CHAPTER 6

FREE AMINO ACIDS DURING TAIL REGENERATION IN THE HOUSE LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

Regeneration of a complex structure such as a lizard's tail is a conglomerate of diverse metabolic and morphogenetic activities. It has been increasingly realized that protein metabolism gets extensively geared up in accordance with the regressive (dedifferentiation), as much as with the progressive (blastema formation, differentiation, growth) events of regeneration (Thornton and Bromley, 1973). Amino acids, the acknowledged building blocks of proteins would predictably manifest fluctuations in their concentrations in event of extensive macromolecular degradations and syntheses. Keeping this in view, the current study deals with a preliminary evaluation of free amino acids in the regenerate during various stages of tail regeneration in the house lizard, Hemidactylus flaviviridis.

MATERIALS AND METHODS

The lizards, H.flaviviridis, collected from the

University campus, were maintained in the laboratory on a diet of insects. Autotomy was induced by pinching off the normal tail leaving two or three basal segments distal to vent intact. The animals were sacrificed at different stages of tail regeneration (Shah and Chakko, 1968b). Pieces of normal tail and regenerates pooled from 5-10 lizards were homogenized in 1% picric acid for deproteinization and the precipitates were separated by centrifugation at 3000 r.p.m. The picric acid from the supernatant was removed by passing the liquid through a 2 cm high resin bed of Dowex 2 x 8 (200-400 mesh) in chloride form. The filtrate was concentrated on a rotary vacuum evaporator. Glutathione, which is present in the tissue and is known to interfere with the chromatogram, was removed by sodium sulphite treatment (as described in Beckman Manual A-IM.3). The samples were analyzed in a Beckman Model 120 C Amino acid analyzer in Beckman resin AA 15 and PA 35, using sodium citrate buffer and ninhydrin as a colour developer (See Table 1, Figs.1 & 2). The concentrations of different amino acids were calculated at 570 nm wave length, except for proline which was calculated at 420 nm wave length. The concentration of amino acids were expressed in µmoles amino acid per 100 gms of wet tissue.

Table 1

Operating conditions required for the separation of free amino acids.

Requirements	Analysis of Basics	Analysis of Acidics & Neutrals
Column Size	23 x 0.9 cm	69 x 0.9 cm
Resin Type	PA - 35	AA - 15
Height of resin column	5.5 cm	56 cm
Duration of run	70 min	175 min
Flow rates:		
Buffer	68 ml/hr	68 ml/hr
Ninhydrin	34 ml/hr	34 ml/hr
First Buffer	pH 5.25 ± 0.01 (0.35N)	pH 3.25 ± 0.01 (0.2N)
Second Buffer	-	pH 4.30 ± 0.02 (0.2N)
Buffer Change (Min.after Start)	None	85 min
Operating temperature		
Bath tank	55.5°C ± 0.1°C	55.5°C ± 0.05°C
Column jacket outlet	55.2°C ± 0.1°C	55.2°C ± 0.05°C
Approximate column pressure	40 psi	130 psi

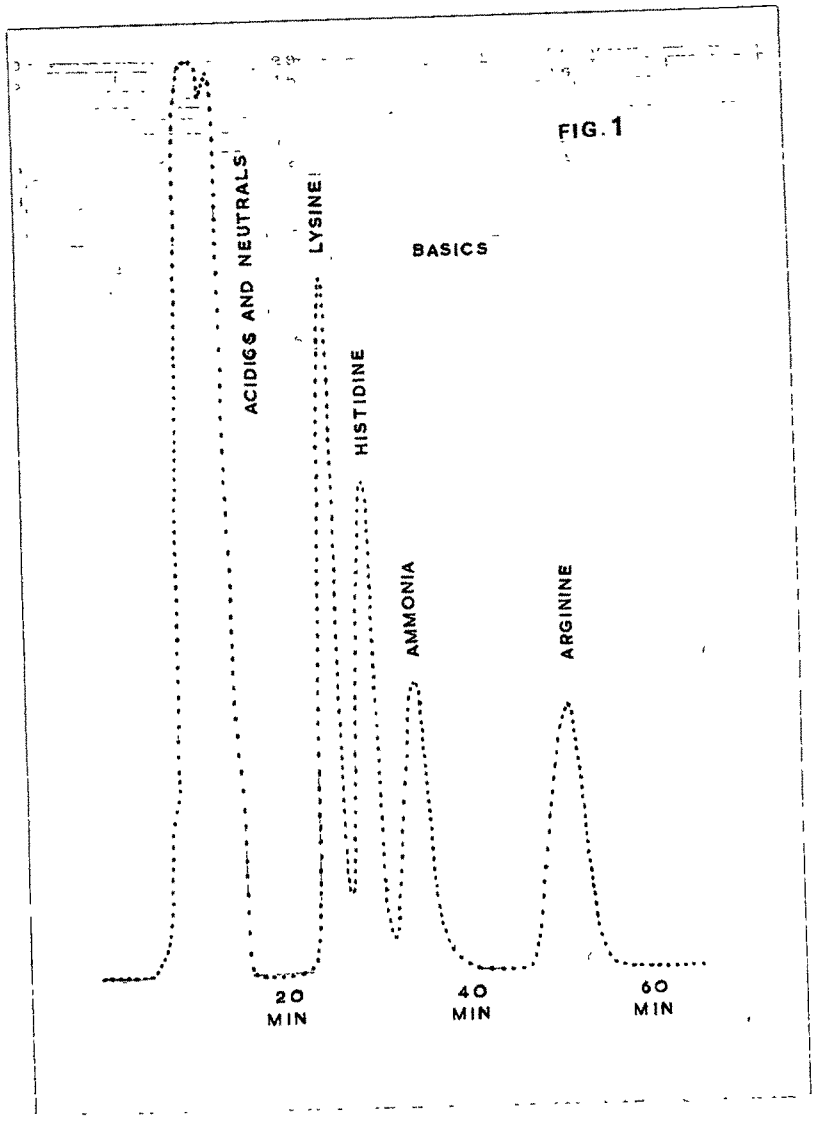

Fig. 1 : Pattern of separation of free amino acids.

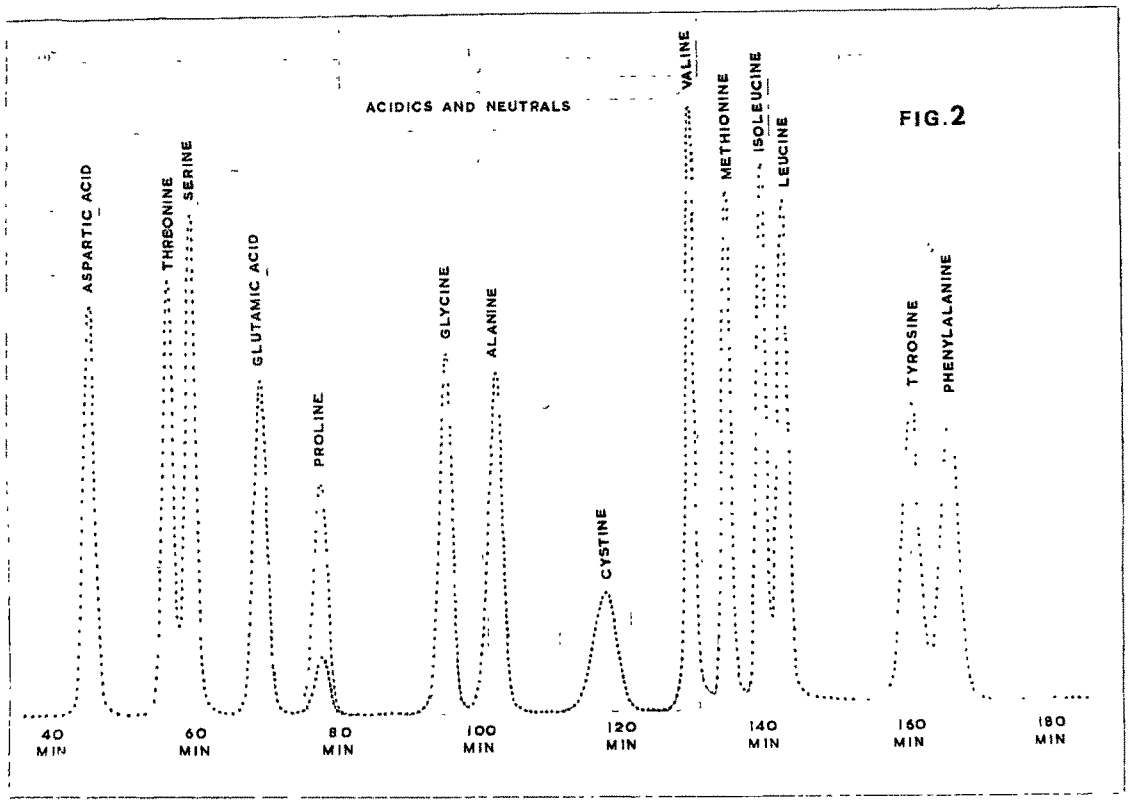

Fig. 2 : Pattern of separation of free amino acids.

Nutritional state of the lizards is difficult to control (Herbert et al., 1966). In this experiment an attempt was made to compensate for this factor by selecting well fed (by examining presence of food in the stomach) and healthy animals only.

RESULTS

Seventeen free amino acids, viz., aspartic acid, threonine, serine, glutamic acid, proline, glycine alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine were detected and their concentration in normal tail and regenerates at different phases are recorded in Table 2 and Figs. 3, 4 & 5. In most of the runs, peaks of threonine and serine appeared simultaneously, hence their combined concentration has been presented. During early phases of regeneration (blastema and differentiation) aspartic acid, glutamic acid, proline, valine, isoleucine, leucine, tyrosine and phenylalanine showed elevations while threonine + serine, glycine, methionine, lysine and histidine showed a depletion in their concentration from their corresponding values observed for the normal tail.

Table 2

Free amino acids during tail regeneration in the lizard, H.flaviviridis.

Amino acids	Normal	Phases of tail regeneration			
		Blastema	Differen-tiation	Growth	Fully regene-rated
Aspartic acid	8.006	38.964	32.736	8.202	6.792
Threonine+Serine	132.120	82.093	105.520	149.382	260.449
Glutamic acid	93.520	194.810	265.009	157.168	138.938
Proline	7.025	16.508	23.431	11.249	8.532
Glycine	185.718	76.553	123.972	338.621	169.733
Alanine	47.770	40.388	56.096	26.210	56.767
Cystine/2	3.796	Trace amount	4.901	5.032	3.321
Valine	7.041	16.733	12.323	5.648	6.260
Methionine	27.390	5.291	3.027	3.976	98.196
Isoleucine	2.715	7.815	4.806	2.601	3.209
Leucine	4.699	13.285	10.252	5.638	6.895
Tyrosine	2.712	7.416	6.065	1.421	4.429
Phenylalanine	2.840	8.249	6.165	2.310	2.828
Lysine	75.392	12.364	20.897	52.397	86.607
Histidine	108.313	19.954	19.085	62.167	91.115
Arginine	11.475	Trace amount	11.326	8.663	12.508

Values are expressed in µmole amino acid/100 gms of wet tissue.
All estimations were carried out in duplicate.

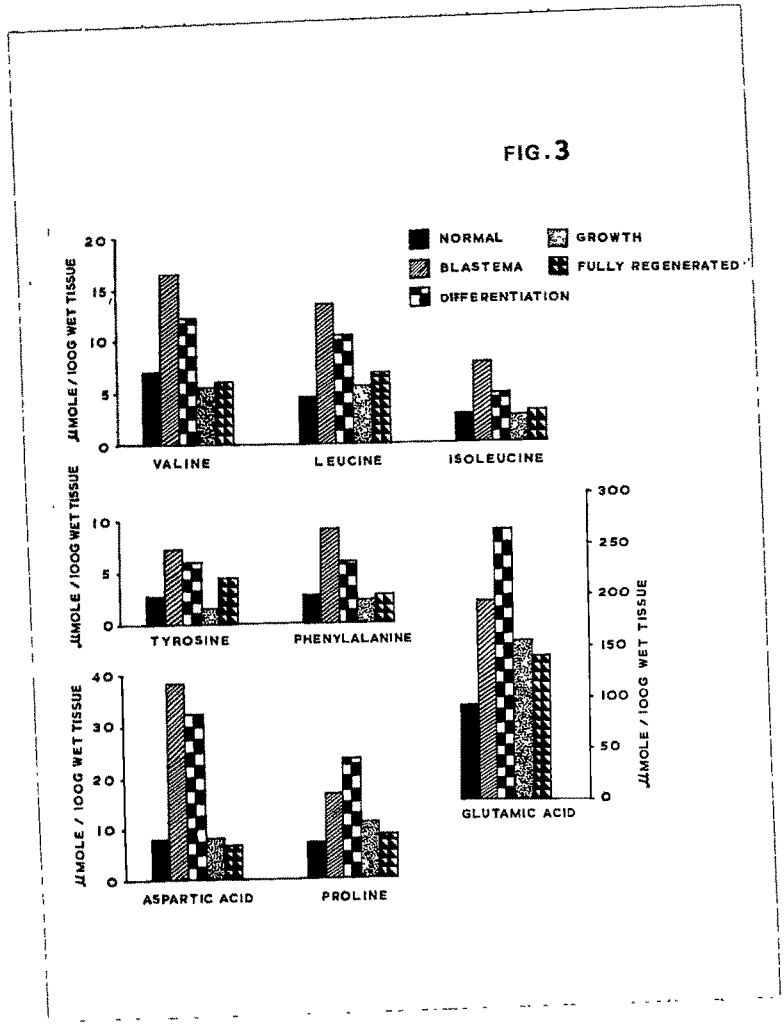

Fig. 3 : Graphic representation of levels of free amino acids in normal and regenerating tail, during different phases of tail regeneration in lizard, H. flaviviridis.

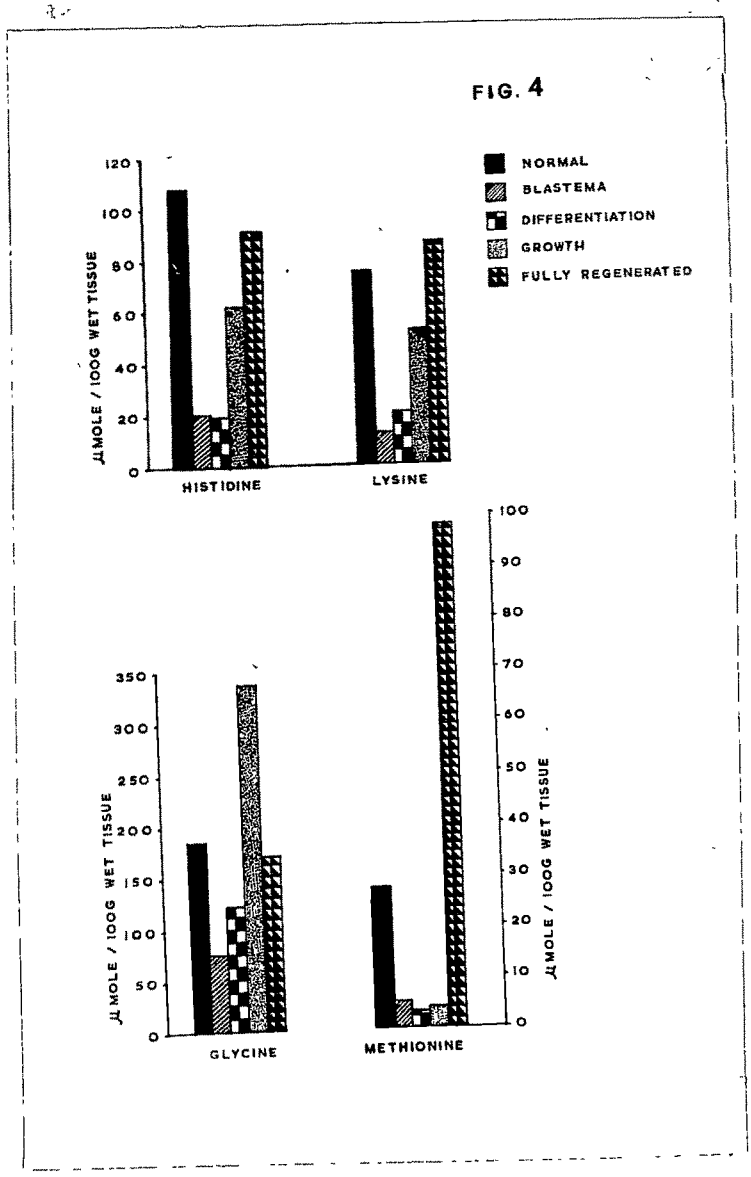

Fig. 4 : Graphic representation of levels of free amino acids in normal and regenerating tail, during different phases of tail regeneration in lizard, H. flaviviridis.

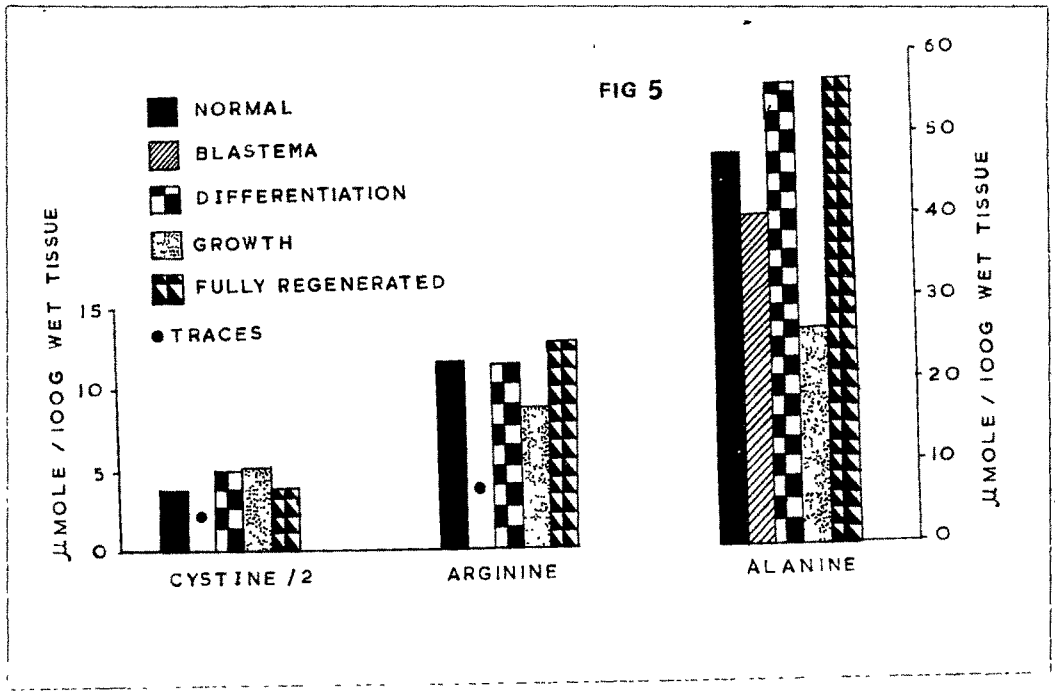

Fig. 5 : Graphic representation of levels of free amino acids in normal and regenerating tail, during different phases of tail regeneration in lizard, H. flaviviridis.

DISCUSSION

There are several reports in recent literature dealing with tissue culture and biochemical embryology which suggest that amino acids are of paramount importance in cell differentiation (Deuchar, 1966). Since the free amino acids are generally recognized as the principal currency for the protein synthesis, their adequate supplies to the developing cells are essential. Young growing animals have a characteristically high distribution ratio of tissue amino acids to those of extracellular fluid (Noll et al., 1957; Christensen et al., 1958). Tumour and tumour bearing animals have also shown higher levels of certain free amino acids (Zamecnik et al., 1948; Roberts and Frankel, 1949). Increase in levels of certain free amino acids during liver regeneration in rats, following hepatectomy has also been reported (Christensen et al., 1948; Roberts and Simonsen, 1962).

A significant quantitative difference in nitrogen of free amino acids, related to different phases of axolotl limb regeneration was observed by Vladeimirova (1934). This has been confirmed by Deuchar et al. (1957) in the regenerating tail of Xenopus tadpoles.

Orechowitsch and Bromley (1934) reported a rise of amino acids during early phase of blastema formation, due to the proteolytic activities in the injured tissues of the regenerating axolotl tail. Considerably high activities of hydrolytic enzymes have also been observed during lizard tail regeneration (Shah and Chakko, 1966; Radhakrishnan, 1972; Shah and Hiradhar, 1976; and Chapter 4). Presently observed elevation in concentration of aspartic acid, glutamic acid, proline, valine, isoleucine, leucine, tyrosine and phenylalanine (see Table 2, Fig. 3) during the blastemal phase from the normal level observed is probably contributed from the broken ends of the stump tissue where action of proteolytic enzymes is known to occur. Depletion of threonine + serine, glycine, methionine, lysine and histidine (Table 2, Fig. 4) during blastema formation suggests their utilization in formation of specific proteins during this phase. Blastemal phase is characterized by active cellular division and proliferation which later leads into differentiation of tissues in the regenerate (Shah and Chakko, 1968b). These activities would undoubtedly demand greater turnover of amino acids which may be utilized for formation of new proteins. Such is the case as observed in the elevation of certain free amino acids (aspartic acid, glutamic acid, proline, valine, isoleucine, leucine,

tyrosine, phenylalanine) during blastema and differentiation phases of tail regeneration during the course of the current study. Significant increase in their protein content in the regenerate during the blastemal and differentiation phases of the lizard tail regeneration, reported by Shah et al. (1977a) strengthens the above suggestion. The presently observed fluctuating levels of free amino acids in the regenerating tail of the lizards could be correlated with their involvement in the syntheses of new proteins. Similarly in amphibian regeneration also new proteins are synthesised which are electrophoretically separable (Schmidt, 1968).

Leucine is significantly involved in the embryonic development (Rose et al., 1948; Deuchar, 1966). Using tritiated leucine, in the study of amphibian limb regeneration, Anton (1961, 1965) reported that leucine uptake is much higher during blastema and differentiation phases, though relatively lesser in the latter phase. Leucine is strongly ketogenic and can be used for lipogenesis (Schmidt, 1968). Lipid oriented metabolism is suggested in the early phases of regeneration in amphibia (Schmidt, 1966a,b) as well as in reptiles (Chakko, 1968; Shah and Hiradhar, 1977). In light of high incidence of leucine

in the blastema and differentiating regenerates, its involvement in lipid oriented metabolism of the regenerate cannot be ruled out. Leucine is also incorporated into new proteins such as myosin of the muscle (Schultz and Herrman, 1958; Lucy and Rinaldini, 1959). Myogenesis is one of the prominent features of the differentiating tail regenerate (Shah and Chakko, 1968b). Hence involvement of leucine in this process too is quite understandable.

Manning and Meister (1966) and Hay (1965) reported proline as a precursor of hydroxyproline. Hydroxyproline is almost exclusively found in collagen (Gould, 1970). With these facts in view, the presently observed elevated levels of proline during differentiation, reflects its involvement in the collagen synthesis which is known to occur at this stage.

Association of nucleic acids with cell proliferation, and growth, and their importance in the processes of protein synthesis is quite well known. Increased levels of nucleic acids were found in H.flaviviridis during blastemal and differentiation phases (Shah and Chakko, 1972). In the syntheses of purine and pyrimidine bases, aspartic acid, glycine and glutamic acid are known to be

involved. The fluctuating levels of these amino acids during regeneration (Table 2) point to their possible involvement in the syntheses of nucleic acids.

Elevated levels of amino acids like serine + threonine, glutamic acid, methionine, tyrosine observed in the fully regenerated tail are probably due to their requirements to bring about the changes that are occurring in the histo-architecture of the regenerate. The sulfur released from sulfur containing amino acids such as methionine can be used in the mucopolysaccharide (MPS) synthesis (McGilvery, 1970). The fully regenerated tail has shown fairly high concentration of sulphated MPS in the cartilagenous neural canal (Shah and Hiradhar, 1975). Thus the involvement of methionine in such a reaction in the regenerating tail appears quite reasonable.

From the fore-going account it is suggested that fluctuations in levels of free amino acids observed during different phases of lizard tail regeneration are for providing required building blocks for syntheses of new types of proteins and/or other macromolecules characteristic of the specific phase.