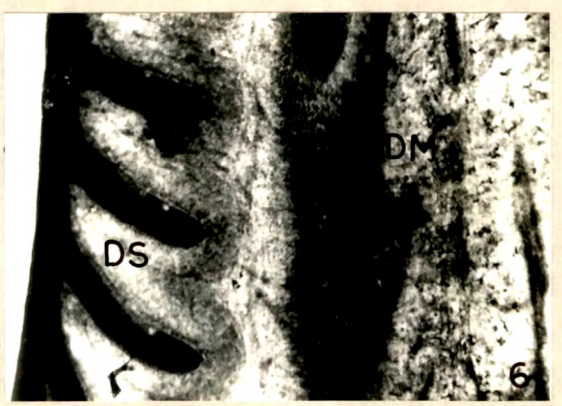
EXPLANATION TO FIGURES

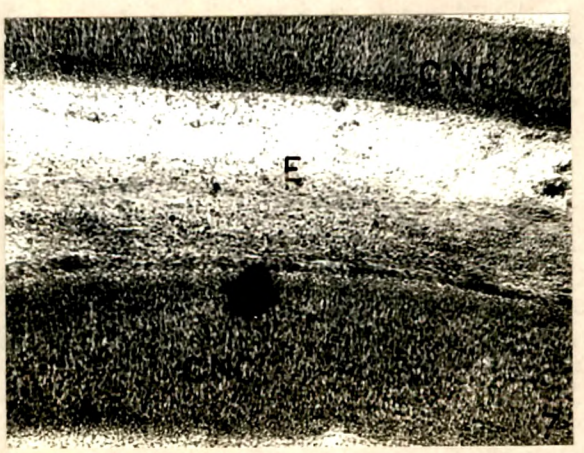
Fig. 5. L.S. of differentiating tail showing GDH activity. CNC - Cartilagenous neural canal; DM - Differentiating muscles; DS - Differentiating scales; E - Ependyma; NC - Nerve cord; VC - Vertebral column.

Fig. 6. Differentiating scales (DS) and differentiating muscles (DM) showing enhanced GDH activity. 50 X.

Fig. 7. Cartilagenous neural canal (CNC) with Ependyma (E) showing GDH activity. 50 X.


DS
E
CNc
DM
5


DM
DS
6


E
7

WOUND HEALING PHASE

Appreciable high enzyme activity could be noticed in the wound epithelium as well as in the cut ends of the tail tissues.

PREBLASTEMIC AND BLASTEMIC PHASES

A furthermore increased activity could be easily discerned in both the stratified blastemic epithelium as well as in the mesenchymal cells of the blastema.

DIFFERENTIATION PHASE

All throughout differentiation, the various differentiating elements such as skin, muscles cartilage etc. maintained a continually high enzyme activity. However, during the late differentiation phase the enzyme activity was seen to record a slightly lower level.

GROWTH PHASE AND FULLY REGENERATE TAIL

During the growth phase the activity of GDH was seen gradually dwindling, a begining of which was already made during the late differentiation phase. With the completion of the growth phase and the

attainment of a more or less a fully regenerate condition, the various tail tissues were seen to attain a level and concentration of the enzyme activity, very much comparable to that in the corresponding normal tail tissues.

DISCUSSION

Whereas the presence of the enzyme GDH in the normal tail could be looked upto as the representation of a normal complement, the increased concentration during wound healing, blastemic and differentiation phases is quite significant.

Though the increased presence of GDH in the wound epithelium could be correlated with the possible synthetic activity of this tissue, the incidence of a high enzyme concentration at the cut end of the stump tissues should be looked at from a different angle. As a significant level of lytic activity is known to reside at the cut surface of the stump tissues during wound healing in regenerating tissues (Needham, 1952), the presently observed GDH activity

could also be implicated in the lytic process leading to cellular demolition and dedifferentiation.

The essential feature of blastemal cells which are in an undifferentiated multipotential condition is the high mitotic activity leading to a necessity for high protein turn over not only to form the structural composition of protoplasm but also to form the structural framework of the various cellular entities. With the advent of differentiation, the process of cell multiplication now gets coupled with a simultaneous process of differentiation, and this morphological complexity adds a new dimension to the biochemical process, in the form of a demand for an increased magnitude of protein turnover necessitated by the requirements of the various differentating elements such as skin, muscle, cartilage etc., to elaborate their own specific types of proteins. Also required are the basic proteins for the formation of nucleoproteins of these cells. Further, all previous studies on lacertilian tail regeneration have shown a

tremendous increase in the activities of various enzymes, both catabolic as well as anabolic. Such an increase in enzyme concentration would also entail a high rate of protein synthesis. When viewed in this light of a tremendous exigency of protein biosynthesis, the high threshold level of GDH is rather self explanatory and indicative of its participation in synthesis of the aminoacids of the glutamate family. This contention gains validity from the observed activity of ICDH and its significance discussed by Shah and Ramachandran (1976). Similar possibilities of aminoacid biosynthesis had been suggested earlier by the observed subcellular metabolic adaptations during regeneration in the tail of Mabuya carinata (Shah and Ramachandran, 1970, 1973, 1976; Ramachandran, 1972). These studies on glycolytic and TCA cycle enzymes had revealed the operation of a short pyruvate centred metabolic cycle with pyruvate and oxalocetate as the important coparticipants; two very important products serving as the starting points for the biosynthesis of pyruvate and aspartate families of aminoacids. Moreover, some

of the intermediary products of glycolysis such as phosphoglycerate, and phosphoenolpyruvate too serve as important biochemical entities for the production of aminoacids of the serine family, as well as the aromatic ones respectively. It could be cited, at this juncture, that the glycolytic pathway was shown to be continually in operation during the various phases of regeneration (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975). From these it could be safely assumed that the biochemical adaptations of the regenerating system especially during the blastemic and differentiation phases are so directed, as to build up a large enough pool of necessary aminoacids so that the quantitative as well as qualitative production of proteins characteristic of the regenerating tissues, and enzymes found therein, could be efficiently and smoothly maintained. At the cellular level, the active synthesis of aminoacids is marked by a proportionate release of H^+ ions which if not counterbalanced by exchange with a monovalent cation like K^+ could lead to a fall in pH to an alarmingly low level (Stern and Nanney, 1965). It is interesting in this connection

to note that a gradual increase in K^+ concentration has been reported during blastemic and differentiation phases, after an initial drop during the preblastemic period (Shah and Hiradhar, 1974). The gradual decline in the activity of GDH noticeable during the growth phase is suggestive of the reduced necessity of protein biosynthesis and corresponds well with the similar decrease in activity reported earlier with respect to other enzymes. Finally, with the completion of the process of regeneration the GDH activity also settled down to a more or less normal level in the various tissues of the fully regenerated tail.